

# Changes in bleached enamel microhardness after application of fluoride and CPP-ACP

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## Abstract

**Background and Aim:** Tooth bleaching decreases enamel microhardness. The aim of this study was to evaluate the changes in microhardness of bleached enamel after applying fluoride and CPP-ACP (casein phosphopeptide –amorphous calcium phosphate).

**Materials and Methods:** In this in vitro experimental study, sixty extracted human molars were divided into six groups. The flatted occlusal surface was covered with nail varnish. Group 1 and 2 served as positive and negative control groups. Group 2, 3 and 4 underwent bleaching procedure. Specimens of group 3 and group 5 were covered with sodium fluoride varnish and group 4 and 6 underwent CPP-ACP therapy. Subsurface enamel microhardness was measured on the occlusal surface with 300 micron distance from the buccal or lingual surface. One way ANOVA and Tukey post hoc were used for statistical analysis.

**Results:** Microhardness of bleached enamel was significantly lower than the other groups. CPP-ACP or fluoride had no significant effect on sound enamel microhardness. The bleached specimens treated by fluoride or CPP-ACP had a significant increase in their microhardness compared with the untreated bleached enamel. Although application of CPP-ACP and fluoride increases the enamel microhardness, there were no significant differences between them.

**Conclusion:** Fluoride and CPP-ACP components increase bleached enamel microhardness.

**Key Words:** Subsurface enamel – Teeth Bleaching – Microhardness – CPP-ACP – Fluoride varnish

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## Introduction

There has been more attention paid towards esthetics in dentistry in the recent decades [1]. Bleaching which is one of the conservative esthetic treatments in restorative dentistry has many appropriate and remarkable results. Apart from the acceptable esthetic results, many studies have determined that tooth bleaching decreases

the enamel's calcium, phosphate and fluoride content and leads to diminished mechanical properties such as microhardness and strength. Surface softening, mineral loss and increase in the probability of erosion or caries and decrease in fracture resistance or decrease in resistance to dental erosion after bleaching has been reported [2]. Wiegand A et al. showed that after tooth

bleaching with 10% and 15% carbamide peroxide, possible morphologic changes such as erosion, decalcification and porosity on the surface of the enamel exist [3].

Some studies have shown that bleaching increases the permeability of the tooth enamel [4, 5].

Many believe that local use of fluoride is effective in remineralization, and prevents mineral loss. Fluoride compounds may reconstruct of microstructural defects of the tooth which is a consequence of tooth bleaching by absorption and sedimentation of salivary components such as calcium and phosphate [6].

Casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) which is prepared from milk casein protein contains calcium and phosphate in amorphous form and transfers calcium and phosphate to pellicle which results in a high concentration of calcium and phosphate ions on enamel surface and enhances remineralization [7-8]. The aim of the present study was to evaluate the effect of fluoride and CPP-ACP materials on enamel microhardness after tooth bleaching.

### Materials and Methods

In this in vitro experimental study, 60 sound extracted human third molars were collected during six months and were immersed in 0.2% thymol solution. The teeth surfaces were debried to remove the remaining soft tissue and plaques. Subsequently, the teeth were placed in acrylic resin up to the CEJ level. Since a completely smooth surface of the tooth is necessary to measure the microhardness; the occlusal surfaces of the samples were prepared parallel to the horizontal surface. The prepared smooth surface was covered by three layers of acid resistant nail varnish to prevent penetration of any substances. Then, the specimens were randomly divided into six groups of ten specimens. Then, the teeth were placed in artificial saliva (Bioxtre, Biohealth care, Bel-gium) in incubator at 37°C temperature for 7 days and the artificial saliva was changed every two days. The experimental groups were as follows: the specimens in group

1 received no intervention to serve as positive control. The specimens in group 2 were bleached according to the manufacturer's instructions with 38% carbamide peroxide (OpalsenseUhadent USA) twice a day for 7 days. These specimens were sealed tightly from the CEJ area by nylon and thin copper wire and covered by bleaching gel with 1mm thickness for 15 minutes. After 15 minutes, the samples were rinsed and dried with air and water spray for 10 seconds and then placed in artificial saliva for 7 days to serve as negative control. Specimens in group 3 were bleached with the same procedure carried in group 2. After bleaching procedure they were covered by 5% sodium fluoride varnish (Opal, Ultradent USA) for 5 minutes. Then the samples were embedded in artificial saliva in incubator at 37°C temperature for 7 days. Specimens in group 4 were bleached with the similar steps and then treated with CPP-ACP paste (GC Corp, Tokyo, Japan) for 15 minutes and then were placed in artificial saliva in incubator at 37°C temperature with no rinsing procedure for 7 days. Specimens in group 5 went on similar circumstances as group 4 except bleaching step. Specimens in group 6 went on similar circumstances as group 3 except bleaching. Then the acid resistant nail varnish was wiped from the occlusal surface of all specimens by cotton wool and acetone and the microhardness was evaluated by microhardness Vickers machine (Festz Germany). The data were analyzed by SPSS software (version11.5) with the use of one Way ANOVA and Tukey HSD tests.

### Results

The mean and standard deviation subsurface enamel microhardness for different studied groups have been presented in Table 1. Group 2 which was only bleached had the least enamel microhardness and group4 which was treated with CPP-ACP after bleaching procedure had the highest one. According to the Kolmogrov-Smirnov test, normal distribution and also variance equality, variance analysis was performed

between these groups and there was a statistical significant difference between at least two of the groups ( $p=0.0001$ ). In order to define between

which of the two groups this difference was seen, Tukey test was used and the results are demonstrated in Table 2.

**Table 1:** Mean and Standard Deviation of Enamel Microhardness in Different Study Groups

Group	Control	Bleached	Bleached and Treated with Fluoride	Bleached with CPP-ACP	Treated with CPP-ACP	Treated with Fluoride
Mean	394.90	329.20	403.30	428.00	419.20	490.00
Standard Deviation	23.20	22.27	14.47	18.87	20.46	22.48

**Table 2:** P Values Based on Tukey Test Results

Group	Control	Bleached	Bleached and Treated with Fluoride	Bleached with CPP-ACP	Treated with CPP-ACP	Treated with Fluoride
Control	-	0.000	0.941	0.009	0.104	0.645
Bleached	0.000	-	0.000	0.000	0.000	0.000
Bleached and Treated with Fluoride	0.941	0.000	-	0.095	0.519	0.989
Bleached with CPP-ACP	0.009	0.000	0.095	-	0.929	0.320
Treated with CPP-ACP	0.104	0.000	0.519	0.929	-	0.875
Treated with Fluoride	0.645	0.000	0.989	0.320	0.875	-

## Discussion

Present Study, measured the subsurface microhardness of the enamel to evaluate the penetration power of applying remineralizing agents used on the bleached enamel. The subsurface enamel has higher permeability in comparison to surface enamel. Therefore it has an important role in the permeability of the enamel and its susceptibility to caries. Evaluation of subsurface enamel hardness may clarify the ambiguities regarding bonding to adhesive agents or susceptibility to tooth decay. Some studies have shown that using 10% carbamide peroxide and 35% hydrogen peroxide increase the enamel's permeability [9-10] while others have demonstrated that 10% carbamide peroxide decreases the permeability of the enamel [11-12]. In the second group which the specimens were bleached only to serve as negative control the microhardness of the enamel showed a noticeable decrease which

is in agreement with other studies even though the concentrations of the bleaching materials were different. Although there have been studies in which 35% and 38% hydrogen peroxide have caused decrease in the calcium content of the enamel, 10% carbamide peroxide has shown no significant decrease in the amount of enamel calcium [13]. Also it has been shown that 10% carbamide peroxide did not change the microhardness of the enamel [14] indicating that the difference between the findings of the studies may be a consequence of different concentration of bleaching agents, different time period for applying of bleaching agents or the type and nature of the tooth itself. In the third group which the specimens were bleached and then fluoride varnished, the microhardness had a remarkable and significant increase compared with negative control (group2) which was consistent with the findings of Bizhang et al stating that the micro-

hardness of the bleached teeth after fluoride therapy increases [15]. Borges et al. showed an increase in the enamel's microhardness of the bleached teeth which underwent fluoride therapy afterwards in comparison to fluoride therapy before bleaching [16]. In forth group, in which the teeth were first bleached and then treated with CPP-ACP paste, a higher increase in the enamel's microhardness was observed in comparison to the third group. Yamaguchi et al. also reported an increase in the microhardness of the calf's demineralized teeth after appliance of CPP-ACP paste and regarding the evaluation of the effect of this substance on the enamel of the calf, they showed that there is high concentration of inorganic components in the CPP-ACP paste that enhances the enamel's remineralization [8]. In fifth group which CPP-ACP paste was used alone with no bleaching step, the microhardness increased in comparison to positive and negative controls which is in agreement with Rahiotis et al.'s study on the dentin [17]. Tantbirojn et al studied the effect of Coca and CPP-ACP paste on enamel's surface hardness change and showed that the samples treated with this remineralizing paste revealed a higher microhardness compared to the control group. The increase in the surface microhardness is due to the rich source of amorphous calcium phosphate present in CPP-ACP. This substance leads to secretion of calcium and phosphate on the supersaturated enamel surface and therefore, increases and accelerates the remineralization process and surface microhardness [18]. In Rehder Neto et al.'s study, which assessed the effect of remineralization factors such as regular tooth pastes, calcium sodium phosphosilicate containing tooth pastes, CPP-ACP materials with or without fluoride; it was concluded that appliance of CPP-ACP increased the demineralized enamel's microhardness in comparison to other groups except fluoride which is in agreement with the present study (19). Panich evaluated the effect of Cola and CPP-ACP on the microhardness of the enamel, revealing the fact that CPP-

ACP increases the enamel microhardness. Uysal et al. showed the suppressing effect of CPP-ACP and fluoride gel on the enamel's demineralization around the orthodontic brackets [21]. Honorio et al.'s evaluated the effect of CPP-ACP and regular fluoride containing tooth pastes on white spot enamel lesions, and concluded that no significant difference is present between fluoride and CPP-ACP, all of which are congruent with the present study [22]. In sixth group which the fluoride therapy was performed alone, microhardness increased significantly in comparison to the negative control which is due to the presence of fluoride in addition to artificial saliva and reconstruction of minerals as fluorapatite. However, it should be pointed that the microhardness of the mentioned group had no significant difference with that of others. According to the findings of the present study, the null hypothesis or the non effective role of fluoride components and CPP-ACP materials on the microhardness of subsurface enamel was rejected and the H1 hypothesis about the effective role of remineralizing agent such as fluoride components and CPP-ACP materials on subsurface enamel microhardness was accepted.

### Conclusion

It can be concluded from the findings of the present study that

- 1-Enamel bleaching with 38% carbamide peroxide decreases the microhardness of the subsurface enamel.
- 2-Fluoride therapy with 5% sodium fluoride gel and application of CPP-ACP increase the microhardness of the subsurface enamel.
- 3- Application of CPP-ACP causes a higher increase in the subsurface microhardness of the enamel in comparison to 5% fluoride varnish.

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