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The effect of two remineralizing agents and natural saliva on bleached enamel hardness

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ABSTRACT

Background: In order to compensate the adverse consequences of bleaching agents, the use of fluoride-containing remineralizing agents has been suggested by many researchers. The aim of this study was to compare the effect of applying two remineralizing materials on bleached enamel hardness and in comparison to natural saliva.

Materials and Methods: In this experimental study, 30 enamel samples of sound human permanent molars were prepared for this study. Microhardness (MH) of all specimens was measured and 35% hydrogen peroxide was applied 3 times to the specimens. After completion of the bleaching process, MH of samples was measured and then enamel specimens were divided into three groups each of 10, specimens of groups 1 and 2 were subjected to daily application of hydroxyl apatite (Remin Pro) and casein phosphopeptide amorphous calcium phosphate fluoride (CPP-ACPF) (MI Paste Plus) pastes, respectively, for 15 days. In group 3, the specimens were stored in the operators' natural saliva at room temperature in this period of time. Final MH of all groups was measured. The data were analyzed using repeated measures ANOVA (α = 0.05).

Results: The hardness significantly decreased in all groups following bleaching. Application of either Remin Pro, CPP-ACPF or natural saliva increased the hardness significantly. The hardness of the three test groups after 15 days were statistically similar to each other.

Conclusion: The hardness of enamel increases eventually after exposure to either MI Paste Plus, Remin Pro or natural saliva.

Key Words: Bleaching agents, casein phosphopeptide-amorphous calcium phosphate nanocomplex, enamel, hardness, surface properties, tooth remineralization

INTRODUCTION

Bleaching is currently a very popular method for tooth whitening. Although, home bleaching is a simple and effective technique, in-office bleaching is more preferred by the patients and dentists.[1,2]

Several studies have shown that bleaching agents may have significant impacts on surface morphology, chemical composition, microhardness (MH), and even fracture toughness of tooth surfaces.[2,3] In order to compensate the adverse consequences of bleaching agents, use of fluoride-containing remineralizing agents has been suggested by some researchers.[4-6]

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Casein phosphopeptide amorphous calcium phosphate fluoride (CPP-ACPF) containing calcium, phosphate, and fluoride ions, commercially is available as MI Paste Plus (GC America, RECALDENT, Alsip, USA). This product has been proven to be a clinically effective as a remineralizing agent. This nanocomplex is able to penetrate the pellicle and dental plaque which in turn provides an opportunity for calcium, phosphate, and fluoride ions to precipitate on the tooth surface. Maturation of these ions decreases the risk of demineralization and enhances remineralization of the enamel surface.[7,8] The results of previous studies have shown that the application of this complex on bleached or eroded enamel can increase MH and decrease enamel surface roughness.[4-6]

A new product with the brand name of Remin Pro (VOCO, Cuxhaven, Germany) has also been introduced which is a combination of hydroxyapatite (HA), fluoride, and xylitol. The manufacturers claim that this product is capable of enamel surface remineralization.[9] Heshmat *et al*. evaluated and compared the effects of Remin Pro and CPP-ACPF on the surface roughness of bleached enamel and found that these two materials had same efficacy for improving the surface roughness.[10] No study was found on the effect of CPP-ACPF on enamel MH.

On the other hand, the results of some studies show that calcium, phosphate, and fluoride present in the human saliva have a reparative effect on enamel erosions by depositing mineral content. In other words, enamel has a potential to remineralize in the presence of natural saliva.[11,12] *In situ* studies demonstrated that home bleaching agents have no adverse effect on the superficial enamel MH.[13]

Considering the lack of information regarding Remin Pro and absence of a study which compares with CPP-ACPF and natural saliva, we designed this *in vitro* study in order to evaluate the effect of Remin Pro, CPP-ACPF and natural saliva on the MH of bleached enamel.

MATERIALS AND METHODS

This experimental study was conducted on extracted human teeth. Thirty intact permanent molars without any crack and caries were collected, tissue appendages, and debris removed from their surfaces and disinfected in 0.1% thymol solution for 48 h. Enamel specimens with 2.5 mm × 5 mm × 5 mm dimensions were prepared using a coarse diamond disk. (SS White, New Jersey, USA) from the buccal and lingual surfaces of teeth; in order to match the specimens, each enamel specimens were polished individually using a 4000 grit disk (Soflex, 3M ESPE, Minnesota, USA) attached to a low speed hand piece in 30 s for each specimen

Vickers' MH of all enamel specimens was measured.[14,15] According to the Vickers' method, a diamond point applied pressure to the surface with a 50 kgf load and 1 mm/min loading rate, creating a square-shape indentation. This measurement was performed at three different points with the distance of 100 μm between them on each specimen. Following this procedure, Pola Office Gel (SDI, Victoria, Australia) containing 35% hydrogen peroxide was applied to the specimens with an approximate thickness of 1 mm (how did you apply and check 1 mm) 3 times (each time for 8 min)according to the manufacturer's instruction. Immediately after completion of bleaching and irrigation of specimens, MH of samples was measured. After random allocation of enamel specimens into three groups each of 10, specimens of groups 1 and 2 were subjected to Remin Pro paste and CPP-ACPF paste, respectively, twice a day each time for 5 min for 15 days. Groups 1 and 2 specimens were stored in 100% moisture during the experiment using artificial saliva Saliva Kin Spray (HALITUS, Portuguese, Sao Paulo) (Ĩ) without remineralizing materials. In group 3, after the measurement of pre- and post-bleaching MH, the samples were stored for 15 days in the natural saliva at 37°C which changed daily. It should be noted that the saliva was collected daily from a caries free person who had no systemic disease which could negatively affect the quality and quantity of saliva. For a collection of saliva every morning, chewable paraffin saliva stimulants were used at similar time periods each day. The pH of saliva was measured by using pH indicator kits (GC Corporation, Shenzhen, China) and found to be within the natural pH range (7.2). Finally, MH of all specimens was measured.

Based on some study, the Vickers' test is still a routine method of measuring hardness.[14,15] MH of specimens was measured at 3 points using Vickers machine (Wolpert UH930 Wilson, Aachen, Germany) and the mean value was considered as the MH value of the specimen.

Data were analyzed using repeated measures ANOVA. MH changes before and after bleaching were considered as the repeated factor measure, type of material was considered as the between subjects comparison variable and level of significance was set at $P < 0.05$.

RESULTS

The means, standard deviations, maximum and minimum values of primary MH after bleaching and after the intervention are shown in Table 1.

The differences of MH observed between each two measurements are shown in Table 2.

Changes in MH of the three groups were not significantly different ($P = 0.159$). In all three groups, MH decreased after bleaching and at the final stage, MH increased in all three groups compared to the post bleaching and initial values significantly ($P < 0.001$). Based on the data displayed in the tables, final MH of all groups was higher than their initial values. The hardness of the three groups was statistically similar to each other after 15 days.

Table 1: Values of MH (kg/mm²) before and after interventions

Group	MH		
	Mean ± SD	Minimum	Maximum
G1			
Initial	297.6910±39.978	254.37	366.30
Bleached	270.9010±37.513	206.04	321.94
Final	397.0450±44.277	350.54	463.60
G2			
Initial	307.4990±32.268	254.37	350.54
Bleached	287.8080±34.018	245.20	350.54
Final	389.0550±37.426	335.78	441.26
G3			
Initial	345.3050±50.545	264.06	401.16
Bleached	328.2660±41.887	264.06	401.16
Final	383.6000±35.631	335.78	441.26

SD: Standard deviation; MH: Microhardness.

Table 2: Differences in MH (kg/mm²) changes at different steps of experiment in the three groups

Group	MH			
	Initial-bleached (Δ)	Bleached-final (Δ)	Initial-final (Δ)	*P*
Group 1	26.79±11.21	−126.144±19.74	−99.354±16.78	<0.001
Group 2	19.691±7.23	−101.247±19.35	−81.556±21.87	<0.001
Group 3	17.039±8.56	−55.334±18.76	−38.295±42.61	<0.001
P	0.159	0.159	0.159	

MH: Microhardness; Δ: Differences between MH of interventions.

DISCUSSION

This study evaluated the effects of CPP-ACPF and Remin Pro on MH of bleached enamel and showed that these materials, as well as natural saliva, are capable of improving the MH of bleached enamel during 15 days.

Various studies have investigated the effect of bleaching agents on enamel surface roughness. Demineralization and loss of calcium from the tooth surface usually occurs in the organic tissue and HA crystals during bleaching treatment.[14] Studies have shown that the effects of bleaching agents depend on their composition, concentration, exposure time, and acidity.[16-18] In this study, Pola Office bleaching agent containing 35% hydrogen peroxide at a pH of 4.2 was used that significantly decreased the MH, as expected, and is in agreement with previous studies.[19]

Differences in mechanical properties of enamel, changes related to tooth age, drug effects, absorbed fluoride content, orientation and density of HA crystals, moisture of specimens, and methodology of studies can affect enamel demineralization as the result of exposure to bleaching agents.[1] Aside from the pH of bleaching agents, storage conditions of specimens can also be responsible for the variability of results in extra oral studies.[2]

Although some researchers believe that these changes are reversible and have no evident clinical complications,[14] many studies suggest using supplemental treatments such as application of fluoride and other remineralizing agents with the aim of compensating for the complications of bleaching agents.[4,20,21]

Oxidation-reduction reactions of bleaching agents dissolve the organic and nonorganic constituents of teeth. Reduction in MH of enamel is due to the loss of mineral content and in other words, demineralization. Thus, the MH test is recommended for assessment of the effects of bleaching agents on enamel.[3,22]

Extensive studies have indicated that CPP-ACP can remineralize the subsurface layer of decayed and eroded enamel.[7,21,23] According to Shadman *et al*. study, CPP-ACP application reduces the shear bond strength of some dental adhesives with different pH to enamel, due to depositioning of calcium and phosphate and producing a hyper mineralized enamel surface by CPP-ACP.[24]

The manufacturers of Remin Pro and CPP-ACPF have not provided accurate instructions on the time period for using these agents. Thus, based on previous studies on CPP-ACPF, we evaluated CPP-ACPF, Remin Pro, and natural saliva for 15 days.[6,8]

Evidence shows that application of CPP-ACP after tooth bleaching significantly improves enamel MH and remineralization.[5,21] Khoroushi *et al*. evaluated the efficacy of CPP-ACP for increasing the flexural strength of bleached enamel.[6]

It seems that casein phosphopeptide incorporated in CPP-ACPF easily bonds to the biofilm and saturates calcium and phosphorus ions exactly at the required spot. In other words, these ions penetrate into enamel crystals and increase the density of HA crystals.[6-8] Data regarding Remin Pro is scarce since it has recently been introduced to the market. Heshmat *et al*. investigated the effect of CPP-ACPF and Remin Pro on the surface roughness of bleached enamel and reported that both materials decreased the surface roughness of the bleached enamel to the same extent.[10] Remin Pro contains HA particles much similar to calcium and phosphate ions in CPP-ACPF that are deposited on the bleached enamel surface and increase the MH of teeth. Artificial saliva devoid of the remineralizing agents was used for the storage of groups 1 and 2 specimens during the study period to eliminate the effect of confounding factors. Previous studies have shown that artificial saliva has no effect on the MH and surface roughness of enamel.[3,10,22] Thus, we intended to observe whether natural saliva could show a different behavior.

In our 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 natural saliva. It could be argued that natural 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 the fact that the microporosities formed on the subsurface enamel due to bleaching provides susceptible areas for re-deposition of these materials with higher mineral content, similar to that which occurs in arrested caries. In the oral environment, perfect conditions exist for enamel remineralization and demineralized enamel is more susceptible to remineralization. Following enamel demineralization by the bleaching agents, the ionic exchange is facilitated leading to greater absorption of minerals replacing those lost during bleaching.[25] This phenomenon explains the MH value greater than that of sound enamel.

It should be noted that in the oral environment, the effects of bleaching agents are attenuated due to the outward movement of tubular fluid and continuous salivary flow that prevent the complete penetration of the bleaching agent into the tooth structure compared to the extra oral *in vitro* environment.[6]

Natural saliva is saturated with calcium and phosphate ions. Such super-saturation provides the necessary conditions for the remineralization and protects the teeth from demineralizing agents.[24,26] Amaechi and Higham compared the effects of natural saliva, artificial saliva, and a remineralizing agent on the MH of eroded enamel during 28 days and reported that natural saliva has the ability to remineralize enamel just like the fluoride-containing remineralizing agent.[22] In another study, Justino *et al*. reported that presence of saliva can prevent the demineralizing effect of bleaching agents on tooth enamel.[23,25] Although the above-mentioned studies, as well as our study, confirm the remineralizing effect of natural saliva, many authors suggest the use of remineralizing agents following bleaching.

Shannon *et al*., in their *in-vivo* study on bleached teeth noticed that the MH of bleached teeth in natural saliva did not change after 2 weeks, but showed a significant increase in 4 weeks.[27] The difference in results between this study and our findings in terms of final MH levels after approximately 2 weeks could be that the current study was performed extraorally. In such a condition, samples were immersed in saliva and not exposed to a dynamic current, hence increasing the chance for precipitation of the mineral content. It could be said that in addition to the important role of saliva, its composition, pH, flow rate, time, and duration also play a role in the efficacy of it in this respect.[28] Since 25-75% of patients develop tooth hypersensitivity following bleaching procedures, use of these substances is recommended for a period oftime in these patients to resolve the clinical symptoms because it has been reported that the tooth hypersensitivity in 20-30% of these patients continued after the application of placebo.[29] In other words, the potential of remineralizing agents for increasing the MH of bleached enamel can justify

their recommended use for the following bleaching treatments in order to enhance the beneficial effects of natural saliva in increasing the MH of teeth and prevent the adverse effects of mineral loss and tooth hypersensitivity.[22]

Future studies are recommended to assess the effects of remineralizing agents in shorter time periods as well as their synergistic effects with natural saliva.

Scanning electron microscopy images in such studies can explain the pattern of deposition of the remineralizing agent on bleached enamel and surface topography of enamel. However, in our study, the specimens had to remain intact before the bleaching treatment, after the bleaching and after the intervention since they were used as controls for themselves which is considered as one of the limitations of our study.

The results of long-term follow-up shall also be reported in the future.

CONCLUSION

According to the findings of this study, remineralizing products such as MI Paste Plus and Remin Pro can increase the hardness of enamel surfaces which have been decreased following bleaching agent's application. There were no differences in the hardness of enamel subsequent to applying MI Paste Plus, Remin Pro, or natural saliva after 15 days.

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Conflicts of interest
The authors of this manuscript declare that they have no conflicts of interest, real or perceived, financial or non-financial in this article.

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