ORIGINAL RESEARCH PAPER

Volume-8 | Issue-4 | April-2019 | PRINT ISSN No 2277 - 8179

INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

EFFECT OF CASEIN PHOSPHOPEPTIDE-AMORPHOUS CALCIUM PHOSPHATE(CPP-ACP) AND FLUORIDE GEL(APF)APPLICATION ON ENAMEL MICROHARDNESS AFTER HOME BLEACHING: AN INVITRO STUDY

Dental Science

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ABSTRACT

Purpose: This in vitro study evaluated the effect of bleaching products and the effect of subsequent applications of CCP-ACP and fluorides on the hardness of enamel during and after tooth bleaching.

Methods: The crowns of 30 extracted intact human molars were decoronated and sectioned longitudinally; the buccal part was embedded in acrylic resin, the occlusal part was ground flat, exposing enamel and dentin, and then polished. Baseline Vickers microhardness (VHN) of enamel was determined. The specimens were randomly divided into 6 groups of 5 specimens. Group 1 – Deoblanc;Group 2 – Zoom DayWhite;Group 3 – Deoblanc + CPP-ACP;Group 4 – Deoblanc + APF;Group 5 – Zoom DayWhite + CPP-ACP;Group 6 – Zoom DayWhite + APF. The teeth were bleached for 21 consecutive days.After each day of bleaching, the tooth specimens are treated with CPP- ACP(Tooth Mouse) for 3 minutes using an applicator brush. VHN was measured for 1,2,3 and 5 groups(21st day).Immediately after 21st day, group 4 and 6 received APF 1.23% for five minutes.14 days post fluoridation(35th day), again VHN values were measured for the 6 groups.The results obtained were statistically analysed by Kruskal Wallis test and Wilcoxon Signed Ranks Test**.**

Results:Groups 3 and 5 showed significant increases in enamel microhardness following treatment with no statistically significant difference with the baseline value($p>.05$) followed by Group 4 and group 6($p<.05$).

Conclusion:The results of the current study indicated that the use of CPP-ACP following bleaching improved enamel microhardness than fluoride gel.

KEYWORDS

Bleaching, Vickers hardness number, Casein phosphopeptide amorphous calcium phosphate, fluoride.

INTRODUCTION

Vital tooth bleaching has become one of the most popular offerings of esthetic dentistry for the most conservative treatment of discolored teeth.(1,2). Bleaching may be performed as in-office bleaching or at-home bleaching alternatively, patients may apply a bleaching agent at home using a gel and a customized tray(3). Night Guard Vital Bleaching (NGVB) is probably the most widely used bleaching technique because of its relative low cost, ease of use, , safety and high success rate.(3)Despite the fact that patients can bleach at their own pace, at-home bleaching technique, with its various concentrations of bleaching materials and regimens(10%, 15%, or 20% carbamide peroxide (Langsten et al., 2002)), has become the gold standard by which other techniques are judged. However, disadvantages include, since active patient compliance is mandatory and the technique suffers from high dropout rates (Leonard et al., 2003). Also, the color change is dependent on the diligence of use, and results are sometimes less than ideal, since some patients do not remember to wear the trays every day. In contrast, excessive use by overzealous patients is also possible, which frequently causes thermal sensitivity, reported to be as high as 67% (Haywood, 1992)(4).

Many studies have investigated the effects of bleaching on the enamel morphology and surface texture morphological alteration of the enamel surface – increased porosity of superficial enamel structure, demineralization and the decreased protein concentration, organic matrix degradation, modification in the calcium:phosphate ratio, and calcium loss – thereby supporting the hypothesis that bleaching agents are chemically active components potentially able to induce structural alterations in human dental enamel .(3,5–9)

Tooth-bleaching agents might adversely affect the tooth structure with increasing concentrations, ion release from both enamel and dentin is increased, and the microhardness of enamel decreased significantly with bleaching.(10–12)

In order to compensate the adverse ceffects of bleaching agents, use of fluoride-containing remineralizing agents has been suggested by some researchers.(13,14)

The use of fluoride during or after bleaching has been shown to be beneficial(17–19) By forming a calcium fluoride layer on enamel, which inhibits demineralization or a decrease in MH values, it has been proposed that fluoride may act as a remineralizing agent(20)In addition, studies have shown that application of fluoride on softened enamel results in remineralization(20,21).

Casein phosphopeptide amorphous calcium phosphate fluoride (CPP-ACPF) containing calcium, phosphate, and fluoride ions has been proven to be a clinically effective as a remineralizing agent. This nanocomplex is able to penetrate the dental plaque and pellicle which in turn provides an opportunity for calcium, phosphate, and fluoride ions to precipitate on tooth surface. Maturation of these ions decreases the risk of demineralization and enhances remineralization of enamel surface(15,16)Previous studies have shown that the application of this complex on eroded or bleached enamel can increase microhardness and decrease enamel surface roughness(13,14)

This study evaluated and compared the effect of subsequent applications of CPP-ACP and fluoride solution on the hardness of bleached enamel subsurface postbleaching.

MATERIALS AND METHODS

- **Preparation of enamel specimens**
- 30 extracted intact human mandibular molars were taken for the study and was stored in artificial saliva for 10 days prior to sectioning. The artificial saliva contained 0.103 g/L of CaCl2, 0.019 g/L MgClz * 6H2O, 0.544 g/L KH2PO4, 2.24 g/L KCl, 4.77 g/L HEPES buffer acid and KOH was added to adjust the pH to 7.0. After the storage period, baseline subsurface Vickers hardness (VHN; kg/mm2) was determined.Then the roots of 30 extracted intact human mandibular molars were cut 2mm from the CEJ and the crown portion was sectioned longitudinally in the mesiodistal direction in half.The buccal half of the tooth crown was taken for the study.The bottom surface (2mm of root portion from CEJ) of each tooth was embedded in acrylic resin.The occlusal portion was ground flat, exposing enamel and dentin and then polished with up to 400 grit water cooled carborundum paper discs (Norton, India). The specimens were randomly divided into 6 groups of 5

specimens and each group was assigned to a specific bleaching agent

Table :1

Materials Used	Content	Manufacturer
DEOBLANC	22% CarbamidePeroxide with Pottasium Nitrate	Azure laboratories
ZOOM DAYWHITE	14% HydrogenPeroxide	Discus Dental , Ontario, USA
ARTIFICIAL SALIVA	0.103g/L of CaCl2, 0.019g/L MgCl2* 6H2O, 0.544g/L KH2PO4,2.24 g/L KCL, 4.77 g/L HEPES buffer acid and KOH	Biogenics Research Lab,Trivandrum,Kerala

- Group 1 – Deoblanc
- Group 2 – Zoom DayWhite
- Group 3 – Deoblanc + CPP-ACP
- Group 4 – Deoblanc + APF
- Group 5 – Zoom DayWhite + CPP-ACP
- Group 6 – Zoom DayWhite + APF

Prior to bleaching, the buccal enamel surfaces were dried with cotton pellets.The buccal surfaces of the specimens were covered with 1mm layer of the bleaching gel.The specimens were bleached for eight hours and kept in a humid atmosphere at 37ºc.The teeth were bleached for 21 consecutive days.After each day of bleaching, the tooth specimens are treated with CPP- ACP(Tooth Mouse) for 3 minutes using an applicator brush.After 3 minutes, the paste was rinsed off with an air water spray for 10 sec. VHN was measured for 1,2,3 and 5 groups(21st day).Immediately after 21st day, group 4 and 6 received APF 1.23% for five minutes.14 days post fluoridation(35th day), again VHN values were measured for the 6 groups.The values are analysed .

Test Method

Microhardness measurements- Enamel hardness was measured using Shimadzu HMV-2 TAW microhardness tester with a load of 100g at time of 14 seconds.For each specimen, four indentations were made on the top surface at a minimum distance of 1mm from each other and from the specimen margin.Readings of the four indentations were averaged to determine the hardness value for each specimen.Baseline and post Vickers hardness numbers(VHN) were obtained for all specimens

Statistical Analysis

Kruskal Wallis test and **Wilcoxon Signed Ranks Test** was used to measure any statistically significant differences in VHN groups.

RESULTS

No significant difference among the baseline groups.(p=0.256) was seen.There was significant difference among group 1,2(bleach only)and 3 and 5(bleach+CCP-ACP) (p=0.020)on 21st dayAlso there was significant difference among 1,2,3,4,5 and 6(APF) groups on 35th day(p=0.001)

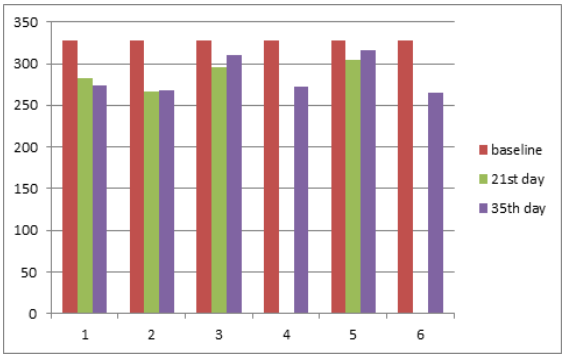


Fig:1

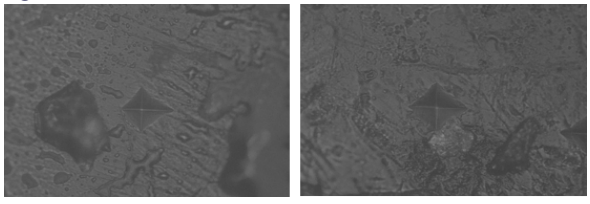
- Baseline v/s group 1 – (p=0.043) **statistically significant**
- Baseline v/s group 2 – (p=0.043) **statistically significant**
- Baseline v/s group 3 – (p=0.893) no statistical difference
- Baseline v/s group 5 – (p=0.345) no statistical difference
- Baseline v/s group 4 – (p=0.043) **statistically significant**
- Baseline v/s group 6 – (p=0.043) **statistically significant**

Table:2

GROUPS	BASELINE (MEAN VALUE)	21ST DAY (MEAN VALUE)	35TH DAY (MEAN VALUE)
1	328.12	304	274.192
2		266.57	268.57
3		295.63	310.23
4		_	272.16
5		304.59	316.78
6		_	265.576

Surface microhardness measurement images

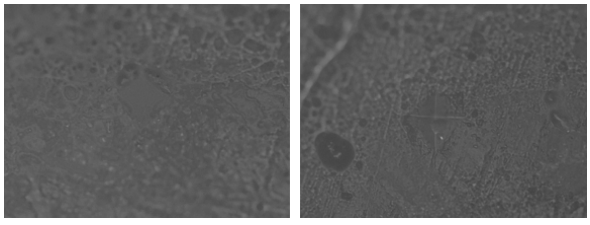
Fig:3



DEOBLANC **ZOOMDAYWHITE**

Fig:4

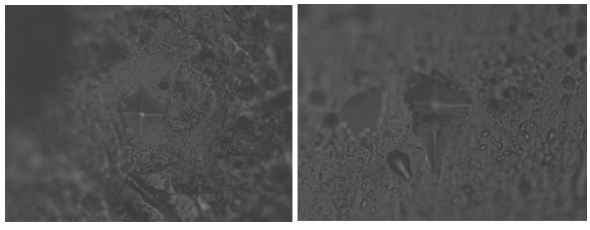
DEOBLANC



CPP-ACPTREATED **FLUORIDE TREATED**

Fig:5

ZOOMDAYWHITE



CPP-ACPTREATED **FLUORIDE TREATED**

DISCUSSION

Bleaching agents have effect on the chemical and morphological structure of hard tissues of teeth. Hydrogen peroxide being an oxidative agent has the ability to produce highly reactive peroxide and superoxide ions. Although bleaching is a complex process, the main reaction is oxidation. As a result of oxidation in the enamel and dentin organic and inorganic substance, changes in microhardness and morphological characteristics are found.(22)

Measurement of surface microhardness is the most common technique used for evaluating the effects of peroxide and bleaching products on enamel and dentin.This study used the Vickers microhardness test to determine the changes on the tooth surface.This study indicated that the application of pastes containing CCP-ACP and fluoride restored the microhardness lost during the bleaching procedure.Although microindentation hardness tests do not provideany specific information about the changes within a substance, these tests are commonly used to detect changes in enamel and dentin surface following demineralization and remineralization experiments. (18)

In our study, both bleaching agents(Group1 and 2) showed significant reduction in surface microhardness of enamel.

Several studies estimated the relationship between the concentrations of carbamide peroxide or hydrogwn peroxide and the decrease in microhardness of enamel.(17,23). The present study revealed a significantly greater reduction in enamel microhardness for both groups containing 22% CP and 14% HP. Results from our study are partially consistent to the study by De Abreu et al., which showed that

hydrogen peroxide bleaching agents caused decrease in enamel microhardness during bleaching treatment.(24).

In order to simulate the clinical situation and to standardize the experimental conditions , the samples were stored in artificial saliva before, in-between, and for 2 weeks after bleaching treatments. Amorphous calcium phosphate gel like ACP, CPP-ACP is known to be an important factor for enamel remineralization. In this study, the microhardness of enamel treated with CPP-ACP(Group3 and 5) applied as topical coating was equal or slightly higher following treatment in comparison to baseline levels. Amorphous calcium phosphate helps restoring the necessary mineral balance in the mouth in an easy and efficient way and decrease adverse side effects from tooth bleaching.(17,25). Also, adding ACP CPP to carbamide peroxide bleaching agents can increase microhardness of bleached enamel.(26). Two-week storage in artificial saliva with daily ACP (Group3 and 5)treatment showed both agents to have a potential remineralization effect. Since there was no significant difference between the baseline and post-treatment values, microhardness returned after a period of remineralization, which was an indication that enamel hardness was completely recovered. The postbleaching treatment with CPP-ACP significantly increased and restored enamel surface microhardness. .This result agrees with the study by Alkhtib(2013)(27) and contradictory to the study done by Cunha et.al(2012)(28)CPP-ACP stabilize amorphous calcium phosphate, it also binds to adsorbed macromolecules of the biofilm on the tooth surface and serves as a reservoir for calcium and phosphate ions.

All the groups that received fluoride 14 days post bleaching (Group4 and6) showed a significant increase in microhardness when compared to groups that did not receive fluoride .In this study, 1.23% APF was used.This result was contadictory to study done by Da costa(13)which showed that microhardness recovery did not occur 14 days after treatment with fluoride gel and agrees with Borges et al. which showed the application of fluoride gel significantly enhanced the microhardness of the bleached enamel.(25). The rehardeneing may be explained by the incorporation of fluoride into the tooth surface, creating a layer of calcium fluoride on the enamel surface that increases hardness values. In the current study, the application of fluoride solution restored the VHN of enamel to a value inferior to the baseline value.

CONCLUSION

The results of the current study indicated that the use of CPP-ACP following bleaching improved enamel microhardness than fluoride gel.

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