The Effects of Titanium Tetrafluoride and Sodium Fluoride on Calcium Loss from Bleached Enamel

Hande KEMALOĞLU: Ege University, Faculty of Dentistry, Department of Restorative Dentistry, 35100 Bornova, Izmir, Turkey; handedalgar@gmail.com

Zeynep ERGÜÇÜ: Ege University, Faculty of Dentistry, Department of Restorative Dentistry, 35100 Bornova, Izmir, Turkey; zergucu@yahoo.com

Hüseyin TEZEL: Ege University, Faculty of Dentistry, Department of Restorative Dentistry, 35100 Bornova, Izmir, Turkey; tezelhuseyin@gmail.com

Corresponding author:

Dr. Hande Kemaloğlu

Address: Ege University School of Dentistry, Department of Restorative Dentistry

35100 Izmir – Turkey.

Phone: +90 232 311 2887 Fax: +90 232 388 0325

Email: handedalgar@gmail.com
Abstract

Background: Application of topical fluoride agents has been shown to be the most effective method in treating demineralized enamel after in-office bleaching treatments. Thus, this study aimed to examine the effects of two different post-bleaching fluoridation agents (1.5% titanium tetrafluoride and 2.1% sodium fluoride) on the calcium loss of enamel after an acidic challenge.

Methods: Ten maxillary premolars were sectioned into four pieces each, to be assigned to one control and three treatment groups. After bleaching the specimens in three test groups with 38% hydrogen peroxide, two out of the three test groups were treated with fluoride agents with different concentrations; 1.5% titanium tetrafluoride and 2.1% sodium fluoride. Then all specimens were subjected to a demineralization process four times for four days to be tested after 16 days for their calcium loss with atomic absorption spectrophotometer. The calcium released to buffer after the 4th, 8th, 12th and 16th days were compared with Repeated Measures ANOVA and Bonferroni as Post Hoc Tests.

Results: The calcium released from the fluoride-applied groups was lower when compared to the 38% hydrogen peroxide and control group. At the end of the 16th day the amount of calcium released from titanium tetrafluoride-treated samples were less than sodium fluoride-treated samples.

Conclusions: Fluoride application decreased the amount of calcium released after bleaching with 38% hydrogen peroxide. Titanium tetrafluoride treated samples released less calcium than sodium fluoride treated samples

Clinical Relevance: The application of topical 1.5% titanium tetrafluoride and 2.1% sodium fluoride agents might be beneficial after the bleaching process with 38% hydrogen peroxide.

Key words: Demineralization, Sodium Fluoride, Titanium Tetrafluoride
Background

Bleaching has been accepted as one method of treating discolored teeth. Recently, novel in-office bleaching products that use high concentrations of hydrogen peroxide (HP) have made in-office treatments easier. However, the effects of these products on enamel are still an open issue and need to be elucidated.

When vital teeth are bleached, due to the direct contact between the bleaching agent and the outer enamel surface, enamel surfaces of the tooth crown can be affected by high levels of HP in bleaching agents, causing structural and morphological changes. There are many studies on reduction of microhardness as well as the ones on loss of calcium from bleached enamel.\(^1\text{-}^4\)

Demineralization is the loss of mineral content from tooth surface. Dental caries process begins with the loss of calcium ions (Ca\(^{2+}\)) from the surface apatite crystals of dental hard tissues. In normal conditions, there is equilibrium between the loss (demineralization) and the retake of calcium (remineralization).\(^5\) When this process shifts towards the demineralization in unfavorable conditions, the first clinical sign of dental caries appear.

Different topical fluoride applications such as sodium fluoride (NaF), acidulated phosphate fluoride (APF) and stannous fluoride (SnF\(_2\)) are widely used in enamel caries prevention. However, unlike the commonly used agents, titanium tetrafluoride (TiF\(_4\)), has been discussed to have greater effect on enamel remineralization in literature. Furthermore, their use after bleaching procedures has been shown to be also beneficial.\(^2\text{-}^6,^7\)

As topical fluoride is applied following bleaching, mineral loss is significantly reduced, microhardness is restored and the resistance of enamel to demineralization is increased.\(^4,^7,^8\)

Fluoride has been admitted to remineralize lesions by increasing resistance to acid attacks by forming a calcium fluoride layer to inhibit demineralization.\(^6\) In addition, formation of a glaze layer have been shown when enamel surfaces were treated with low pH TiF\(_4\).\(^9,^{10}\) However, there is no data available in literature on the preventive effect of these applications to further demineralization. Thus, the aim of this present study was to examine the effects of two different post-bleaching fluoridation agents (NaF and TiF\(_4\)) on the Ca\(^{2+}\) loss of enamel after an acidic challenge. The hypothesis was that there would be less Ca\(^{2+}\) loss from the bleached enamel surfaces, which were fluoridated with NaF and TiF\(_4\) solutions after being subjected to acidic attacks, thus these fluoride agents will reduce the susceptibility of bleached enamel to demineralization.
Methods

The study was approved by “Ege University Faculty of Medicine Research Ethics Committee” and written informed consent was received from participants.

Sample Preparation

Ten maxillary premolars extracted for orthodontic purposes at Ege University Faculty of Dentistry were selected for this in vitro study. All participants have given written consents prior to extraction process. Extracted teeth were rinsed in tap water, 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. Ten selected teeth were stored in 0.1% thymol for one week in +4ºC to eliminate the reproduction of microorganisms and then rinsed in distilled water. Each tooth was sectioned bucco-lingually or bucco-palatinally into two halves with a diamond disc. These halves were then sectioned longitudinally into two parts, so that four specimens were obtained from each tooth. These specimens were later, randomly assigned to one of the four groups, on the condition that each part of every tooth would be in one of the four different groups. Then, teeth were covered with wax except for the enamel surface (Table 1, Fig 1).

Bleaching Procedure

All specimens in three of the test groups were treated with a bleaching agent of 38% hydrogen peroxide (HP) (Opalascence Xtra Boost, Ultradent, USA) according to manufacturers’ instructions. The untreated specimens in the fourth group were used as a control group and kept in artificial saliva during the test period. A thick layer (~1 mm) of 38% HP (pH ≅ 7) was applied to enamel surfaces of the specimens in the test groups (Table 1). To have the optimum effectiveness, the bleaching gel was stirred/agitated every 5 minutes and refreshed every 15 minutes. Total time of application was 45 minutes a day. This procedure was repeated every other day for 3 days. After removing the whitening gel, the teeth were rinsed, dried and kept in artificial saliva until the next procedure.

Fluoride Pretreatment

Two out of the 3 test groups were treated with two different fluoride agents with approximately the same concentrations; 1.5 % TiF₄ (Aldrich Chem. Co, Milw, WI, USA) (pH= 1.2) and 2.1% NaF (Merck,
Switzerland) (pH=1.2). They were applied for 60 seconds utilizing a pipette while the third test group was left untreated and kept in artificial saliva during test period after the bleaching process.

Demineralization Process

Immediately after the application of the bleaching and fluoride agents for the prescribed time, the specimens were rinsed with a water spray and dried with blasts of air. Then the enamel was covered with standard “o” shaped wax so as to expose a standard round window area (6.83mm²) and acetic acid buffered with 0.34M sodium acetate (pH=4) was used as a demineralization buffer. Salt of calcium monohydrate [Ca\((H_2PO_4)_2H_2O\)] was dissolved to obtain 10 mmol/l Ca\(^{2+}\) and 20 mmol/l PO\(_4^{3-}\) in the solution.

Each specimen was treated with 50ml of solution in the polyethylene test tubes. The specimens were demineralized in four consecutive periods of four days. At the end of the 4\(^{th}\) day, each specimen was taken out from each test tube and placed in new tubes, which contained fresh buffer solution (Fig 1). The previous solutions were kept in their tubes to be tested afterwards for their Ca\(^{2+}\) concentration using atomic absorption spectrophotometer, as performed in previous studies.

Calcium analysis with atomic absorption spectrometer (AAS)

0.1ml of each demineralization solution was diluted with 4.9 ml of distilled water. To prevent the interaction of magnesium and phosphate ions, 50000 mg/l of lantana chlorine (LaCl\(_2\)) was added to each test tube to end up with 10% of LaCl\(_2\) in each buffer solution. The same procedure was applied to blank and standard solutions of calcium. The amount of calcium concentration of the samples was detected with an atomic absorption spectrophotometer, Varian Spectra-10 plus AA (wavelength: 422.7nm; slit 0.5nm) (Varian, Victoria, Australia). The calcium released to buffer after the 4th, 8th, 12th and 16th days were compared with Repeated Measures ANOVA and Bonferroni as Post Hoc Tests.
Results

The Ca\textsuperscript{2+} concentrations of the samples were measured at the end of the 4\textsuperscript{th}, 8\textsuperscript{th}, 12\textsuperscript{th} and 16\textsuperscript{th} days (Table 2, Fig 2). The loss of Ca\textsuperscript{2+} in the groups Control, 38% HP, 38% HP + NaF and 38% HP + TiF\textsubscript{4} were evaluated cumulatively every 4 days, and in the end of the 16th day, 15.07±1.81 µg/ml, 22.44±2.52 µg/ml, 13.67±1.86 µg/ml and 9.12±2.40 µg/ml were obtained in total, respectively.

The loss of Ca\textsuperscript{2+} in each of the test groups was compared with that of the control group using the “Repeated Measures ANOVA”. A statistically significant difference was observed among the groups after 4, 8, 12, and 16 days and total Ca\textsuperscript{2+} loss (p<0.05). The Bonferroni test was used as post-hoc test to identify possible statistically significant differences between the groups (Table 3).

After bleaching, there was significantly less Ca\textsuperscript{2+} released in the fluoride-treated group than for the bleached-only group (38% HP) and control group.

When the NaF and TiF\textsubscript{4} treated samples were compared, there were no significant differences between the amounts of Ca\textsuperscript{2+} released from the specimens after the 4\textsuperscript{th} and 8\textsuperscript{th} days. After the 12\textsuperscript{th} and 16\textsuperscript{th} days, the amount of Ca\textsuperscript{2+} in the buffer solution was less for TiF\textsubscript{4}-treated than for NaF-treated samples (p<0.05). Thus, it might be suggested that TiF\textsubscript{4}-treated samples were more acid-resistant than NaF-treated samples (Table 3).
Discussion

High concentrations of HP that promoted enamel surface alterations soften the superficial layer of enamel surface, increase surface porosity and Ca\(^{2+}\) release more than low concentrations of HP and CP. Thus, 38% HP has been recruited for the present study to investigate the Ca\(^{2+}\) released from the bleached enamel surfaces after an acidic challenge. With this designated high concentration, it was aimed to observe the utmost Ca\(^{2+}\) release after a further demineralization process.

Alterations in the inorganic component of hydroxyapatite might be an indicator of the changes in Ca\(^{2+}\) levels of enamel. Rotstein et al\(^1\) demonstrated that most of the bleaching agents might cause various changes in the levels of calcium, phosphorus, and potassium in dental hard tissues; whereas Tezel et al.\(^4\) demonstrated that 35% and 38% HP caused Ca\(^{2+}\) loss from the enamel surfaces. In the present study Ca\(^{2+}\) released from the enamel of the specimens treated with 38% HP was significantly higher than the untreated control group (p<0.05). Based on the Ca\(^{2+}\) values, this result bears out the fact that high concentrated bleaching agents caused surface alterations after an acidic challenge.

The application of highly concentrated fluoride favors the formation of the calcium-fluoride like layer.\(^13\) 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 supports the remineralization of enamel. The results of a previous study confirmed that phosphates and proteins from saliva coated the calcium-fluoride layer on the enamel as a pH-controlling reservoir. This layer acted to decrease demineralization and promote remineralization.\(^14\)

Al-Qunaian et al\(^15\) investigated the effects of whitening agents on caries susceptibility of human enamel and reported that no significant differences in caries susceptibility were observed between the untreated control specimens and those specimens treated with 10% CP, 20% CP with fluoride and 35% HP. There were no significant differences between the treated and controlled specimens for teeth treated with 10% CP or 35% HP. However, specimens treated with the whitening gel containing 20% CP with fluoride had significantly reduced caries susceptibility when compared with their untreated controls. It was claimed that this effect, could be related to fluoride incorporation in 20% CP gels containing fluoride and the results were in agreement with laboratory studies that fluoride enhanced enamel remineralization.

In the present study, fluoride agents were applied to the bleached enamel and subjected to further demineralization. When the test groups which were bleached with 38% HP were compared, the decrease in
Ca\textsuperscript{2+} losses of 1.5% TiF\textsubscript{4} treated group was detected to be the lowest (Table 1; Fig 1). Regarding this result, it can be assumed that TiF\textsubscript{4} may be effective in preventing the bleached enamel surface against the acid attacks. Furthermore, no Ca\textsuperscript{2+} release was detected from three specimens of TiF\textsubscript{4} group during the first four days, and also there was no Ca\textsuperscript{2+} release from two specimens during the second 4-day interval (Table 2). We assume that this effect might be a result of the glaze formed after topical TiF\textsubscript{4} application. It is known that formation of glaze layer takes less than 10 seconds after the application of TiF\textsubscript{4}.\textsuperscript{16} The ability of TiF\textsubscript{4} to protect enamel strongly against the action of acid is a synergistic effect of glaze formation and increased enamel fluoride content. The high fluoride content and great reduction in solubility found in TiF\textsubscript{4} treated enamel suggest that a fluoride reaction with the enamel is involved.\textsuperscript{17} In a previous study, Tezel et al.\textsuperscript{11} reported that TiF\textsubscript{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.\textsuperscript{18} 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 acid attacks has increased after by 1.5% TiF\textsubscript{4} treatment.

The comparison of the Ca\textsuperscript{2+} losses from the test groups which were bleached with 38% HP revealed that, the decrease in Ca\textsuperscript{2+} losses of 2.1% NaF treated group was also lower indicating that NaF could also prevent enamel surfaces against acid attacks. When NaF treated group was compared to the control group, it has been seen that the Ca\textsuperscript{2+} lost from the NaF group was significantly different during the first four days (p<0.05) (Table 3). However, when NaF treated group was compared to 38% HP group, the difference was statistically significant during the whole test period (16 days) (p<0.05). In the present study, although NaF was effective against acid attacks on enamel surfaces, its influence was not as strong as TiF\textsubscript{4} (Table 3; Fig 2). Tveit et al.\textsuperscript{19}, assumed that complexes were formed between TiF\textsubscript{4} and hydroxyapatite, based on a strong binding of the titanium compound and the oxygen atom of the phosphate group. Mundorff et al.\textsuperscript{17} suggested that TiF\textsubscript{4} acted with enamel both chemically, by decreasing enamel solubility and physically due to the formation of protective glaze on the enamel surface. vanRijkom et al.\textsuperscript{20} compared the erosion-inhibiting effect of the topical fluoride treatment based on the deposition of CaF\textsubscript{2}-like material using 1% NaF and 4% TiF\textsubscript{4}. It was concluded that the reduction of Ca\textsuperscript{2+} loss was more stable for TiF\textsubscript{4} than the NaF group and the reduction appeared to be smaller for the longer acid exposure times.

Generally, fluoride uptake of demineralized enamel is higher when compared to sound enamel.\textsuperscript{21} It is assumed that the applied fluoride can easily penetrate through the porous structure of the demineralized
enamel and that can retain to higher number of possible retention sites. 22 According to the results of a study 23, the bleached and fluoridated enamel was more resistant against erosive attacks than the bleached/unfluoridated and unbleached/unfluoridated enamel. In the present study, at the end of the 16th day, the calcium released from the bleached/fluoridated specimens was lower than the control group (unbleached/unfluoridated) and the difference was significant in TiF$_4$ treated specimens (Table 3). This result is noteworthy in a way that bleached and fluoridated teeth can be more resistant to acid attacks than sound teeth in which the results need to be investigated with further studies.

Based on the results of the present study, the hypothesis, which states that there would be less Ca$^{2+}$ loss from the enamel surfaces that were treated with NaF and TiF$_4$ after an acidic challenge was accepted. It was shown that topical fluoride applications decreased the amount of Ca$^{2+}$ released from the 38% HP treated enamel surfaces to further demineralization.

It may be concluded that post-fluoride supplements may be beneficial for reducing the risk of demineralization caused by acid attacks after bleaching processes and remineralize the bleached enamel surfaces. In addition, TiF$_4$ treated samples released less Ca$^{2+}$ than NaF treated samples which indicates that TiF$_4$ may act better than NaF solution in preventing acid attacks.

Competing Interests
The authors declare that they have no competing interests.

Authors’ contributions
HK participated in the design of the study, collected data and involved in drafting the manuscript
ZE have analyzed data, revised the manuscript and given the approval of the version to be published
HT conceived of the study, participated in its design and performed the statistical analysis

Acknowledgements
We have one to acknowledge.
References


10. Büyükyılmaz T, Sen BH, Ogaard B: **Retention of titanium tetrafluoride (TiF4), used as fissure sealant on human deciduous molars.** Acta Odontol Scand 1997, **55:** 73-78.


Figure Legends

**Figure 1.** Flowchart showing the processes applied to specimens until calcium ion (Ca$^{2+}$) measurements with atomic absorption spectrophotometer.

**Figure 2.** The calcium ion (Ca$^{2+}$) concentrations of the specimens measured at the end of the 4$^{th}$, 8$^{th}$, 12$^{th}$ and 16$^{th}$ days (µg/ml).
Four pieces were obtained from each tooth.

Group 1: Control
Group 2: 38% HP
Group 3: 38% HP + Haf
Group 4: 38% HP + TiF

Buffer solutions (freshened every 4 days)

Days 1-4
Days 5-8
Days 9-12
Days 13-16

AAS
Figure 2

Ca²⁺ (µg/ml) over different days (4, 8, 12, 16) for different treatments:
- Control
- 38% HP
- 38% HP + NaF
- 38% HP + TiF₄

Error Bars: 95% CI
Additional files provided with this submission:

Additional file 1: Tables.docx, 17K
http://www.biomedcentral.com/imedia/8991523791288835/support1.docx