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# Effects of CPP-ACP and Remin-Pro on Surface Roughness of Bleached Enamel: an Atomic Force Microscopy Study

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**Objectives** Bleaching agents can change the organic and mineral contents of the tooth structure. The aim of this study was to evaluate the effects of two remineralizing agents on surface roughness of bleached enamel.

**Methods** In this experimental study, 24 premolars were collected. The testing area was a window measuring 3 × 4 mm. First the surface roughness of specimens was measured by atomic force microscopy (AFM). Then, the teeth were bleached. Surface roughness was measured again. Specimens were randomly divided into 3 groups. No remineralizing agent was applied in the control group (A). Casein phosphopeptide amorphous calcium phosphate (CPP-ACP) and Remin-Pro were used in groups B and C, respectively. After 15 days, the surface roughness was measured. The changes in surface roughness were analyzed by paired t-test, and comparison between the groups was done by the Welch and Games-Howell post hoc tests.

**Results** The surface roughness increased after bleaching (P<0.000). Surface roughness in groups B (P=0.03) and C (P=0.04) was significantly lower than that in group A. There was no significant difference in the level of surface roughness reduction between groups B and C. The Welch test revealed that the mean change in surface roughness values after remineralization in groups B and C was significantly higher than that in group A (P=0.001 and P=0.002, respectively). The difference between groups B and C was not significant (P=0.97).

**Conclusion** CPP-ACP and Remin-Pro reduce the surface roughness of bleached enamel more effectively than the saliva.

**Keywords** Tooth Bleaching; Tooth Remineralization; Microscopy, Atomic Force

## Introduction

At present, tooth whitening is one of the most requested treatments performed by various mechanical or chemical methods; among which, bleaching is widely used as a low-risk and effective treatment. Hydrogen peroxide is the main product used in the bleaching process, which can easily pass through the organic matrix of dentin and enamel due to its low molecular weight, and oxidize the chromatic molecules.[1]

Although bleaching does not cause any macroscopic change in the tooth surface, microscopic changes can be caused by increased enamel porosities, reduced enamel hardness, increased surface roughness, and changes in calcium and phosphate content. One of the main problems in this treatment is the increase of surface roughness of the bleached enamel, which can have side effects such as absorption of pigments and reducing the enamel resistance to stress.[2-4]

Several studies have shown that use of either 10% carbamide peroxide or 30% hydrogen peroxide can cause morphological changes in the tooth surface and increase the surface roughness of the enamel.[5-7] Rough surfaces increase the accumulation and adhesion of bacteria and result in surface discoloration.[8] Toothbrushing after bleaching can reduce the mineral content and lead to tooth hyper-sensitivity. In order to solve this problem, a variety of materials have been proposed that have the potential to remineralize enamel lesions.[9, 10] Tooth Mousse is a water-based material containing casein phosphopeptide amorphous calcium phosphate (CPP-ACP). Evidence shows that CPP-ACP releases calcium and phosphate in the oral environment and increases the amount of these ions in the dental plaque. This phenomenon leads to saturation of the enamel by these ions and its remineralization.[11] It has been shown that CPP-ACP can neutralize the effects of acidic drinks. Furthermore, if CPP-ACP is applied on the bovine enamel before and after bleaching, it can reduce the bleaching effects on the enamel hardness and surface roughness.[12] Remin-Pro is another effective product, which is a water-based material containing hydroxyapatite, sodium fluoride (1450 ppm) and xylitol. The manufacturers claim that this substance improves tooth remineralization by filling the porosities and creating a protective biofilm on the tooth surface and preventing the accumulation of bacterial plaque.[9] Several methods have been proposed for evaluation of surface roughness after bleaching and remineralization treatments such as atomic force microscopy (AFM). The purpose of this study was to investigate the effect of CPP-ACP and Remin-Pro on

surface roughness of bleached enamel by using AFM.

## Methods and Materials

An experimental study was designed in which 24 premolars with no obvious cracks, fractures, discoloration, restoration or caries, extracted due to orthodontic or periodontal reasons were collected and after cleaning with ultrasonic scaler, they were disinfected for one hour in sodium hypochlorite (Rakhsha, Iran). The teeth were stored in deionized water until the study was initiated. The study protocol was approved by the ethics committee of Shahid Beheshti University of Medical Sciences (IR.SBMU.RIDS.REC.1394.106).

The crown of the teeth was cut at the cementoenamel junction by a diamond disc (D & Z-Germany) under water coolant. Then, the crowns were sectioned mesiodistally into two halves (bur No. 836; SWS, Switzerland) under cold water irrigation.

In the next step, a 3 × 4 mm window (at the center of the buccal surface of each tooth) was marked as our testing area and other areas were covered with two layers of nail polish. The specimens were adhered to glass plates with cyanoacrylate glue (Pishgam Iran CIA, Iran).

Prior to any experiment, the surface roughness of all specimens was measured in the testing area using AFM (Nanowizard 11-JPK, Berlin, Germany). In this method, the surface of the sample is scanned by a microscope with a head composed of a single atom of diamond and the data are transported to a computer .The placement of the atoms in three axes is processed by a computer and then the topography of the surface is determined.[13]

The Pola Office bleaching gel (SDI, Australia) containing 37.5% hydrogen peroxide was applied on the testing area of each specimen according to the manufacturer's instructions. A thick layer of office bleaching gel was placed directly on the enamel surface of all specimens for 24 min (3 times, every time for 8 min).

After each application, the bleaching agent was removed from the surface of the tooth by a surgical aspirator tip, sterile gauze, and deionized water (Pars Chemical, Iran).

After bleaching, the surface roughness of all specimens was measured and recorded by AFM (Fig. 1).

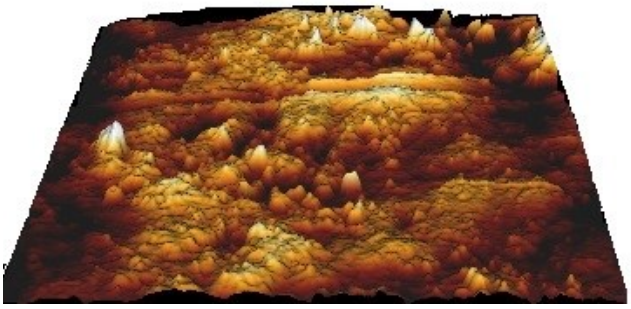


**Figure 1- Surface roughness after bleaching at ×10.05 magnification (10.05 µm)**

The specimens were then randomly divided into 3 groups (n= 8) and treated as follows:

A: Control group: After bleaching, samples in this group were restored in artificial saliva (pH = 7, prepared at the chemistry laboratory of Tehran University, Tehran, Iran) without any other treatment until the end of the study. Meanwhile, artificial saliva solution was changed on a daily basis.

B: CPP-ACP group (Tooth Mousse; GC, Japan): CPP-ACP paste was applied on the surface of the bleached enamel.

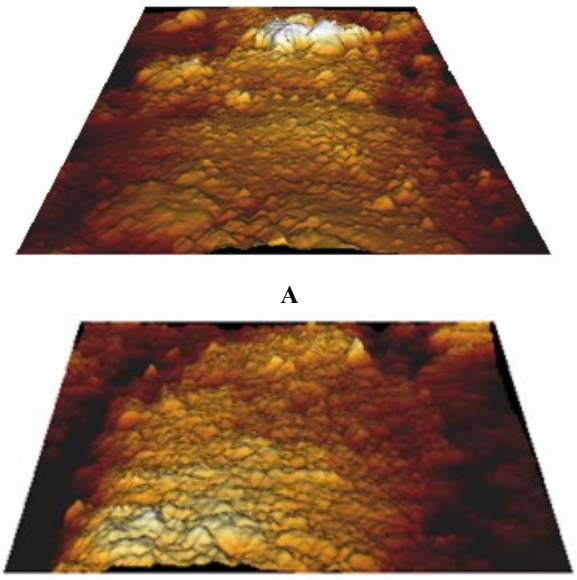
C: Remin-Pro (Voco, Germany): Remin-Pro was applied on the surface of the bleached enamel.

Sufficient amount of paste was applied by a micro-brush on the testing area (without any rubbing). After 5 min, the paste was washed with deionized water (it was repeated twice a day for 15 days). During the treatment, the samples in groups B and C were immersed in artificial saliva after each remineralization treatment.

The pH level of the artificial saliva was 7 with the following formulation (numbers in parenthesis are based on weight percentages):

KCl (0.04) + NaCl (0.04) + CaCl2.2H2 (0.09) + NaH2Po4.2H2 (0.069) + MgCl2.6H2O (0.008) + urea (0.05) + Ascorbic acid (0.01) + H2O (10cc) + Methyl-p hydroxyBenzoat (0.15) + PEG 6000 (7) + Glucose (0.1)

After 15 days, the surface roughness of all specimens was measured again and recorded by AFM (Fig. 2).



**A**

**B**

**Figure 2- Surface roughness after treatment with CPP-ACP (A). Surface roughness after treatment with Remin Pro (B) at ×10.05 magnification (10.05 µm)**

Mean and standard deviation values of surface roughness were reported before bleaching
(T1), after bleaching (T2) and after remineralization (T3). The normal distribution of surface roughness data was checked in the 3 study groups by the Shapiro-Wilk test, which confirmed the normal distribution of data (P>0.05).

Paired t-test was used to compare the mean change in surface roughness after bleaching (T2-T1) and after the

remineralization cycle (T3-T2), separately for each study group.

The changes in surface roughness values during the remineralization process were calculated (T3-T2), and these changes were compared between the study groups by the Welch (robust test for ANOVA) and Games-Howell post hoc test.

Although no significant differences were detected between the groups in surface roughness values before the remineralization cycle (T2), but due to small differences in T2 values between the study groups, the effect of surface roughness values at T2 was adjusted by ANCOVA to compare the surface roughness after the remineralization cycle (T3). Data analysis was carried out using SPSS 21.

## Results

Descriptive statistics for surface roughness in the study groups are presented in Table 1.

**Table 1-** Descriptive statistics for surface roughness in the study groups

| Groups | Before bleaching (T1) | After bleaching (T2) | After remineralization (T3) | (T2-T1) | P-value (T2-T1) | (T3-T2) | P-value (T3-T2) |
|---|---|---|---|---|---|---|---|
| **A (control)** | 37.40±5.71 | 82.76±31.36 | 77.84±30.33 | 45.36±25.72 | 0.002 | -4.92±2.57 | 0.001 |
| **B (CPP-ACP)** | 35.87±4.62 | 75.19±26.46 | 21.16±3.73 | 44.94±22.08 | 0.001 | -56.72±25.67 | <0.0001 |
| **C (Remin Pro)** | 36.72±4.41 | 81.66±26.43 | 24.94±3.78 | 39.33±21.90 | 0.001 | -54.03±23.27 | <0.0001 |

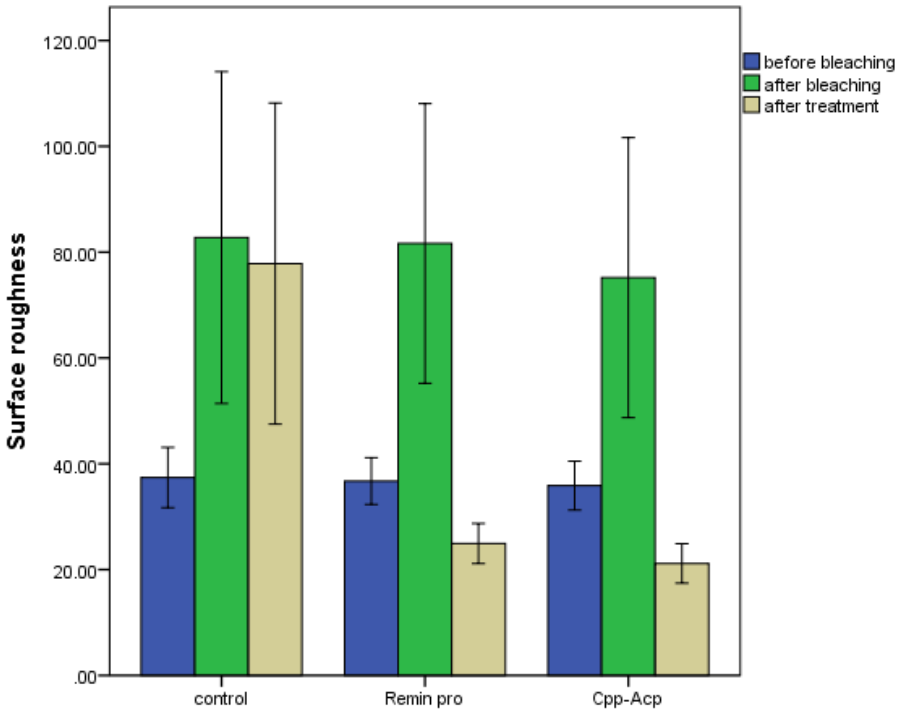



**Figure 3-Comparison of surface roughness before and after bleaching and after remineralization in the study groups**

Based on these findings, the following results were obtained:

The mean values of the surface roughness of all samples in each group increased significantly after the bleaching process (P<0.05). The mean values of the surface roughness of group A (control) and two bleached specimens in groups B and C (after remineralization therapy) significantly decreased (P<0.05).The Welch test revealed that the mean change in surface roughness after the remineralization cycle (T3-T2) in both the CPP-ACP and Remin-Pro groups was significantly higher than that in the control group (P=0.001 and P=0.002, respectively), but the difference between the CPP-ACP and Remin-Pro was not significant (P=0.97).

ANCOVA showed that the mean surface roughness values after the remineralization cycle in both CPP-ACP and Remin-Pro groups were significantly higher than the control group (P<0.00 and P <0.00, respectively), and the difference between the CPP-ACP and Remin-Pro was not significant (P>0.05).

## Discussion

The purpose of this study was to examine the effects of CPP-ACP and Remin-Pro on surface roughness of

bleached enamel by AFM. According to the results of the study, it was found that CPP-ACP and Remin-Pro compared with saliva were more effective in reducing the surface roughness of bleached enamel, but the effect of these two agents on the reduction of surface roughness after bleaching treatment was similar.

The bleaching agents affect the mineral content of the teeth[14] such that the surface of the enamel under the microscope after bleaching is somewhat similar to etched enamel, which has a higher porosity than the intact enamel. According to Ernst et al, the porosity generated on the enamel surface depends on the concentration of the agent used, and 10% or 16% concentrations cannot create large porosities, while 35% concentration of the bleaching agent makes the enamel surface more porous.[1] In the present study, based on the study of Heshmat et al.[9] and de Freitas et al.[15] a bleaching agent with 37.5% hydrogen peroxide was used; the acidic pH of this substance and its oxidizing effect increase demineralization and surface roughness. However, by repositioning of mineral ions during the treatment phase, the porosities on the enamel surface can be partially filled.[1]

In this study, both CPP-ACP and Remin-Pro were applied on the samples twice a day, for 5 min, during 15 days. This methodology was the same as that of Kamath et al.[16] and Heshmat et al.[9] In order to improve the conditions, according to Reynolds[17] (2009), CPP-ACP was also used in the same way as the Remin-Pro. The results of the present study are in agreement with the study of Esfahani et al.[18] since both studies confirmed the remineralizing potential of these two substances. However, in the study by Esfahani et al.[18] the remineralizing property of the CPP-ACP was reported to be more than Remin-Pro. The reason for this difference can be related to the methodology, number of samples, and different parameters that were examined. Since Esfahani et al.[18] aimed to investigate the long-term effects of remineralization, the duration of treatment with CPP-ACP and Remin-Pro was 8 h per day for one month, and more samples were studied in each group. However, in the present study, the duration of application of these two materials on the enamel was different. Also, the parameter studied in this study was surface roughness which was measured by AFM; while, Esfahani et al.[18] calculated the surface microhardness by the Vickers hardness tester.

Rahiotis and Vougiouklakis[19] reported similar results to the present study on the remineralization efficacy of CPP-ACP in vitro. The results showed that application of CPP-ACP on the dentin surface decreased demineralization and induced remineralization.

Poggio et al.[12] used AFM and reported results consistent with the results of the present study. They showed the protective effect of CPP-ACP against enamel demineralization. The results of Zhou et al.[20] which investigated the effect of CPP-ACP on primary enamel lesions in primary teeth were consistent with the results of the present study, which was based on the reduction of enamel surface roughness after using CPP-ACP. They also observed that CPP-ACP increased the size of enamel hydroxyapatite crystals and strengthened the enamel microscopic structure, and increased the nano-hardness and elastic modulus.

Various methods have been used to evaluate the level of enamel surface roughness in different studies. For example, Heshmat et al.[9] used profilometry. Surface roughness of less than 10 nm is difficult to be detected reliably by profilometry, and it is important to smoothly polish the specimens.[21] Rajan et al.[22] used a polarizing microscope to investigate the effect of four remineralizing agents. Another study also used scanning electron microscopy (SEM) to evaluate the effects of office bleaching on the surface morphology of the enamel. The use of this method requires special conditions and in some cases, the gold coating of the surface of the samples may cause some major changes in the specimen surface properties. In addition, the SEM micrographs do not provide numerical values of the morphological parameters of the sample surface. The information obtained from this method is only related to two dimensions. These limitations were obviated by the introduction of new techniques such as AFM which is able to measure and record the surface properties of many materials at an atomic level. One of the most important advantages of this method is that there is no need to prepare the specimens as for the SEM and information is provided in three spatial axes of X, Y and Z.[23] In the present study, as in the studies of Poggio et al.[12] Hegedüs et al.[24] and de Freitas et al.[15] AFM was used to evaluate the changes in the enamel surface roughness after bleaching.

Overall, the findings of the present study are consistent with previous studies on the remineralizing properties of CPP-ACP and Remin-Pro, and confirm the positive effect of these two substances on the bleached enamel.[1, 9, 12, 16, 25]

## Conclusion

CPP-ACP and Remin-Pro reduce the surface roughness of bleached enamel more effectively than the saliva.

## Conflict of Interest

No Conflict of Interest Declared ■

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