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Influence of Potentially Remineralizing Agents on Bleached Enamel Microhardness

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Clinical Relevance

Dentists should be aware of the enamel demineralization potential of 35% hydrogen peroxide. The addition of fluoride and calcium can enhance remineralization of surface and subsurface bleached enamel.

SUMMARY

This study investigated the effect of the addition of calcium and fluoride into a 35% hydrogen peroxide gel on enamel surface and subsurface microhardness. Twenty extracted human third molars were sectioned to obtain enamel fragments and they were divided into four groups (n=20) according to the bleaching treatment. Group 1 received no bleaching procedure (control). Group 2 was treated with a 35% hydrogen peroxide gel (Total Bleach), Groups 3 and 4 were bleached with Total Bleach modified by the addition of sodium fluoride and calcium chloride, respectively. The microhardness of the enamel surface was assessed using a Vickers microdurometer immediately after the bleaching treatment. The specimens were sectioned in the central portion, polished and evaluated to determine the microhardness of the enamel subsurface to a depth of 125 µm, with an interval of 25 µm between measures. There were significant differences among the groups. In terms of surface microhardness, the bleached group exhibited the lowest means, and the calcium-modified bleached group exhibited the highest means. Regarding subsurface microhardness, there were no significant differences among the groups for the depth and interaction factors. The bleached group exhibited the lowest means, and the calcium-modified bleached group presented the highest means. It was concluded that the bleaching treatment with 35% hydrogen peroxide significantly reduced the surface and subsurface microhardness of the enamel, and the addition of

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fluoride and calcium in the bleaching agent increased the microhardness means of the bleached enamel.

INTRODUCTION

The in-office technique of external bleaching is a popular method used to whiten discolored teeth. The development of high-concentrations of hydrogen peroxide-based bleaching gels has facilitated these in-office bleaching procedures. The significant advantage to using this technique is obtaining immediate results without requiring patient compliance.

Although previous studies have reported the efficacy of bleaching agents that use high-concentrations of hydrogen peroxide, there is still controversy as to whether these agents could adversely affect dental hard tissues.[1-5]

Studies that investigated the effect of bleaching gels on enamel microhardness using the in-office technique are scarce and the results have been controversial. Oltu and Gürgan[6] observed a reduction in enamel microhardness when 35% carbamide peroxide bleach was used; nevertheless, phosphoric acid was applied onto the enamel before the bleaching agent, which could affect the results. Lewinstein and others[7] and Attin and others[8] showed a reduction in surface enamel microhardness after the bleaching treatment. Al-Salehi and others[9] reported that the reduction in bleached enamel microhardness was inversely proportional to the concentration of hydrogen peroxide. However, Park and others[10] observed no alterations in 30% hydrogen peroxide bleached enamel microhardness.

Sulieman and others[3] have reported that, following treatment with 35% hydrogen peroxide, there was no evidence of deleterious effects on enamel or dentin. They believe that studies reporting adverse effects on bleached enamel and or dentin do not reflect the bleach itself; instead, they reflect on the pH of the formulation used.

The loss of demineralization and calcium are alterations that can occur in the organic composition of bleached hydroxiapatite.[5,9,11-14]

Attempts to enhance the remineralization of bleached enamel have been tried; however, the results are controversial. The addition of fluoride and calcium in the bleaching agent did not significantly enhance the enamel microhardness.[2] In a study by Burgmaier and others,[15] they also did not observe any improvement in fluoride uptake in bleached enamel. On the other hand, previous studies showed that post-bleaching fluoridation prevented mineral loss and restored the softened dental tissues.[7,16] It was also found that, by adding dicalcium phosphate dehydrate to a whitening agent, it reduced caries susceptibility when compared with a gel without fluoride[17] and that enamel treated with a fluoridated bleaching agent exhibited higher caries resistance than non-bleached enamel.[18]

Current knowledge of the effects of in-office bleaching agents on the surface and subsurface microhardness of enamel is still limited and controversial, as well as the addition of remineralizing agents in bleaching agents. Therefore, this study investigated the effect on the enamel surface and subsurface microhardness of adding calcium and fluoride to a 35% hydrogen peroxide gel. It was hypothesized that the addition of remineralizing agents in the bleaching agent would improve the microhardness of enamel.

METHODS AND MATERIALS

Preparation of the Specimens

Twenty extracted, sound human third molars obtained after receiving informed consent according to the São Paulo State University Ethics Committee were used. The teeth were stored in distilled water at 4°C for up to three months. The crowns were separated from the roots, and each crown was sectioned into four quarters using a water-cooled diamond disc (Labcut 1010 low-speed diamond saw, Extec Corp, Enfield, CT, USA).

The tooth fragments were positioned in an acrylic resin cylinder and fixed using sticky wax with the labial surface exposed. The enamel surfaces were ground and polished on a polishing machine (Struers DP-10, Panambra Industrial e Técnica SA, São Paulo, SP, Brazil) using #600, #1200 and #2400-grit aluminum oxide abrasive papers and a 0.4 µm alumina polishing suspension. These procedures were conducted to form parallel planar surfaces, which are fundamental for microhardness testing.

The specimens were randomly divided in four groups, according to the bleaching treatment. Group 1 received no bleaching treatment (control). Group 2 was bleached with a 35% hydrogen peroxide gel (Total Bleach, Clean Line, Taubaté, SP, Brazil). The bleaching agent was modified by adding 0.2% fluoride sodium and 0.2% calcium chloride and was used in Groups 3 and 4, respectively.

The bleaching agents' pH was measured using a pH Meter (Digimed DM-20, Digicrom Analítica Ltda, São Paulo, Brazil) with an electrode (Digimed DME-CV8) that was previously calibrated to the analysis of the gels using pH 4.01 and 6.86 solutions. The conventional gel presented a pH of 6.34, the NaF-modified gel showed a pH of 6.15 and the $CaCl_2\ 2H_2O$-modified gel exhibited a pH of 6.48.

The bleaching agents were applied in one 30-minute session and changed every 10 minutes.

Microhardness Measurements

Vickers microhardness, at a load of 50g, with an indentation time of 10 seconds, was determined using a microhardness tester (FM-700, Future-Tech, Tokyo, Japan). Three indentations were performed on the surface of each specimen, with a distance of 100 µm between them, and averaged.

To determine the subsurface enamel microhardness, the specimens were sectioned in the central portion, using the water-cooled diamond disc, and the halves were fixed in an acrylic resin cylinder using sticky wax.

The inner portion of the specimens was exposed to allow for polishing, as described previously. After polishing, indentations were performed in the specimens from the surface to a depth of 125 µm, with intervals of 25 µm in-between. Three indentations were performed at each depth, with a distance of 100 µm between them and the total was then averaged (Figure 1).

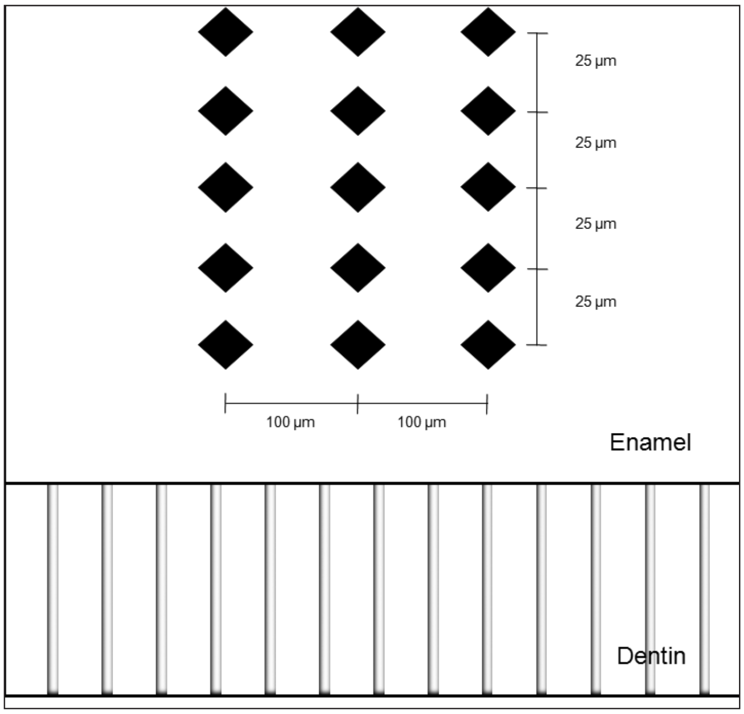

Figure 1. *Schematic diagram of enamel subsurface microhardness measures.*

Statistical Analysis

The enamel surface microhardness data were statistically analyzed using the one-way ANOVA test. The enamel transverse section microhardness analysis was performed using the two-way ANOVA test (bleaching agent and enamel depth factors). The Tukey's post-hoc test was used at a 5% significance level.

RESULTS

Surface Microhardness

The application of one-way ANOVA revealed significant differences for the different groups (p=0.00). The Tukey's test was then applied and showed that the bleached group exhibited the lowest microhardness means and the group bleached with the calcium-added agent showed the highest values (Table 1).

Subsurface Microhardness

The application of two-way ANOVA revealed significant differences for the bleaching gels tested (p=0.00), but there were no significant differences in hardness in the cut sections from the surface toward the DEJ (p=0.88) and in the interaction (bleaching agent X enamel depth) factor (p=0.48).

Table 1 presents the means and standard deviation data of all enamel depths measured and the results of the Tukey's test for the bleaching gels tested. The bleached group showed the lowest means, followed by the group bleached with the fluoride-added agent, and finally, the control group. The group bleached with the calcium-added agent exhibited the highest mean values.

Table 1: *Means and Standard Deviation (SD) Data for the Tested Groups and Results of Tukey's Test for the Surface and Subsurface Vickers Microhardness (5%)*

Groups	Surface* Mean (± SD)	Subsurface* Mean (± SD)
Bleached	302.39 (± 29.27)[a]	304.19 (± 43.54)[a]
Fluoride	340.90 (± 34.82)[b]	341.94 (± 54.33)[b]
Control	369.75 (± 45.93)[b]	360.88 (± 38.77)[c]
Calcium	402.24 (± 40.84)[c]	389.67 (± 37.26)[d]

**Different lower case letters in the same column indicate statistically significant difference.*

DISCUSSION

The adverse effects of the in-office bleaching treatment on enamel microhardness were previously studied, and the results were controversial. Some authors reported significant alterations in bleached enamel microhardness,[7-9,19-20] but, in other studies, the alterations were not significant.[3,10]

In the current study, the 35% hydrogen peroxide bleaching treatment significantly reduced the enamel surface and subsurface microhardness. Although this effect was previously attributed to the pH of the bleaching gel,[3] the bleaching agent used was not acidic enough to cause demineralization of the enamel.

The exact mechanism by which hydrogen peroxide affects dental tissue has not yet been fully understood. The microhardness alterations are probably attributed to the reaction ability of the bleaching agent in relation to the organic phase of enamel, consequently affecting its mineral content. The strong oxidizing effect of hydrogen peroxide on the organic matrix plays a predominant role in the post-bleaching alterations observed in bleached teeth; this can be enhanced by the low pH of the bleaching agent, leading to a decrease in enamel and dentin microhardness.[19,21-22]

The results of the current study showed no significant alteration according to the evaluated depth, that is, the effects of the bleaching treatment on the enamel surface were similar to the effects on the enamel subsurface. Therefore, the authors of the current study can suggest that the potential deleterious effects that occur on the enamel surface extend to the subsurface, as was also observed by Attin and others.[8] In fact, peroxide can penetrate the dental structure, due to its low molecular weigh, exerting its oxidative effect also on the subsurface of enamel where the organic content is higher.[21]

The use of fluorides following bleaching has been shown to restore the surface hardness of softened, bleached enamel.[7] In addition, the attempt to reduce the demineralization of bleached enamel has been successfully accomplished by means of adding fluorides to bleaching agents.[23-24] A fluoridated bleaching gel can also reduce the time needed for bleached enamel hardness to recover, compared with unfluoridated gel[20] by means of the fluoridated hydroxyapatite and calcium fluoride formation, which are favorable to the remineralization process of the tooth surface.[25] In the current study, the addition of fluoride to the bleaching gel significantly enhanced the microhardness of the enamel compared to the bleaching group.

The bleaching treatment with high-concentrations of hydrogen peroxide can reduce the Ca^{2+} concentration of the enamel surface,[9,13-14] thus decreasing its microhardness.[26] Although these alterations are reversible and may not have clinical significance,[11,13,23] knowledge of their adverse effects to bleached enamel is important, so that bleaching procedures are carried out correctly, respecting the time and concentration of agents, according to the manufacturers' instructions and as substantiated by scientific knowledge, thereby ensuring the safety of the treatment.[9]

Additionally, previous attempts to increase the remineralization potential of the dental structure were made by adding calcium compounds to chewing gum[27] and toothpastes,[28-29] thus obtaining satisfactory results. It was also observed that the addition of small amounts of calcium to acid solutions can reduce enamel loss.[30]

In the current study, adding calcium chloride to bleaching gel increased enamel microhardness, as opposed to the results obtained by de Oliveira and others,[2] who found no remineralizing effect in bleached enamel using 10% carbamide peroxide with calcium.

In addition to inhibition of the deleterious effects of bleaching agents on enamel mineral content, the benefits of using remineralizing agents in bleaching agents could include the reduction of enamel solubility and reduced sensitivity due to mineral deposition in enamel crystallites.[31]

This hypothesis was confirmed, as there was a significant increase in both surface and subsurface enamel microhardness for the groups bleached with fluoride and calcium-containing bleaching gels. However, the current *in vitro* study did not take into account the remineralizing action of saliva and, therefore, future clinical studies should be performed to confirm the beneficial action of remineralizing agents in bleaching gels, as well as the optimal concentrations of these compounds.

CONCLUSIONS

According to the limitations of the current study, it can be concluded that the 35% hydrogen peroxide bleaching agent resulted in a reduction in both surface and subsurface enamel microhardness and that the addition of fluoride and calcium in the bleaching agent significantly increased the microhardness of bleached enamel.

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