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Susceptibility of Enamel Treated with Bleaching Agents to Mineral Loss After Cariogenic Challenge

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1. Introduction

As patients and consumers demand not only a healthy mouth but also a perfect appearance, vital bleaching of teeth has gained interest. It has been accepted as one of the most effective methods of treating discolored teeth and considered to be a conservative approach towards obtaining esthetic or cosmetic results rather than other methods such as veneering or crowning. Procedures that utilize different concentrations of carbamide peroxide (CP) or hydrogen peroxide (HP) have been commonly used by dentists as “in office” or by patients as “home bleaching” applications. Other than these, over the counter products have been widely used by patients; however they cannot be considered as one of the “bleaching treatments”.

The efficacy of bleaching is influenced by many factors like the type, concentration of the bleaching agent, time it is applied, application method used (heat, light, laser, etc.) cause of the stain and the condition of teeth. Procedures apparently rely on an extended period of contact between the bleaching agent and the teeth to accomplish the bleaching. The decomposition of hydrogen peroxide results in oxygen and per-hydroxyl free radicals that oxidize the stained macromolecules and break them down into smaller lighter colored fragments. Then the fragments diffuse across the tooth surface resulting in the bleaching effect (Haywood, 1992; Chen et al., 1993). The oxidation reaction should not exceed the saturation point in which the organic and inorganic elements of enamel and dentin are damaged. Otherwise, the crystals of mine matrix proteins lead to adverse changes in the morphology of the tooth surface and weakened structure (Haywood & Heymann, 1989; Goldstein&Garber, 1995). Studies (Seghi RR&Denry, 1992; Justino et al., 2004; Flaitz & Hicks, 1996; Spalding et al., 2003; Türkun et al., 2002; Bitter, 1992; Lopes et al., 2002; Hegedüs et al.,1999; Rotstein et al.,1996; Potocnick et al., 2000; Tezel et al., 2007.) have shown that bleaching agents can cause structural alterations on enamel surface and that the biomechanical properties of the enamel can change. In addition Basting and others (Basting et al., 2001) reported the possibility of formatting of active caries lesions after bleaching process since they diffuse through the enamel by demineralization.

2. Effects of bleaching agents on enamel surface

Calcium and phosphorus are present in the hydroxyapatite crystals, the main building block of dental hard tissue. Changes in Ca/P ratio indicate alterations in the inorganic components of hydroxyapatite. In the previous studies it has been shown that the bleaching agents cause calcium loss in hard dental tissues, change Ca/P ratio and surface alterations depending on their concentration. The linear relationship between decrease in enamel hardness and Ca^{2+} and PO_4^{3-} loss shows that hardness measurements can be used as an indication of the degree of enamel mineralization which relates to enamel caries. Ingram and Ferjerskov (Ingram & Ferjerskov, 1986) observed that macroscopically the degree of chemical attack roughly correlated with the appearance of discrete white spot lesions where approximately 7 μg or more calcium had been removed from the experimental area (1.77 mm^2). This means that when approximately 3.95 μg of calcium loss is observed in a surface of mm^2 , the surface cannot be remineralized.

Additionally, *in vitro* studies have shown a close correlation between the bleaching agent effects and the enamel surface changes (Zalkind et al., 1996; Titley et al., 1988). There are also some reports that bleaching agents promote chemical and microstructural changes in enamel similar to initial caries lesions but it has been noted that these alterations have no clinical significance. Demineralization process begins with the loss of calcium ions from the surface apatite crystals that form the bulk of three calcified dental tissues. Under normal circumstances, this loss of calcium (demineralization) is compensated by the uptake of calcium (remineralization) from tooth's microenvironment. This dynamic process of demineralization and remineralization take place more or less continually and equally in a favorable oral environment. In an unfavorable environment, the remineralization rate does not sufficiently neutralize the rate of demineralization, and thus caries occurs (Davidson et al., 1973). It has been reported that most of the bleaching agents caused changes in the levels of calcium phosphorus, sulfur and potassium in dental tissues and that bleaching agents may have a possible influence on active caries lesions in enamel and dentin (Rotstein et al., 1996; Basting et al., 2001). It is still a question whether the enamel would be more susceptible to cariogenic challenge after the bleaching process. Little is known about this issue. The controversial results of existing reports and the continuous appearance of new bleaching products that are on the market demand more research in this field.

This is particularly evident in a study demonstrated by Tezel et al (Tezel et al., 2007) who showed different amounts of Ca^{2+} loss from enamel surface after bleaching with three different concentrations of bleaching agents. Premolar teeth were divided into four pieces and each piece was bleached with one of the bleaching agents (38% HP, 35% HP with light and 10% CP) leaving one as a control. Then the specimens were covered with wax as to expose a round window area on 6.83 mm^2 and immersed in an artificial caries solution of acetic acid buffered with 0.34 M sodium acetate (pH=4). The buffer solution was refreshed every four days till the 16th day. The previous solutions were kept to be tested afterwards for their Ca^{2+} loss with atomic absorption spectrometer (AAS). The loss of Ca^{2+} in the groups 38% HP, 35% HP with light activation, 10% CP and the control were evaluated cumulatively every four days and at the end of the 16th day, 27.52±5.22 $\mu\text{g}/\text{ml}$, 25.15±4.99 $\mu\text{g}/\text{ml}$, 19.53±4.03 $\mu\text{g}/\text{ml}$ and 18.35±4.00 $\mu\text{g}/\text{ml}$ were obtained in total, respectively (Table 1, Fig. 1).

	N		Days 1-4	Days 5-8	Days 9-12	Days 13-16	Total
38% HP	10	Mean	5.22	4.91	6.46	10.93	27.52
		Std Dev	1.78	1.02	2.43	2.96	5.22
		Min.	1.37	3.23	2.81	5.54	20.88
		Max.	6.97	6.34	11.02	14.67	38.09
35% HP	10	Mean	5.72	4.42	6.27	8.74	25.15
		Std Dev	1.41	0.90	2.01	4.98	4.99
		Min.	3.86	3.23	2.81	3.72	19.13
		Max.	8.21	6.34	10.11	19.23	34.98
10% CP	10	Mean	4.10	3.61	4.17	7.64	19.53
		Std Dev	1.69	1.90	1.51	3.55	4.03
		Min.	1.99	1.37	1.89	4.63	14.20
		Max.	3.73	6.84	4.56	10.04	12.57
Control Group	10	Mean	3.48	3.42	3.89	7.55	18.35
		Std Dev	1.32	1.38	1.34	1.96	4.00
		Min.	1.37	1.37	1.89	4.63	12.76
		Max.	5.72	6.34	6.46	11.02	25.56

Table 1. Release of calcium (Ca²⁺) from the specimens after treatment with bleaching agents in mm² (µg/ml).

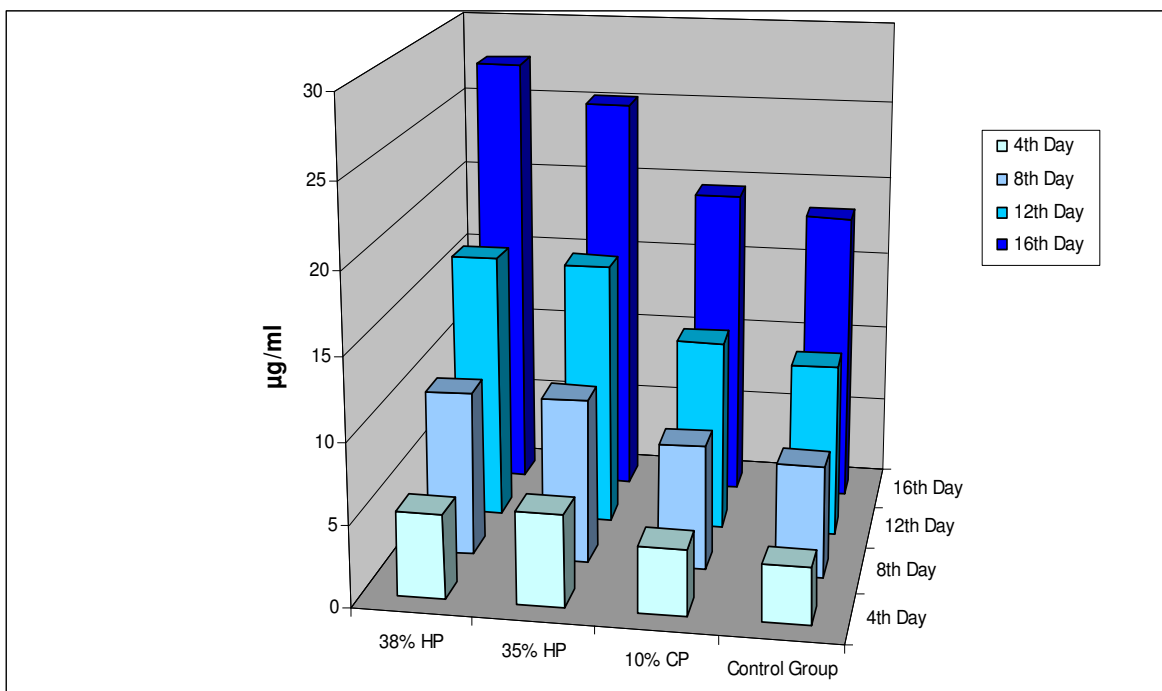


Fig. 1. Release of Ca²⁺ from the specimens to the buffer solution after treatment with bleaching agents on the 4th, 8th, 12th and 16th days cumulatively (µg/ml).

According to the statistical analysis, all the bleaching agents increased the Ca^{2+} in the solutions and all the agents other than CP were statistically different from the control group. And starting from the fourth day, the amount of Ca^{2+} release varied for the control and test groups. These differences are considered to be due to the variety of concentrations of the bleaching agents which were applied to the enamel. When 38% and light activated 35% HP groups were compared to the control group, it was observed that the difference Ca^{2+} loss was statistically significant and the high concentration of HP agents changed the surface morphology of the enamel. Thus, the resistance of enamel surfaces decreased in these groups when there was an acid attack. The use of HP or CP as bleaching agents reportedly decreased the Ca/P ratio on the enamel surface. It should also be mentioned that, HP was the only material that significantly reduced the Ca/P ratio in all these tissues. Most of the bleaching agents examined, caused changes of calcium, phosphorous, sulfur and potassium in the tissues. It was important to examine the effects of the oxidation agents on all the dental hard tissues because contact could occur during internal and/or external bleaching procedures, or they could inadvertently come into contact with dentin in carious lesions, enamel defects or abrasions.

In the group to which 10% CP was applied, a difference of Ca^{2+} loss was observed when it was compared to the control group after the 16th day, but this difference was not statistically significant (Table 2).

Materials	Days 1-4	Days 5-8	Days 9-12	Days 13-16	Total
38% HP X 35% HP	-	-	-	-	-
38% HP X 10% CP	-	*	*	*	*
38% HP X Control	*	*	*	*	*
35% HP X 10%CP	*	-	*	-	*
35% HP X Control	*	-	*	-	*
10% CP X Control	-	-	-	-	-

* Statistically significant differences between the groups ($p < .05$)
 - No statistically significant differences ($p > .05$)

Table 2. Statistical differences of the test groups in the end of the 4th, 8th, 12th and 16th days.

The reason why there occurred a close demineralization to the control group was due to low HP concentration of 10% CP. In accordance with this study, Haywood *et al* (Haywood *et al.*, 1990) reported no change in the surface morphology of the enamel with 10% CP, whereas, McGuckin *et al* (McGuckin *et al.*, 1992), Bitter & Sanders (Bitter & Sanders, 1993) observed slight surface modifications of teeth treated with 10% CP.

Tezel *et al* (Tezel *et al.*, 2007) also examined that there was a sudden rise of Ca^{2+} discharge on the 16th day (Table 1). When they compared the results, they have observed that Ca^{2+} releases on the 4th, 8th and 12th days were similar (Fig 1). The data had been measured twice with atomic absorption spectrophotometer, and the same results were obtained each time. So the sudden rise could not be related to the inaccuracy of the measurements. Thus, they have assumed that the organo-inorganic structure of enamel got weaker by the 12th day, reaching a critical point on the 16th day when sudden Ca^{2+} discharge had been observed.

In addition, it has been shown that the control and CP are similar in demineralization and HP is so different. The 10% CP disassociates into 3% HP and approximately 7% urea. The HP further degrades into oxygen and water, while the urea degrades into ammonia and carbon dioxide. The ammonia and carbon dioxide elevate the pH. Hence, a CP solution will supply urea and may raise the pH of solution when used clinically. In addition it was pointed out that after 15 minutes of treatment with CP the pH of saliva increases to greater than baseline because of chemical reactions to neutralization of acidic CP by saliva

Tezel *et al* (Tezel et al., 2007) claimed that the Ca^{2+} loss as a result of bleaching process can be named as "erosion" since the erosion is defined as "the physical result of a pathologic, chronic and localized loss of dental hard tissue which is chemically etched away from tooth surface by acid and/or chelation without bacterial involvement" (Imfeld, 1996). However it should also be mentioned that with in the presence of dental plaque, the outcomes could be very different.

There are published studies showing that the bleaching treatments can cause loss of mineral content which may widen the space between the enamel prisms. This can influence caries activity in cooperation with the increased surface roughness and gingival plaque formation (Flaitz et al., 1996; Quirynen & Bollen, 1995; Perdigo et al., 1998). As a result, *Streptococcus mutans* adhesion is included in the action which might be related to caries formation if undesirable conditions continue to develop (Hosaya et al, 2003).

Many researchers have reported the effects of different bleaching agents to dental hard tissues. Lewinstein *et al* (Lewinstein et al, 1994) pointed out that the microhardness of bleached enamel is decreased after bleaching with hydrogen peroxide and some have reported that there are no changes in the microhardness values of enamel when treated with 10% CP (Potocnick et al., 2000; Murchinson et al., 1992; Seghi & Denhy, 1992). There are also some studies which reported a decrease in microhardness values after application of these bleaching agents (Attin et al, 1997; Smidt et al., 1998; Rodrigues et al., 2001). It can be concluded that most of the bleaching agents cause alterations on the enamel surface and decrease in microhardness some causing grooves on the surface and even effect the inner surface (Hegedüs et al., 1999; Rodrigues et al., 2001).

The decomposition of HP results in oxygen and per-hydroxyl free radicals, which then oxidize the stained macromolecules and break them down into smaller fragments. Then the fragments diffuse across the tooth surface, resulting in the bleaching effect. To accelerate this reaction light sources such as blue halogen light, light-emitting diode (LED)-laser system, blue plasma arc lamp, argon laser, GAAIAs diode laser, ultraviolet light, ER:YAG laser and CO_2 laser are used (Sydney et al., 2002; Wetter et al., 2004; Zhang et al., 2007) But today, lights and lasers are the preferred activation methods. The use of activation methods has shortened the extensive period of time, which involves the direct contact of the high concentrated bleaching agents with the tooth surface that may cause a certain amount of enamel matrix degradation. A shortened treatment period may eradicate the side effects of high concentrated HP.

Tezel *et al* conducted an *in vitro* study (Tezel et al., 2011) aiming to compare the Ca^{2+} loss after activating the bleaching agents with halogen light and diode laser. The specimens were prepared with the same method of their earlier study. The bleaching agents (38% HP with halogen light activation, 38% HP with diode laser activation, and 10% CP) were applied according to manufacturers' instructions. Then the same artificial caries formation method was used. However this time in the study, inductively coupled plasma mass spectrometry

(ICP-MS) was used to measure the calcium ions released, which is a very precise method that can determine the very low concentrations of ions. When the results were examined, the Ca^{2+} ions released from CP group (12.88 $\mu\text{g}/\text{ml}$) was very close to the control group (11.97 $\mu\text{g}/\text{ml}$) and there was no statistical difference between them ($p>0.05$). The highest value was obtained from the 38% HP group with light activation (16.20 $\mu\text{g}/\text{ml}$) and this result was statistically higher than the other groups ($p<0.05$). Laser activated group (14.10 $\mu\text{g}/\text{ml}$) did not show any statistically different result than the CP group ($p>0.05$). However; it was statistically different than the control group ($p<0.05$) (Table 3, 4 and Fig. 2).

		Days 1-4	Days 5-8	Days 9-12	Days 13-16	Total
10% CP	Mean	3.03	3.28	3.30	3.26	12.88
	Std Dev	0.42	0.58	0.48	0.30	1.48
	Min.	2.55	2.60	2.83	2.80	11.04
	Max.	4.06	4.47	4.16	3.84	14.59
38% HP with light	Mean	3.66	4.19	4.24	4.10	16.20
	Std Dev	0.70	0.44	0.49	0.37	1.67
	Min.	2.54	3.58	3.66	3.69	13.79
	Max.	5.24	5.03	5.10	5.00	19.28
38% HP with diode laser	Mean	3.23	3.66	3.65	3.56	14.10
	Std Dev	0.59	0.55	0.66	0.55	2.16
	Min.	2.60	2.68	2.90	2.53	10.71
	Max.	4.52	4.57	4.67	4.47	17.72
Control Group	Mean	2.87	3.06	3.00	3.03	11.97
	Std Dev	0.30	0.43	0.32	0.20	0.87
	Min.	2.52	2.67	2.65	2.81	10.92
	Max.	3.54	3.82	3.69	3.38	13.56

Table 3. Release of Ca^{2+} from the specimens after treatment with bleaching agents (in $\mu\text{g}/\text{ml}$) (n=10 each group).

As mentioned during the study, the bleaching agents were used in accordance with manufacturers' protocols and these were also followed for the activation methods used. The aim was to follow the clinical protocol. According to the manufacturers' instructions, the chemical bleaching without activation is 15 minutes for three times. In total, the contact time for the bleaching gel throughout the study would have been 135 minutes. Activating the bleaching gel with halogen light reduced the contact time to 45 minutes and for the laser activated group to 36 minutes. It can be assumed that the higher concentration of HP could have caused more Ca^{2+} loss than the CP group, but due to laser activation which shortened contact time of the high concentrated bleaching gel, the calcium loss in the laser group was close to the CP group. The results of the study was in correlation with the other *in vitro* bleaching studies that Ca^{2+} loss was lower when lasers were used for the activation of the bleaching agents compared to halogen light.

Materials	Days 1-4	Days 6-8	Days 9-12	Days 13-16	Total
38% HP with light vs. 10% CP	-	*	**	*	*
38% HP with light vs. 38% HP with laser diode	-	-	-	*	*
38% HP with light vs. Control	*	*	**	*	*
38% HP with laser diode vs. 10% CP	-	-	-	-	-
38% HP with laser diode vs. Control	-	-	-	*	*
10% CP vs. Control	-	-	-	-	-

* Statistically significant differences between the groups for Bonferroni test (p<.05).
 ** Statistically significant differences between the groups for Dunnet C test (p<.05).

Table 4. Statistical differences of the test groups at end of days 4, 8, 12, and 16.

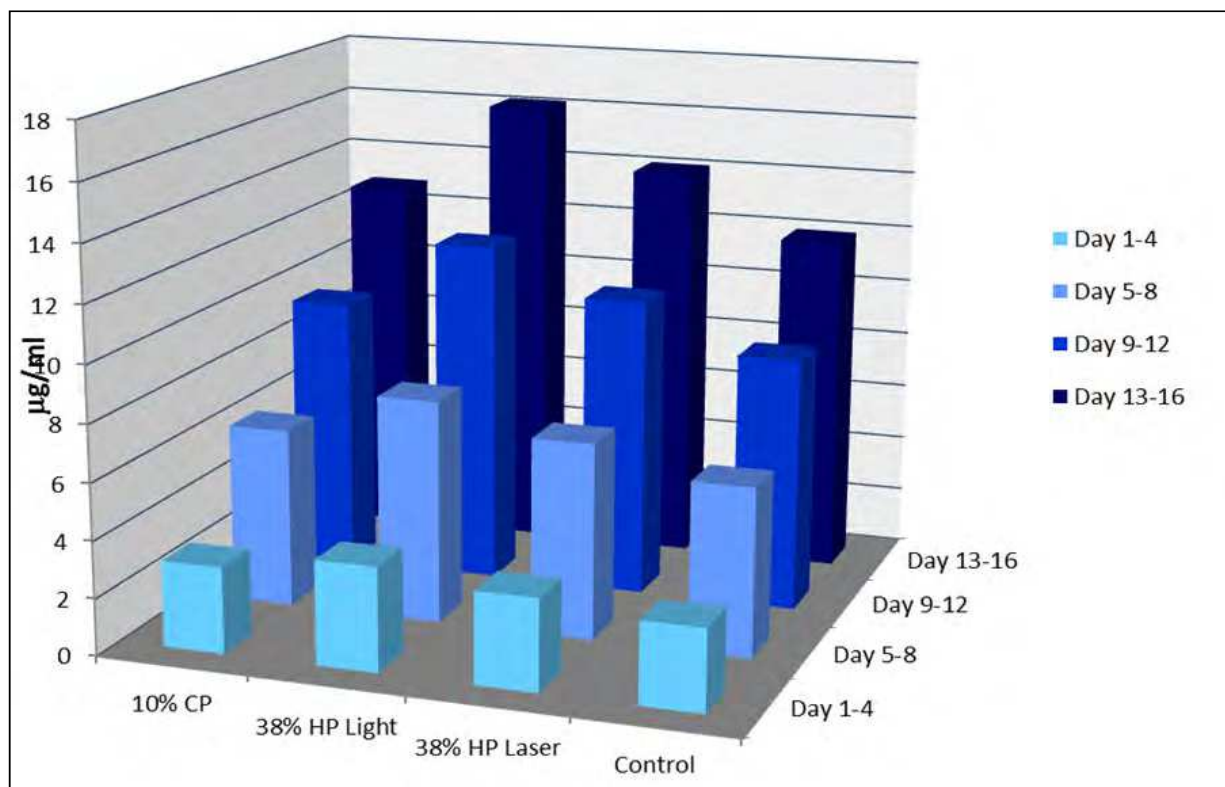


Fig. 2. Release of Ca²⁺ after treatment with bleaching agents (per mm², measured cumulatively).

These mentioned effects of bleaching agents with different concentrations and activation techniques on human enamel surface morphology were observed by Kemaloglu and Tezel using scanning electron microscopy (SEM; Fei, Quanta Feg 250). The purpose was to

compare the changes caused by the bleaching agents visually. Human impacted third molar teeth were rinsed in tap water and were cleaned off plaque and debris with a dental handpiece and brush. The buccal, lingual and occlusal surfaces were checked under a stereomicroscope, and teeth with enamel defects or cracks were rejected. The selected teeth ($n=5$) were stored in 0.9% saline solution for one week and then rinsed in distilled water. Each tooth was sectioned into five parts, so that five specimens were obtained from each tooth. These specimens were randomly assigned to one of the groups, ensuring that each part of every specimen would be in a different group. Teeth were then covered with wax except for the enamel surface. The groups were as follows:

Group 1 (positive control): No agent was used and they were kept in artificial saliva during the test period.

Group 2 : Treated with 37% orthophosphoric acid (ScotchBond Phosphoric Etchant Kit, 3M ESPE, USA) for 30 seconds.

Group 3: Bleached with 10% carbamide peroxide (CP) (Opalescence PF 10% CP, Ultradent Products Inc, South Jordan, USA) for 8 hours a day, throughout 14 days.

Group 4: Bleached with 38% hydrogen peroxide (HP) (Opalescence Boost 38% HP, Ultradent Products Inc, South Jordan, USA) with light activation for 15 minutes. This procedure was repeated every other day for 3 days.

Group 5: Bleached with 38% HP (Opalescence Boost 38% HP, Ultradent Products Inc, South Jordan, USA) with diode laser activation (LaserSmile, Biolase, USA) in 10 Watt-continuous mode for 12 minutes. This procedure was repeated every other day for 3 days.

Following every session, the bleached teeth were rinsed, dried and topical fluoride agent (Flor-Opal 1.1% NaF, Ultradent Products Inc, South Jordan, USA) was applied for 10 minutes. After the application of the bleaching agents for the prescribed time, the specimens were anticipated in artificial saliva for 12 days to mimic the *in vivo* remineralization condition. Then the specimens were rinsed ultrasonically with water for ten minutes and prepared for scanning electron microscope. After dehydration, enamel surfaces were sputter coated with gold (~ 30-35 nm) and photomicrographs of representative areas were taken at 5000x magnifications. The enamel changes were classified as no alterations, mild or slight alterations and altered surfaces (loss of superficial structure).

A representative SEM image of sound enamel surface stored in artificial saliva (positive control group) is shown in Figure 3. There were no remarkable morphologic alterations on unbleached enamel surfaces. The surface was not completely smooth, however the aprismatic surface layer was uniform. Perikymata was evident all over the surface. In addition, pores could easily be seen and there were some areas that had cracks.

In the second group, the acid-etched samples had a rough and uneven surface, which indicates alterations of the prismatic structure of the enamel due to selective dissolution of the apatite crystals (Figure 4). Formation of an irregular meshwork and dissolution in central (intraprismatic) or peripheral (interprismatic) part of the prism take place as a result of demineralization.

Bleached groups showed alterations on surface smoothness and presented different levels of surface changes. Minor changes of the enamel surface occurred in samples treated with 10% CP for 8 hours daily for 14 days (Figure 5). This aspect suggests a slight increase in the

enamel porosity, as compared to the control samples. Mildly changed areas and the noted interprismatic limits are show the surface change.

Mild intraprismatic structure dissolution occurred on the surface treated with 38% HP with light activation (Figure 6). The surface alterations were much more significant than the other bleaching groups. Porosity and concavity of the enamel structure increased due to intraprismatic dissolution.

Minor alterations on surface smoothness and mildly increased porosity occurred in the teeth bleached with 38% HP with laser activation (Figure 7). Interprismatic dissolution could clearly be observed. These changes were similar to the image of 10% CP group (Figure 5), but additionally the deposits on the surface were also noted (Figure 7).

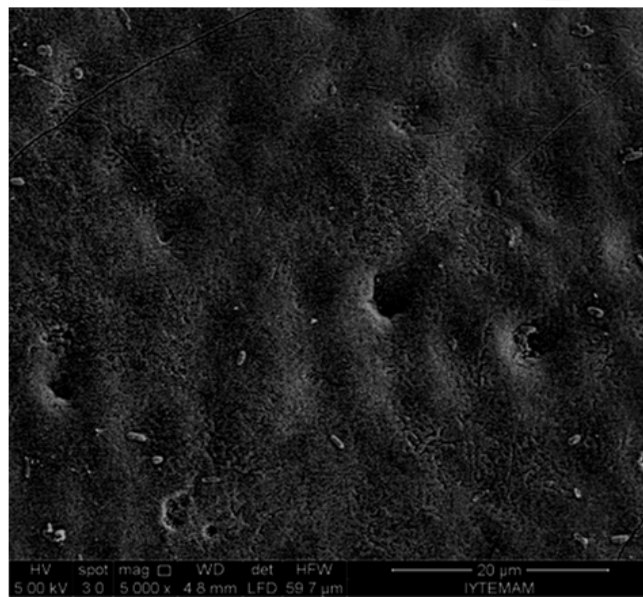


Fig. 3. SEM micrograph of the sound enamel surface

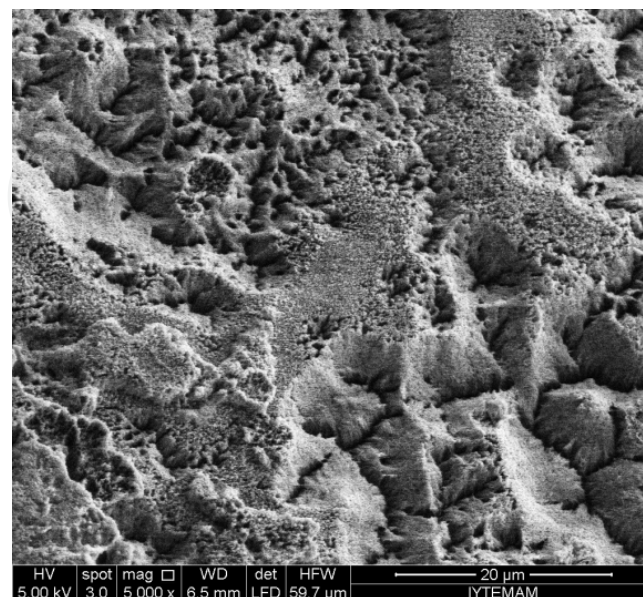


Fig. 4. SEM micrograph of enamel surface treated with 37% orthophosphoric acid

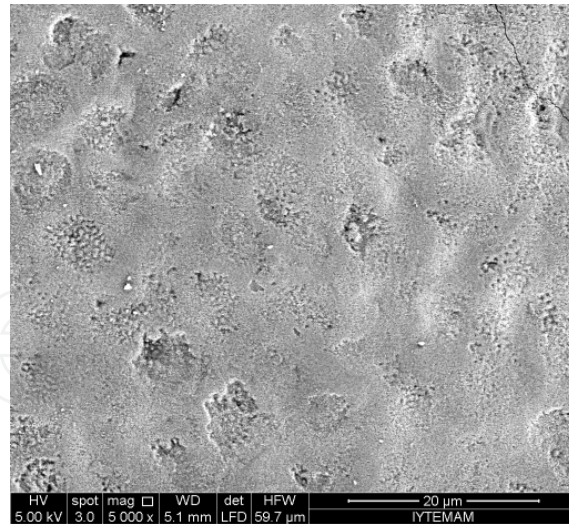


Fig. 5. SEM micrograph of enamel surface treated with 10% CP

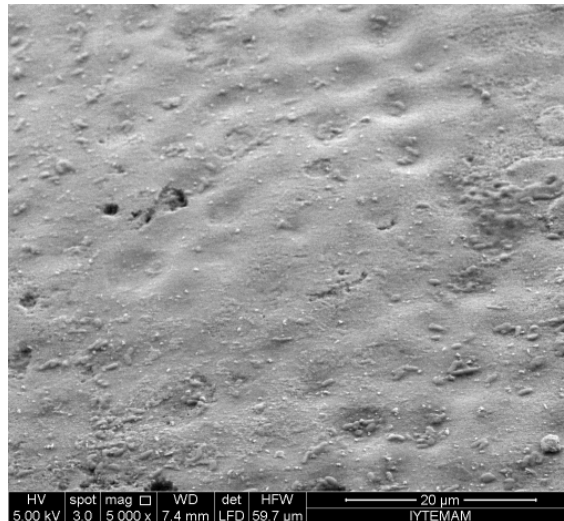


Fig. 6. SEM micrograph of enamel surface treated with 38% HP with light activation

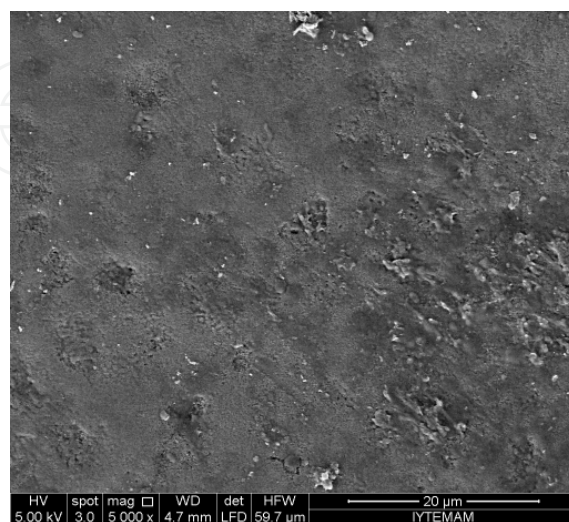


Fig. 7. SEM micrograph of enamel surface treated with 38% HP with diode laser activation

It can be concluded that the Ca^{2+} loss results and surface alterations observed in SEM images were in compliance.

3. Effect of pH values of the bleaching agents on mineral loss of enamel

The physical and chemically soundness of the enamel depends on the pH and the saliva consisting of calcium phosphate and fluoride. Caries lesions develop with the fermentation of carbohydrates by bacteria's, the formation of organic acids and the pH decrease. The critical pH value for the enamel is $\text{pH} = 5.5$ and when the oral pH decreases below this value, the bands between the fibrils and apatite of the enamel dissolve and the inorganic structure is affected (Axellson, 2000). In a study (Mcgucking et al., 1992) the enamel surface of bleached teeth were examined with a scanning electron microscope and a profilometer. The results showed that the enamel surface was affected by different concentrated bleaching agents, but these differences were not related with the pH values of the agents. When Tezel *et al* (Tezel et al., 2011; Tezel et al., 2007) measured the pH values of the bleaching agents used in their study with a pH meter it had been found that the pH was approximately 8 for each group. The pH values of bleaching materials were almost similar in the study, but the Ca^{2+} losses of the groups were found to be different. For this reason, they postulated that the pH values of the bleaching agents might not be effective on the Ca^{2+} loss of the groups and that the results were compatible with the study mentioned above.

4. Effects of topical fluoride agents on enamel surface in cooperation with bleaching agents

Considering enamel abrasion, the calcium/phosphate precipitation from saliva results in hardening of porous enamel which gradually may return to its initial situation if the process continues. The amount of the fluoride in the tooth's surface is also an important factor for the changes that could occur after the bleaching process. It has been shown that fluoride has the potential to inhibit demineralization which means that less surface changes would occur. To avoid these unfavorable effects of bleaching treatments, it is recommended to use fluoride containing remineralization agent incorporation with bleaching agents in order to decrease surface solubility (Akal N et al., 2001; Chen et al., 2008)

Taking this suggestion into consideration Tezel *et al* (Tezel et al., 2011) used artificial saliva to reflect the oral conditions and "after bleaching mousse" containing fluoride to maintain clinical schedule in their study mentioned above. This procedure gave them the potential effects of the topical fluoride agent for remineralization. As mentioned before, fluoride has been admitted to remineralize softened enamel by increasing resistance to acid attacks by forming a calcium fluoride layer to inhibit demineralization (Attin et al., 1997; Ten Cate & Arends, 1977; Featherstone et al., 1982). It accumulates in the plaque fluid and as calcium fluoride on the enamel surface. During the acid challenge, calcium fluoride is dissolved (Axellson, 2000). It may be a question if the calcium loss could be from the dissolved calcium fluoride or not. Fundamentally, the source of calcium for calcium fluoride is from the enamel. Depending on this fact, the measured calcium loss after the acidic challenge should be from the enamel either directly or indirectly from the dissolved calcium fluoride. Nevertheless, further studies are required to estimate this fact.

In another study (Tezel et al., edited to be published) which is still being edited to be published, the effects of different fluoride agents on the caries-like lesion formation and Ca^{2+} loss from the enamel surfaces after bleaching with 38% hydrogen peroxide have been examined. Teeth were divided into four pieces and randomly divided into four groups; three of them being the test groups and the last one being the control. The test groups were then bleached with 38% hydrogen peroxide leaving the control group untreated. Then two out of three test groups were treated with fluoride agents with different concentrations; 1% titanium tetrafluoride (TiF_4) and 1.1% sodium fluoride (NaF). Immediately after the application of the bleaching and fluoride agents for prescribed time, the specimens of each group were subjected to erosive demineralization with acetic acid buffered with 0.34M sodium acetate (pH=4). The specimens were demineralized four times for four days. The amount of Ca^{2+} released from the specimens was detected with atomic absorption spectrometer. When the results were examined it was clearly observed that there was a decrease in the Ca^{2+} release in the fluoride-treated groups after bleaching. When these two groups (TiF_4 and NaF) were compared, it was determined that at the end of the test period (16 days) the amount of Ca^{2+} in the buffer solution of TiF_4 -treated samples was less than that of NaF -treated samples and the difference was statistically significant (Table 5, 6). Regarding this result, it can be assumed that TiF_4 may be effective in preventing the bleached enamel surface against the acid attacks. Furthermore, there was no Ca^{2+} release from three specimens during the first four days, and during the second 4-day interval. This result can be a result of the glaze layer formed just after the application of TiF_4 .

	N		4 th Day	8 th Day	12 th Day	16 th Day	Total
Control Group	10	Mean	3.59	3.20	3.72	4.55	15.07
		Std Dev	0.54	0.59	1.23	0.89	1.81
		Min.	3.01	2.46	2.46	2.74	12.60
		Max.	4.93	4.38	6.58	5.75	18.36
38% HP	10	Mean	5.75	5.18	5.56	5.95	22.44
		Std Dev	1.60	1.52	0.71	0.37	2.52
		Min.	3.83	3.01	4.66	5.48	18.36
		Max.	7.95	8.50	6.85	6.58	27.68
38% HP+ NaF	10	Mean	2.05	3.15	3.94	4.52	13.67
		Std Dev	0.83	0.58	1.01	0.83	1.86
		Min.	0.82	2.19	2.46	3.29	10.68
		Max.	3.01	4.11	5.75	6.03	15.61
38% HP+ TiF₄	10	Mean	1.15	1.94	2.66	3.37	9.12
		Std Dev	0.92	1.54	0.63	0.48	2.40
		Min.	0	0	1.64	2.46	4.93
		Max.	2.46	4.11	3.56	3.83	11.50

Table 5. Ca^{2+} release from the bleached specimens treated with 1% TiF_4 and 1.1% NaF in mm^2 ($\mu\text{g}/\text{ml}$).

In a previous study, Tezel *et al* (Tezel et al., 2002) reported that TiF_4 was found to be more effective than Duraphat (NaF, 2.26% F) or Elmex (amine fluoride, 1.25% F) in preventing artificial enamel lesion formation. Attin *et al* (Attin et al., 1999) reported that, fluoridation was effective in increasing resistance of enamel against demineralization by erosive substances. Similarly, the findings of this present study demonstrated that the resistance of enamel against the erosive demineralization caused by 38% HP application was increased after 1% TiF_4 treatment.

When the Ca^{2+} losses from the test groups which were bleached with 38% HP were compared, there was also a decrease in Ca^{2+} losses of 1.1% NaF treated group indicating that NaF could also prevent enamel surfaces against acid attacks during the first four days ($p < 0.05$). Addition of sodium fluoride to hydrogen peroxide solutions leads to formation of fluoridated hydroxyapatite and calcium fluoride when applied on hydroxyapatite samples (Tanizawa, 2005). In the present study, although NaF was effective against acid attacks on enamel surfaces, its influence was not as strong as TiF_4 (Table 6; Figure 8).

Materials	4 th Day	8 th Day	12 th Day	16 th Day	Total
Control X 38% HP	*	*	*	*	*
Control X 38% HP + NaF	*	NS	NS	NS	NS
Control X 38% HP + TiF_4	*	NS	NS	*	*
38% HP X 38% HP + NaF	*	*	*	*	*
38% HP X 38% HP + TiF_4	*	*	*	*	*
38% HP + NaF X 38% HP + TiF_4	NS	NS	*	*	*

* Statistically significant differences between the groups ($p < .05$)
NS No statistically significant differences ($p > .05$)

Table 6. Statistical differences between test groups.

Different topical fluoride agents (sodium fluoride, acidulated phosphate fluoride, stannous fluoride, amine fluoride or titanium tetrafluoride) to human tooth enamel are widely used in caries prevention. Topically applied fluoride may reduce the solubility of the surface enamel, render the tooth surface harder, more resistant to demineralization, and more prone to remineralization (Skartveit et al., 1990). The inhibiting effect of sodium fluoride on caries is well documented and a protective effect against dental erosion has been shown *in vitro* (Sorvari et al., 1994; Ganss et al., 2001). Fluoride varnishes provide long contact periods between the dental tissues and the fluoride agent resulting in high fluoride uptake and the formation of calcium fluoride deposits that act as fluoride reservoirs (Arends J & Schuthof, 1975; Grobler et al., 1983; de Bruyn, 1987; Petersson, 1993). It has been reported that during the application of titanium tetrafluoride, a glaze layer was formed on the tooth surface (Mundorff et al., 1972). TiF_4 has been shown to reduce artificial caries lesion formation and enamel solubility enabling high fluoride uptake (Tezel et al., 2002; Büyükyılmaz et al., 1997).

Generally, fluoride uptake in demineralised enamel is higher when compared to sound enamel (Attin et al., 2006) It is assumed that the porous structure of the demineralised enamel allows better diffusion and penetration of the applied fluoride and that the porosity

offers a higher number of possible retention sites for the fluoride. The application of highly concentrated fluoride favors the formation of the calcium-fluoride like layer (Attin et al., 1977). This deposit is later dissolved, allowing fluoride diffuse into the underlying enamel, the saliva, or a plaque layer covering the tooth. It is assumed that some of the fluoride is supporting the remineralization of the enamel. The results of a previous study confirmed that the calcium fluoride layer on the enamel was coated by phosphates and proteins from saliva as a pH-controlling reservoir that acts to decrease demineralization and promote remineralization (Rolla&Saxegaard, 1990).

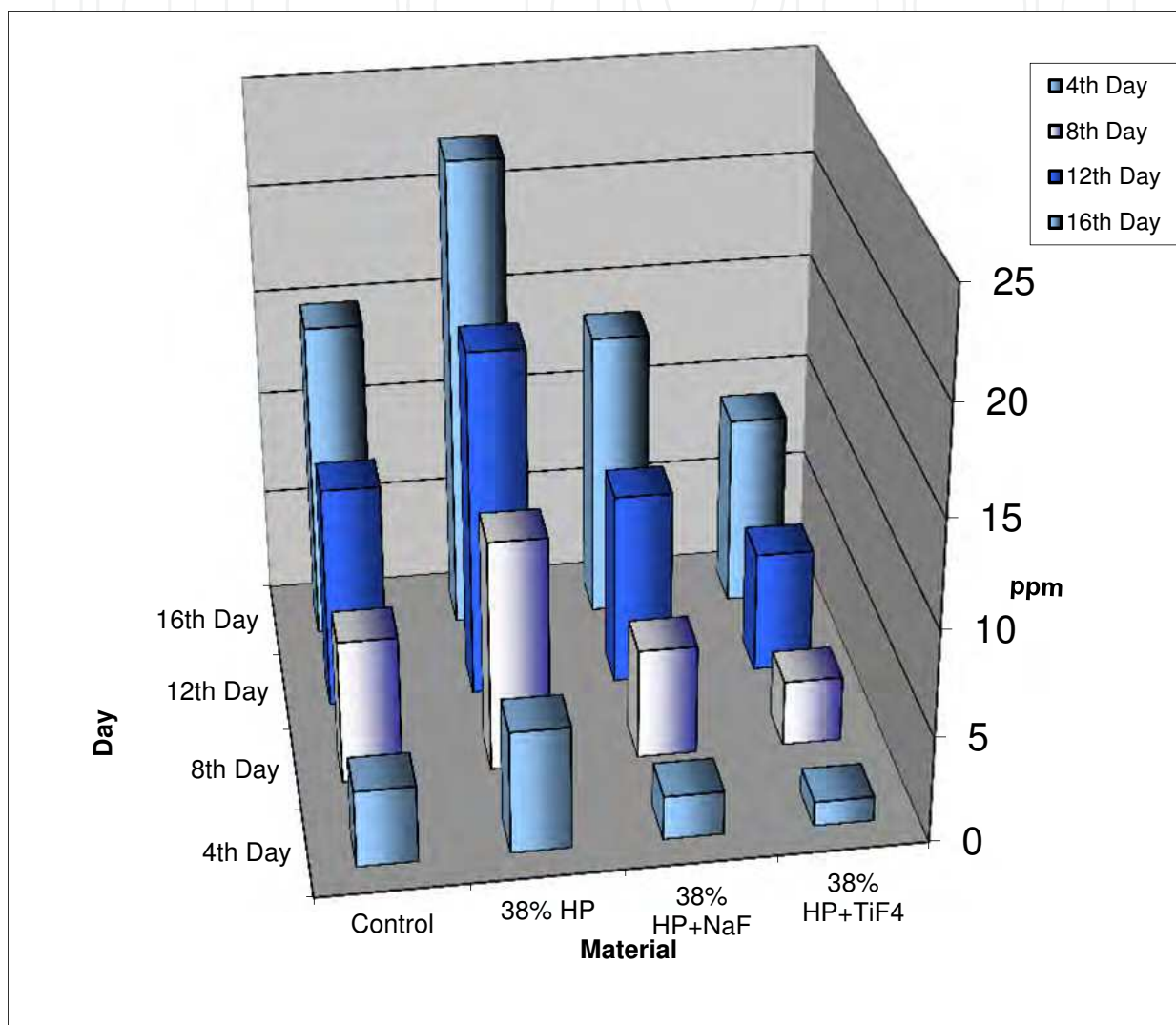


Fig. 8. Cumulative calcium (Ca^{2+}) release from the bleached specimens in the buffer solution after treatment with 1% TiF_4 and 1.1% NaF on the 4th, 8th, 12th and 16th days ($\mu\text{g}/\text{ml}$).

VanRijkom *et al* (VanRijkom et al., 2003) compared the erosion-inhibiting effect of the topical fluoride treatment based on the deposition of CaF_2 -like material using 1% NaF and 4% TiF_4 . It was concluded that the reduction of Ca^{2+} loss was more stable for TiF_4 than the NaF group and the reduction appeared to be smaller for the longer acid exposure times. Recently, Magalhães *et al* (Magalhães et al., 2008) have stated that a TiF_4 varnish showed better results than 2 commercial NaF varnishes in reducing enamel erosion. Based on the results of Tezel

et al's (Tezel et al., edited to be published) study, it was shown that topical fluoride applications decreased Ca^{2+} loss from the 38% HP treated enamel surfaces. It may be concluded that the application of fluoride agents may reduce the risk of erosion-like lesions caused by bleaching and remineralize the bleached enamel surfaces. The findings of this *in vitro* study demonstrated that TiF_4 may act better than NaF solution in preventing acid attacks.

5. Conclusion

As a conclusion, considering the conditions tested, the changes in enamel were directly proportional to the treatment time and peroxide concentration. According to the methodologies used in these studies, higher concentrations of HP caused more Ca^{2+} loss than lower concentrations. The contact time of high concentrated bleaching agents may also be an important factor for Ca^{2+} loss. A recommendation to use activation methods which shorten the contact time of the highly concentrated bleaching agents can be used in the dental office. But it must still be mentioned that 10% CP would be the safest method. In addition, to avoid the unfavorable effects of bleaching treatments, it is recommended to use topical fluoride agents incorporation with bleaching agents to take advantage of remineralization process.

The findings of these *in vitro* studies may not be representative of the *in vivo* condition; in which the oral cavity is continually bathed with saliva that contains various minerals (*i.e.* fluoride, calcium phosphate), lipids, carbohydrates and proteins. They also do not represent unfavorable conditions where the deficiency of saliva or poor oral hygiene that might increase the caries risks. Further studies are needed to clarify the effects of these materials on Ca^{2+} loss of enamel and caries susceptibility.

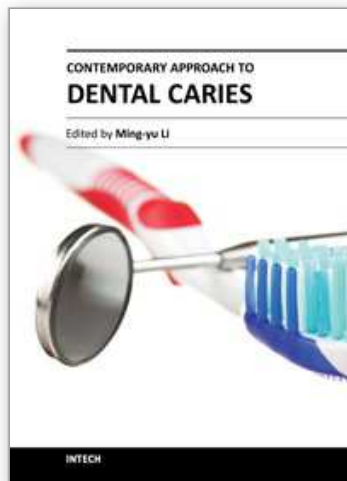
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With an update of the recent progress in etiology, pathogenesis, diagnosis, and treatment of caries, it may be said that the final defeat of dental caries is becoming possible soon. Based on the research in this area in recent decades, "Contemporary Approach to Dental Caries" contained the caries in general, the diagnosis of caries, caries control and prevention, the medical treatment of caries, dental caries in children and others such as secondary caries. This book provides the reader with a guide of progress on the study of dental caries. The book will appeal to dental students, educators, hygienists, therapists and dentists who wish to update their knowledge. It will make you feel reading is profitable and useful for your practice.

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