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IMPACT OF TWO REMINERALIZING AGENTS ON BLEACHED ENAMEL MICROHARDNESS

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ABSTRACT

Objective: To evaluate the influence of fluoride varnish and casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) on bleached enamel microhardness.

Materials and Methods: Thirty human permanent incisors were selected to preparer thirty enamel specimens. All specimens were bleached using 40% hydrogen peroxide bleaching agent (power whiting YF). The bleached enamel samples were divided into three equal groups (n = 10) according to the used remineralizing agent. Group A was treated by CPP-ACP past (GC Tooth mousse), group B was treated by Fl varnish (Biflourid 10) and group C was stored in artificial saliva. The microhardness was assessed using Vicker test (before bleaching, after bleaching and after application of different remineralizing agents). The data was collected and statistically analyze using two-way ANOVA and post hock LSD test at ($P < 0.05$).

Results: Enamel microhardness has diminished significantly in all groups after bleaching. After application of remineralizing agents, there was a significant increase in microhardness. There was no significant difference between groups A and B, while there was significant difference between these groups and group C.

Conclusions: Using fluoride, CPP-ACP or artificial saliva improves bleached enamel microhardness.

KEYWORDS: CPP-ACP, fluoride, bleaching, microhardness, remineralization

INTRODUCTION

Vital tooth bleaching is one of the most effective treatment for discolored teeth.[1-3] Bleaching may be performed professionally at clinic (in-office bleaching), or patients may apply a bleaching gel at home customized tray (home bleaching).[4] In-office bleaching is superior to home bleaching in many ways, including dentist control, soft-tissue protection and guard against material ingestion, less treatment time, immediate results and more patient satisfaction.[5] In-office bleaching utilizes concentrations of up to 40% hydrogen peroxide (HP).[5, 6] There is an argument about the negative

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effect of HP or peroxide containing agents. Some studies have found changes in surface morphology, microhardness deterioration and dental hard-tissue volume loss as a result of bleaching.[3, 4, 7-10]

Enamel fluoridation is recommended after bleaching to get rid of these hazards.[4, 11] A calcium fluoride layer was formed on the enamel surface inhibiting demineralization and improves microhardness.[12, 13] CPP-ACP is a milk-protein derivative, has been introduced for caries prevention and enamel remineralization.[14] The application of a CPP-ACP complex has been recommended following bleaching and professional tooth cleaning, for white spot prevention/removal in orthodontics and as a topical coating for patients suffering from erosion or caries.[15] However, the effect of CPP-ACP on the microhardness of bleached enamel surfaces is uncertain. Hence, this study was conducted to evaluate the effect of fluoride, CPP-ACP application and storage in artificial saliva on bleached enamel microhardness.

MATERIALS AND METHODS

Thirty human permanent maxillary central incisors extracted from diabetic patients scheduled for complete denture construction were collected. After extraction, each tooth was thoroughly washed under tap water to remove any remnants and blood. Tissue appendages and debris were removed from their surfaces using a sharp hand sickle scalar (Prima-Dent International, Frank. F Germany). Teeth were disinfected in o.1% thymol solution for 48 h. Teeth were examined under a stereomicroscope (SZ-PT; Olympus, Japan) at 30 X magnification to ensure that teeth were free of defects, cracks and caries.

Enamel specimens (5 mm × 5 mm) were prepared using a coarse diamond disk (Struers, 230 CA, USA). Each specimen was mounted in polyvinyle chloride cylinder of 1.8 cm diameter and 1cm height using self-polymerizing acrylic resin (Acrostone, Egypt) to ease handling. To create standardized flat surface, each enamel specimen was polished individually using a coarse, medium, fine and super fine grit disks (Soflex, 3M ESPE, Minnesota, USA) mounted on low speed hand piece under running water. Specimens were assigned into three equal groups (n=10) according to the type of remineralizing agents.

Vickers' MH of all enamel specimens was measured using a digital microhardness tester (Yukon 1202, Buehler, USA). According to the Vickers' method, a diamond point applied pressure to the surface with a 200 kgf load for 10 sec, creating a square-shape indentation. Three indentations were made on the surface of specimen and their averages represent final microhardness of the specimen.

Following this procedure, WHITE smile bleaching gel (GC corporation, Tokyo, Japan) containing 40% hydrogen peroxide was applied to the specimens 3 times (each time for 10 min) according to the manufacturer's instruction. Immediately after completion of bleaching and irrigation of specimens, MH of all samples was measured. After random allocation of enamel specimens into three groups (n= 10), specimens of group A was treated by CPP-ACP paste (GC Tooth mousse; GC corporation Tokyo, Japan), twice a day each time for 5 min for 15 days. Group B specimens was subjected to fluoride varnish (Bifluoride 10; Voco GmbH, Cuxhaven, Germany) immediately after bleaching treatment and after one week. Specimens of group C were stored in artificial saliva for 15 days.

The artificial saliva used in the present study was prepared at Analytical Chemistry department, Faculty of Pharmacy, Mansoura University. The composition of artificial saliva: Methyl –p-hydroxybenzoate (2g/L), Sodium carboxymethyl cellulose (10 g/L), KCl (0.625 g/L),$MgCl_2$.$6H_2O$(0.059 g/L), $CaCl_2.2H_2O$ (0.166 g/L), K_2HPO_4 (0.804 g/L) and KH_2PO_4 (0.326 g/L).The PH of artificial saliva was adjusted to 7.4 with KOH, artificial saliva was changed daily. Finally, MH of all specimens was measured.

Results were statically analyzed using Two-way ANOVA and Post Hoc LSD test at ($P < 0.05$) using SPSS software program (SPSS version 22, IBM, Chicago, IL, USA).

RESULTS

Means, standard deviations and post hock LSD results of all groups are presented in table-1 and figure-1. It was obvious that there was no significant difference among measurements before bleaching (base line) and the mean values range from 374.86±14.27 to 380.02±12.8 VHN. Also, after bleaching there was no significant differences between measurements and mean values ranged from 360.78±13.78 to 365.36±9.27 VHN. After bleaching, there was highly significant drop in mean of microhardness ($p<0.001$). After application of different remineralizing agents, there was significant improvement of microhardness ($p<0.05$); in case of fluoride (398.12±11.79VHN) and in case of CPP-ACP (398.98±14.2VHN). Storage in artificial saliva improves microhardness to the base line values (372.94±12.9VHN). The results of post hock LSD showed that there was no significant difference between the effect of fouride and CPP-ACP on microhardness of enamel but there was significant difference between them and group immersed in artificial saliva.

TABLE (1) Means ± standard deviations in VHN and Post Hoc LSD results for all groups.

	Baseline	After Bleaching	After Remineralization
GC Tooth mousse	380.02±12.8 ^{A}	365.36±9.27 ^{B}	398.98±14.2 ^{Ca}
Bifluoride 10	375.15±11.28 ^{A}	360.78±13.78 ^{B}	398.12±11.79^{Ca}
Artificial saliva	374.86±14.27^{A}	364.66±11.7^{B}	372.94±12.9^{Ab}

The same small superscripted letters denote non-significant difference between groups in same column and similar capital superscripted letters denote non-significant difference in same row.

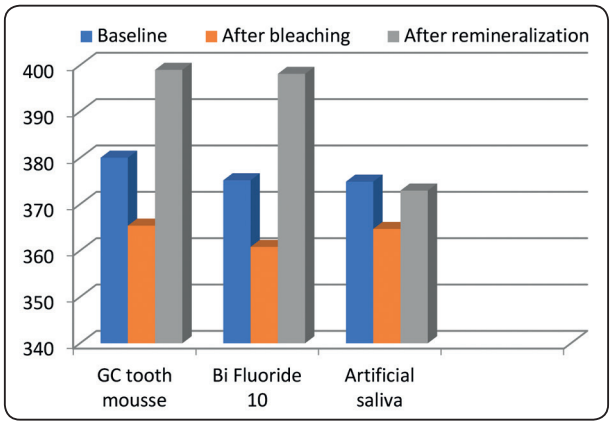

Fig. (1) Abar chart of the microhardness mean values in VHN for all groups.

DISCUSSION

Bleaching is an easy and conservative method for treating discolored teeth than veneers and laminations. Bleaching is a whitening method that can occur in a solution or on the surface. The color producing materials are organic compounds that possess long conjugated chains of alternating single or double bonds. Such compounds in the conjugated system often include hetero atoms, carbonyl, and phenyl rings and are often referred to as chromophore. Chromophore bleaching may occur through dissolution of one or more of the double bonds in the conjugated chain or through the oxidation of other chemical moieties in the conjugated chains.[16]

Hydrogen peroxide bleaching can proceed via the perhydroxyl anion or free radicals depending on pH. Because the peroxide diffuses into the tooth, it can react with organic color materials found inside the tooth structure resulting in color reduction. These oxidation reactions can cause alterations in the enamel structure[17] calcium and phosphate mineral loss,[18] and pulpal sensitivity. The results of these studies which are in accordance with the studies done by Al-Salehi et al.[9], Elfallah et al.[19] showed clearly that the application of bleaching gel lowered the microhardness of the enamel and also showed the changes in enamel are directly proportional to concentration of bleaching agent which are in similar to the studies done by Hegedus *et al.*,[20] The results are in contrast with the study of Basting *et al.*[21]

There was a statistically significant increase in mean surface microhardness after storage in artificial saliva. Saliva not only has a cleaning function and improves oral comfort but also has a buffering action due to the inorganic electrolytes contained in it (calcium, phosphourous and fluoride). These inorganic electrolytes are important participants in the remineralization process of dental hard tissues that could improve the microhardness of the bleached enamel.[22]

This was in agreement with Nathoo et al. who found that enamel alterations due to the effect of 10% carbamide peroxide; revert to almost normal within three months.[23] Carbamide peroxide found to decrease enamel microhardness and deteriorate surface morphology. Smidt et al. reported that "the buffering ability and the remineralization potential of saliva could overcome detrimental bleaching effect. When artificial saliva is used to mimic the natural saliva features, the detrimental effect of bleaching was less apparent".[24]

There was a statistically significant increase in mean surface microhardness of group remineralized by (Bifluoride 10). Preventing bleaching agent demineralizing effects is likely due to the ability of sodium fluoride to remineralize hard tooth structure.[25] Fluoride leads the formation of calcium fluoride deposits on tooth surface.[26] these results are in agreement with Kemaloğlu et al.,[27] Fukuyama et al.[28] and Borges et al.[29] as they concluded that sodium fluoride induct the formation of fluorapatite or fluorhydroxyapatite. This was in contrast to a study by Araujo et al. that said the use of fluoride varnish did not significantly improve the microhardness of bleached enamel.[30]

Casein phosphopeptides are multi phosphorylated from the bovine milk protein casein enzymatic digestion. These peptides stabilize calcium phosphate in solution as amorphous calcium phosphate (ACP), CPP bind to form nanoclusters of ACP in metastable solution, inhibiting their growth to critical size needed for nucleation and phase transformation. CPP-ACP nanoclusters have been shown to locate at the tooth surface. CPP-ACP buffers free calcium and phosphate ion activity by helping to maintain a supersaturating state with regard to tooth enamel which depresses demineralization and stimulate remineralization.[31, 32]

The results of this study showed that there was a definitive increase in microhardness when compared to the post-bleach microhardness. The addition of ACP components to the salivary system at normal oral pH and temperature will cause ACP hydrolysis into apatite within a few minutes, which is approximately 20,000 times faster than normal.[33] In current study, surface MH of teeth increased at the end of the study ,compared to the baseline value, in all three groups after the exposure to the two mineralizing agents as well as artificial saliva. It could be argued that artificial saliva could only have the ability to restore the surface MH to baseline levels, as normally occurs in the oral environment. The only possible explanation for these results could be that, these findings may be due to that the micro porosities formed by bleaching on the subsurface

enamel provides susceptible areas for re-deposition of these materials with higher mineral content, similar to that which occurs in arrested caries. Perfect conditions for enamel remineralization in oral cavity are available. After bleaching, calcium and phosphrus exchange is an easy process leading to more mineral absorption replacing those lost by bleaching process.[34] This phenomenon explains why MH value greater than that of sound enamel.

CONCLUSION

Using Bifluoride 10 and CPP-ACP are recommended for patients after bleaching treatment as they are able to remineralized damaged enamel surfaces and render these surfaces more resistant to acid attack.

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