

Original Research Article

## The Influence of Remineralization after Bleaching - *In Vitro* Study

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**Abstract:** The aim of this study is to compare remineralizing agent applications on micro-hardness of bleached teeth using Vicker's Hardness profiles. 60 extracted human premolar teeth were selected. Teeth were divided into 3 groups. Group A- Control (16% CP), Group B- (16% CP + CPP- ACP), Group C- (16% CP + APF). All specimen were bleached for 15 days with 16% carbamide peroxide for 4 hours. On 15<sup>th</sup> day remineralizing agent application was done with respective agents for 5 minutes. Microhardness testing was done on baseline (prior to bleaching), on 15<sup>th</sup> day (post bleaching), on 30<sup>th</sup> day (post remineralizing agent application). Statistical analysis was done by descriptive and inferential statistics using Student's paired t test, one way ANOVA and Multiple Comparison: Tukey Test and software used in the analysis was SPSS 21.0 version and ( $P < 0.05$ ) is considered as level of significance. After bleaching all samples from all the groups showed decrease in micro-hardness. Further application of APF & CPP- ACP showed reversal of microhardness to the baseline and more than baseline values. APF & CPP – ACP both are equally efficient in regaining the microhardness of teeth.

**Keywords:** Bleaching, Remineralization, CPP- ACP, APF

### INTRODUCTION

The advantage of external bleaching by applying hydrogen or carbamide peroxide to discolored vital teeth has been frequently reported. The application of carbamide peroxide (CP) gels at a concentration of 10%-15% is popular at home bleaching techniques and is suggested as, simple and efficient procedure for whitening teeth.

Although potential adverse effects of carbamide peroxide bleaching is not visible macroscopically or clinically. This technique involves all the steps carried out by patient at home, other than fabrication of bleaching trays. Hence the dental practitioner has very less or no control over the procedure. Different concentrations of Carbamide peroxide is generally used. 16% carbamide peroxide has shown faster and safer effect of bleaching on teeth. Major side effect of extra coronal bleaching is enamel damage [1].

In literature a great deal of controversy concerning the effects of this bleaching agent on the micro-hardness of dental hard tissues, particularly enamel has been noted. Therefore the best combinations resulting in limited side effects, safety and efficacy are

used. To reduce the tooth sensitivity and re-establish surface hardness addition of potassium nitrate, fluoride and amorphous calcium phosphate (ACP) is done in recently developed materials. ACP has been shown to promote enamel remineralisation through buffering of free calcium and phosphate ion activities [2]. Fluoride is incorporated as a remineralizer, which is beneficial during and after bleaching. Fluoride helps in formation of calcium fluoride layer on enamel which reduces demineralization. And helps to keep the post bleaching micro-hardness values as that of pre bleaching values [3].

Fewer studies has shown that application of fluoride on softened enamel above the critical pH helps in remineralization. Fluoride can greatly help dental health by strengthening the tooth enamel, making it more resistant to tooth decay. It also reduces the level of acid that the bacteria produce [4].

The aim of this study is to compare the effect of APF and ACP -CPP application on micro-hardness of teeth post bleaching procedure using VHN. (There are no studies which have shown comparison in between these materials.)

## METHODS AND MATERIAL

60 non carious, extracted for orthodontic and periodontal reasons; human premolar teeth were selected. Extractions were carried out less than 3 months prior to the study. Teeth were stored in artificial saliva at the beginning of study for 10 days.

### Sample preparation

Selected teeth were sectioned horizontally 2 mm below the level of CEJ and were sectioned labio-palatally by using double sided diamond disc (Diamond disc- Super flex double sided fine grit (DFS m.i. Germany). The buccal half of each crown was embedded in acrylic resin till CEJ. The occlusal portion was ground flat till DEJ, exposing enamel and dentin. Specimens were stored in artificial saliva for 10 days at temperature of 37°C prior to the bleaching procedure and during the 2 successive bleaching.

Sample were divided randomly in 3 groups. 20 samples in each group.

GROUP A - 16% Carbamide Peroxide without remineralizing agent application

GROUP B - 16% Carbamide Peroxide with application of CPP-ACP paste

GROUP C - 16% Carbamide Peroxide with application of 1.23% APF gel

Baseline VHN was determined prior to the beginning of bleaching.

Pre-operative micro-hardness testing was done using – Micro-hardness Tester (Vicker's Micro-hardness tester, DHV- 3000, Croma, Pune, India) (Fig 1A). VHN values were determined ( $\text{kg/mm}^2$ ) at a load of 100 gm with indentations for 5 sec. Three indentations were performed on an area 5x3 mm in the central part of the exposed enamel of each specimen, at each indentation, the long axis of the diamond indenter was kept parallel to the buccal surface. Each measurement were taken at least 300  $\mu\text{m}$  from the edge of the enamel, spaced 100  $\mu\text{m}$  apart.

### Bleaching

Enamel surfaces (buccal and occlusal) were dried with cotton pellets. Surfaces were isolated with jig made by vinyl polysiloxane impression material. 1 mm layer of the bleaching gel was placed on the buccal and occlusal surfaces of specimens (16% Carbamide Peroxide, Polanight gel). The specimens were bleached for four hours and kept at 37°C. After each bleaching procedure, the gel was removed with a cotton pellet and the specimens were cleaned and dried with an air & water syringe for five seconds. The specimens were then immersed in artificial saliva at 37°C. The teeth were bleached for 15 consecutive days. VHN values were determined on 15<sup>th</sup> day (Fig 1B).

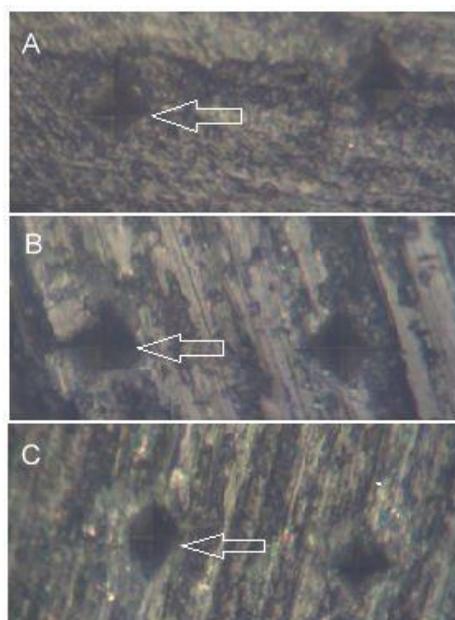


Fig-1: (A,B,C) Pyramidal indentations seen on enamel surface (arrow), surface roughness observed in post bleaching view (B)

### Remineralizing agent application

Immediately after 15<sup>th</sup> day the specimens from Group B (CPP-ACP, G.C. Tooth Mousse) and Group C (1.23% APF, Flurovil) received the allotted agents for 5 min. On 30<sup>th</sup> day (15 Days post fluoride application) (Fig 1 C).

The first measurements (baseline) were taken on the most mesial part of the central area, the other measurements were taken at 15 and 30 days on the most distal part.

**RESULTS AND STATISTICAL ANALYSIS**

Baseline (pre-operative), post bleaching (15<sup>th</sup> day) and post remineralizing agent application (30<sup>th</sup> day) VHN values were compared. (Figure 2) After

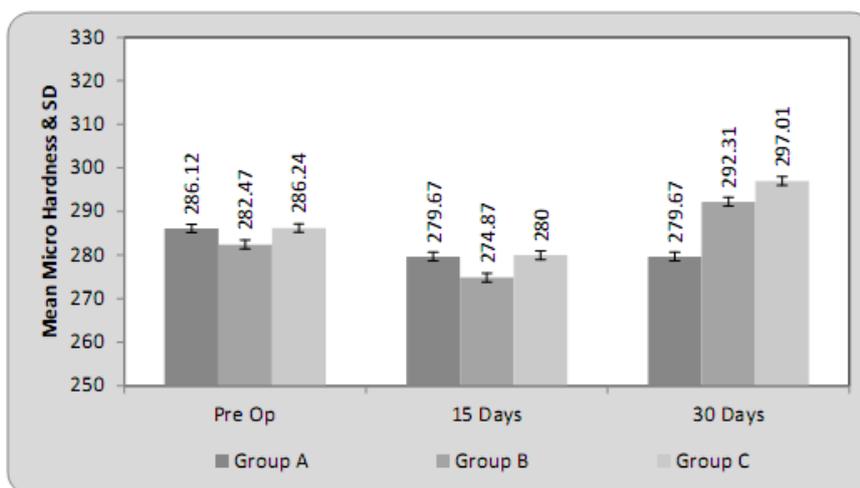
bleaching all samples from all the groups showed decrease in micro-hardness. Further application of APF & CPP- ACP showed reversal of microhardness equal or more than the baseline values (Table 1).

**Table 1: Observations & Results of micro-hardness in all groups (HV- Hardness Value)**

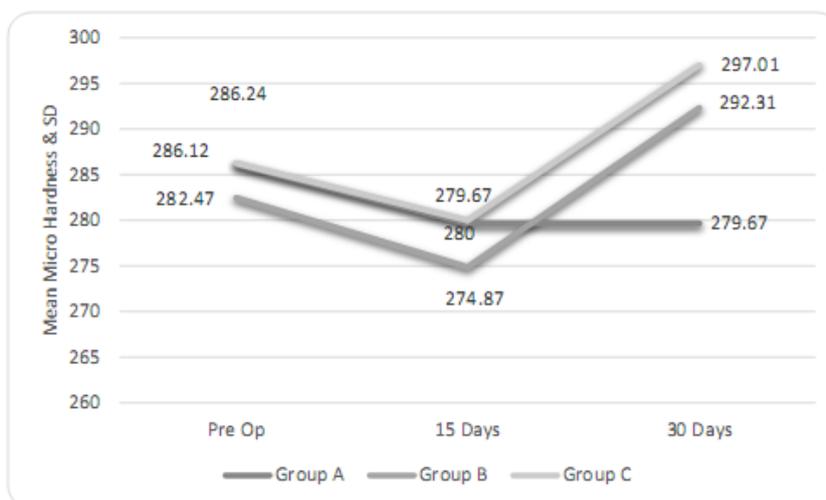
	Group	N	Mean ( HV)	Std. Deviation
Baseline	Control (A)	20	286.12	7.86
	CPP-ACP (B)	20	282.47	6.55
	APF (C)	20	286.24	11.19
15 Days	Control (A)	20	279.67	6.90
	CPP-ACP (B)	20	274.87	7.02
	APF (C)	20	280.00	10.88
30 Days	Control (A)	20	279.67	6.90
	CPP-ACP (B)	20	292.31	8.34
	APF (C)	20	297.01	9.43

Statistical analysis was done by inferential statistics using Student’s paired t test, one way ANOVA and Multiple Comparison: Tukey Test and software used in the analysis was SPSS 21.0 version and ( $P < 0.05$ ) is considered as level of significance.

APF & CPP – ACP both are equally efficient in regaining the microhardness of teeth. There is no significant difference in remineralizing potential between APF and CPP- ACP (Figure 3)



**Fig-2: Comparison of micro-hardness in all the groups**



**Fig-3: Line Diagram showing comparison of micro-hardness in all the groups at different time intervals**

## DISCUSSION

The adverse effects of the bleaching procedure on enamel microhardness have been tested and it has been noted that many of the studies have showed reduction in enamel, microhardness, due to enamel damage on microscopic levels. Some studies have reported that bleaching teeth with 10% Carbamide Peroxide does not alter the surface hardness of enamel [5, 6]. However, current study showed a reduction in enamel microhardness after bleaching gel application of 16% carbamide peroxide. This finding is in agreement with Basting and others [7-10]. This study was taken up to verify the effectiveness of remineralizing agent post bleaching and it has shown to be effective in counteracting the reduction in microhardness. Effectiveness of ACP- CPP and APF are compared. Differences in the method used, like bleaching time and formula, may influence the results. Severe changes have been noted in longer bleaching treatments [11].

As per the results obtained, application of bleaching agents lead to reduction in micro-hardness (Group A- 279.67 HV, Group B – 274.87 HV, Group C – 280.0 HV). 16%CP gel was applied for four hours. Carbamide peroxide is reported to alter the enamel surface on microscopic levels, and decrease in the levels of calcium, phosphate and fluoride. To achieve the maximum duration & benefit, carbamide peroxide is advantageous for night application [12]. In current study bleaching for a lesser time period have led to low amount of reduction in microhardness in enamel VHN testing.

Loss of mineral content changes the enamel hardness. Even though kept in salivary substitutes the microhardness was not reversed in control group (Group A -Baseline– 286.12 HV, Post Bleaching - 279.67 HV). In vitro studies have noted loss of minerals after bleaching with 10-22% Carbamide Peroxide on enamel [13].

In group B (Baseline– 282.47 HV, Post Bleaching - 274.87 HV) application of CPP - ACP showed reversal of microhardness to the baseline and more than baseline values in almost every sample (Post Application - 292.31 HV). ACP works through a method that uses amorphous calcium phosphate compounds complexed with milk proteins in a carbonate solution to crystallize and form hydroxyapatite. This maintains a state of super saturation with respect to tooth enamel. And acts as reservoir of bioavailable calcium and phosphate ions leading to an increased remineralization potential leading to increase in microhardness [14].

In group C (Baseline– 286.24HV, Post Bleaching - 280.00 HV) after application of APF microhardness was restored to that of the baseline (Post Application - 297.01 HV). Application of fluoride to soft enamel leads to remineralization by formation of

fluorapatite and fluoridated hydroxyapatite. Reaction with hydroxyapatite crystals occurs directly or it promotes the transformation of other calcium phosphate phases. The formation of fluorapatite and fluoridated hydroxyapatite can reduce the solubility of hydroxyapatite crystals [8, 15].

Groups that received ACP - CPP and fluoride (Group B, C) showed a significant increase in microhardness compared to the control. (Percentile Gain – Group A- 0%, Group B– 6.18%, Group C – 6.10%). This finding is in agreement with Attin [15], Lewinstein [8] and others [16-20]. Another studies showed reduction in demineralization when fluoride application was done to the enamel post treatment [19, 20]. In the current study, the application of remineralizing agents restored the VHN of enamel and presented positive results. Fluoride and ACP- CPP both showed similar potential for remineralization, as it supplied stabilized bioavailable calcium, phosphate and fluoride in ionic forms. There was no significant difference noted.

This study tried to replicate clinical conditions. Artificial saliva was used as a storage medium for specimen prior to, in-between and after bleaching procedure. Artificial saliva was used in this study, still there was no positive effect in remineralization was seen either during or after bleaching treatment. Study conducted by Justino *et al* [20] showed positive effects of artificial saliva in regaining the microhardness. The saliva and its substitutes provides conditions to maintain the pH above the critical pH which is helpful for remineralization. After comparing the pre- operative, after bleaching and post remineralization microhardness values (Days – 0, 15 and 30) it can be stated that microhardness was restored with the help of remineralizers (At day –30) which was reduced after the bleaching procedure (At day-15).

## CONCLUSION

As per the results of this study it can be concluded that the microhardness of the dental hard tissues were adversely affected by the concentration of peroxide in the bleaching agent. Postoperative treatment with CPP- ACP & APF helped to restore the microhardness of enamel after bleaching. Both (CPP- ACP & APF) showed similar potential and equal percentile gain in remineralization.

## KEY MESSAGES

Bleaching leads to reduction in microhardness of tooth. Post bleaching application of CPP-ACP & APF helps in regaining the microhardness of tooth effectively & equally.

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