Effect of high- or low-concentration bleaching agents containing calcium and/or fluoride on enamel microhardness

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The aim of this in vitro study was to evaluate enamel microhardness following bleaching treatments using either high- or low-concentration hydrogen peroxide (HP) agents containing calcium and/or fluoride. Sixty enamel blocks were bleached with 1 of 6 different bleaching agents (n = 10). The high-concentration HP agents were Whiteness HP Maxx (35% HP), Whiteness HP Blue (35% HP, 2% calcium gluconate), Pola Office+ (37.5% HP, 5% potassium nitrate), and Opalescence Boost (38% HP, 1.1% fluoride ion, 3% potassium nitrate). The low-concentration HP agents evaluated were Pola Day (9.5% HP) and White Class (10% HP, potassium nitrate, calcium, fluoride). High-concentration agents were applied in 3 sessions, whereas low-concentration agents were applied for 14 days. Knoop microhardness measurements were taken on the surface of the enamel before bleaching, at various timepoints during bleaching, and 14 days after the final bleaching treatment. The 2-way analysis of variance test showed that microhardness values were significantly influenced by the bleaching agent ($P < 0.001$) and application time ($P < 0.001$). The Tukey test showed that enamel bleached with Whiteness HP Maxx or White Class presented lower microhardness values than did the enamel treated with the remaining products. There was a reduction in micro-hardness values up to the end of the treatment. The results showed that the composition, concentration, and application protocol for each bleaching agent influenced the enamel microhardness values in that the microhardness decreased over time, regardless of the agent used or the addition of calcium and/or fluoride.

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Although the efficacy of bleaching agents containing high or low concentrations of hydrogen peroxide (HP) in achieving shade change is well established, there have been concerns regarding their side effects on the tooth surface.[1,2] An understanding of the effects of bleaching agents on enamel surface properties, especially microhardness, may help the clinician to determine which bleaching agent, concentration, or application approach (in-office application with high concentrations of HP or at-home bleaching with low concentrations) will promote the best clinical outcome with maximal safety.

Tooth bleaching occurs due to decomposition of applied peroxide into free radicals, such as free oxygen and perhydroxyl. These free radicals subsequently react with the pigment molecules within the enamel and the dentin, reducing the size of the molecules, which results in bleaching.[3] In at-home bleaching, low concentrations of carbamide peroxide (20%) or HP (up to 10%) are applied by the patient. With in-office bleaching, much higher concentrations of HP (up to 38%) are used, thus requiring that the dentist provide closer supervision during the application and the patient remain in the office during the procedure.[4]

While researching the various factors involved in tooth bleaching—such as the contact time between the bleaching agent and the tooth surface, bleaching protocol used, chemical composition and pH of the product, and the peroxide concentration used—some studies have reported changes in the mineral content of the enamel, resulting in reduced microhardness.[5-11] Other research, however, has revealed no change in surface hardness.[12-15]

The addition of calcium, fluoride, or both to bleaching agents potentially could bring beneficial effects by reversing enamel demineralization.[6,8] Some studies have shown less enamel erosion when 35% HP enriched with calcium was used and higher surface hardness when either 35% HP enriched with both calcium and fluoride or 16% carbamide peroxide combined with amorphous calcium phosphate was used.[16-18] Alexandrino et al also showed that the addition of calcium gluconate to a high-concentration HP bleaching agent prevented a decrease in enamel microhardness when compared with a control group.[19] However, other studies have not found such favorable results, in terms of either enamel hardness values or the mineral content of the enamel, when bleaching agents containing calcium and/or fluoride were used.[5,8,20]

While conflicting reports have been published regarding enamel microhardness after use of high- or low-concentration HP agents, with or without calcium and/or fluoride, there

Table 1. Composition, pH values, and application protocols of hydrogen peroxide (HP) bleaching agents used in the study.

Product details	High-concentration HPs				Low-concentration HPs	
	Whiteness HP Maxx	Whiteness HP Blue	Pola Office+	Opalescence Boost	Pola Day 9.5%	White Class 10%
Manufacturer	FGM Produtos Odontológicos	FGM Produtos Odontológicos	SDI (North America)	Ultradent Products	SDI (North America)	FGM Produtos Odontológicos
Lot No.	P72199	150514	132020	DOO6A	P130308Z	140114
Composition	35% HP, thickener, dyes, glycol, inorganic filler, deionized water	35% HP, thickener, violet dye, neutralizing agents, 2% calcium gluconate, glycol, deionized water	37.5% HP, 5% potassium nitrate	38% HP, 1.1% fluoride, 3% potassium nitrate	9.5% HP, <47% additives, 30% glycerol, 20% water, 0.1% flavoring, potassium nitrate	10% HP, neutralized carbopol, 5% potassium nitrate, sodium fluoride, aloe vera, calcium gluconate, stabilizer, moisturizer, deionized water
pH	5.5	7.9	7.6	6.9	6.0	5.6
Application protocol	15 min × 3[a] (each session) = 45 min; 3 sessions	40 min (each session); 3 sessions	8 min × 4[a] (each session) = 32 min; 3 sessions	15 min × 3[a] (each session) = 45 min; 3 sessions	30 min/d; 14 d	30 min/d; 14 d

[a]Number of times the bleaching agent was applied during each session. The agent was reapplied without prior rinsing.

has been no research comparing the effects of in-office and at-home bleaching techniques on enamel hardness. The aim of this study was therefore to evaluate human enamel microhardness before, during, and after the use of bleaching agents containing high or low concentrations of HP (with or without calcium and/or fluoride) when applied with either an in-office or at-home bleaching approach.

Materials and methods

This study was approved by the Research Ethics Committee of the São Leopoldo Mandic Dental School and Research Center, Brazil (registration No. 32278214.8.0000.5374). Thirty freshly extracted sound human third molars were stored in 0.1% thymol solution for up to 6 months. The teeth were cleaned with a rotary brush, pumice, periodontal curettes, and scalpel blades. The teeth showed no evidence of cracks, wear, fractures, or stains under a stereoscopic microscope (EK3ST, Eikonal do Brasil) at 20× magnification.

The coronal portions of the teeth were prepared into 80 enamel slabs measuring 3 × 3 × 3 mm from the lingual, palatal, and buccal surfaces of each tooth. Tooth sectioning was performed under constant cooling from deionized water. The blocks were individually embedded in polyester resin (Maxi Rubber) and planed with a rotary electric polisher (EcoMet 250, Buehler) coupled with a power head (AutoMet 250, Buehler) and self-adhesive aluminum oxide sandpaper (600-1200 grit, Arotec). Polishing was performed using a suspension of alumina 0.3 µm (Alpha Micropolish, Buehler) and felt pads (Arotec). The blocks were ultrasonically cleaned in deionized water for 10 minutes to remove any polishing residue.

To obtain homogenous groups and standardized values of initial hardness, microhardness measurements were performed at baseline (T0) on 80 enamel slabs using a digital microdurometer (Pantec HVS 1000, Panambra) and Knoop-type indenter at a static load of 25 g for 5 seconds. Three microhardness indentations were performed on the enamel surfaces of each block at random locations 200 µm apart, and a mean value was calculated for each block. The 20 blocks with the highest and lowest mean microhardness values were excluded. An average value of microhardness of 435 kg/mm^2 and a 25% coefficient of variation were obtained for the remaining 60 enamel blocks, which were used throughout the study.

The 60 blocks were divided into 6 experimental groups (n = 10). One-way analysis of variance (ANOVA) was applied, obtaining a value of $P = 0.3864$, which indicated that there was no significant difference between groups at baseline. Until the initial microhardness evaluations (T0) and application of bleaching agents, the embedded enamel blocks were stored at 37°C in a bacteriologic incubator (ECB 1.3, Odontobrás) in relative humidity for 30 days.

The bleaching agents used in this study were separated into 2 groups: high- and low-concentration peroxides (Table 1). The pH of the bleaching agents was measured in triplicate using a pH meter (MS Tecnopon Equipamentos Especiales). Bleaching agents were applied following the instructions recommended by the manufacturers. The enamel microhardness of the specimens was measured immediately on completion of the first bleaching session of high- or low-concentration HP (T1). During the experimental phase and after bleaching procedures, the specimens were stored in artificial saliva; the solution was changed every 2 days, as suggested by De Abreu et al and Basting et al.[5,20]

Table 2. Mean (SD) Knoop microhardness values (in kg/mm^2) according to hydrogen peroxide (HP) bleaching agent and time.

Timepoint*	High-concentration HPs				Low-concentration HPs		Sample mean
	Whiteness HP Maxx	Whiteness HP Blue	Pola Office+	Opalescence Boost	Pola Day 9.5%	White Class 10%	
T0	415 (70)	411 (61)	370 (79)	403 (40)	432 (74)	390 (62)	404 (66)[a]
T1	373 (82)	414 (64)	372 (77)	399 (86)	415 (80)	341 (66)	386 (78)[a]
T2	295 (106)	436 (118)	365 (107)	407 (92)	400 (92)	405 (53)	385 (103)[a]
T3	303 (84)	384 (90)	392 (94)	414 (85)	417 (62)	400 (95)	385 (91)[a]
T4	296 (47)	375 (53)	341 (60)	373 (77)	326 (86)	288 (57)	333 (71)[b]
T5	306 (71)	358 (71)	340 (70)	365 (70)	346 (62)	300 (54)	336 (68)[b]
T6	301 (50)	335 (71)	345 (72)	360 (68)	348 (87)	286 (84)	329 (75)[b]
Agent mean	327 (84)[B]	388 (82)[A]	361 (80)[A]	389 (75)[A]	384 (85)[A]	344 (83)[B]	—

Means followed by the same letter (uppercase superscript for bleaching agents [row] and lowercase superscript for times [column]) did not show a statistically significant difference ($P > 0.05$).

*Timepoints for high-concentration HPs: T0, baseline; T1, immediately after initial bleaching; T2, 7 days after initial bleaching and before the second bleaching session; T3, immediately after the second bleaching session; T4, 14 days after initial bleaching and before the third bleaching session; T5, immediately after the third bleaching session; T6, 14 days after the completion of bleaching treatment.

Timepoints for low-concentration HPs: T0, baseline; T1, immediately after initial bleaching; T2, 7 days after starting treatment (immediately before the 8th bleaching application); T3, immediately after the 8th application of the bleaching agent; T4, after 13 days of bleaching (immediately before the 14th application); T5, immediately after the 14th application of the bleaching agent; T6, 14 days after the completion of bleaching treatment.

The teeth receiving the bleaching agents with high-concentration HP (Whiteness HP Maxx, Whiteness HP Blue, Pola Office+ and Opalescence Boost) were stored in artificial saliva for 7 days; the solution was changed every 2 days. The bleaching treatment was repeated twice for a total of 3 clinical sessions intercalated by 7 days of immersion in artificial saliva. During bleaching, the enamel hardness of the specimens treated with these agents was evaluated at the following times: T2, 7 days after initial bleaching and before the second bleaching session; T3, immediately after the second bleaching session; T4, 14 days after initial bleaching and before the third bleaching session; T5, immediately after the third bleaching session; and T6, 14 days after the completion of bleaching treatment.

Teeth receiving low-concentration HP bleaching agents (Pola Day and White Class) were stored separately in artificial saliva. The application of bleaching agents was performed daily for a total of 14 days. During this time, the teeth were removed from the storage solution and dried before the daily application of the agent. Enamel hardness assessments were made at the following times: T2, 7 days after starting treatment (immediately before the 8th bleaching application); T3, immediately after the 8th application of the bleaching agent; T4, after 13 days of bleaching (immediately before the 14th application); T5, immediately after the 14th application of the bleaching agent; and T6, 14 days after the completion of bleaching treatment.

The hardness values obtained at different times for different bleaching agents were tabulated. The data were checked for normality and equality of variance and analyzed using a repeated measures 2-way ANOVA. The Tukey test was used for multiple comparisons. The statistical calculations were performed on SPSS statistical software (IBM) at a significance level of 5%.

Results

The 2-way ANOVA showed no interaction between the factors *bleaching agent* and *time* ($P = 0.341$). The microhardness values were significantly influenced by the type of bleaching agent ($P < 0.001$) and also varied over time ($P < 0.001$). The Tukey test revealed that, at any timepoint, the mean microhardness values of enamel bleached with Whiteness HP Blue, Pola Office+, Opalescence Boost, or Pola Day were not significantly different (Table 2). However, enamel bleached with these agents did exhibit significantly higher mean microhardness values than did the specimens whitened with Whiteness HP Maxx and White Class (whose mean microhardness values were not significantly different from each other).

When the effect of time was evaluated via the Tukey test, microhardness values at timepoints up to and including T3 were significantly higher than those at T4, T5, and T6. Microhardness values did not differ significantly among the timepoints T0, T1, T2, and T3, while values at T4, T5, and T6 were statistically similar to each other.

Discussion

For one of the bleaching agents with a high concentration of HP (Whiteness HP Maxx), lower enamel microhardness values were observed when compared to the other groups. A factor that might be related to this finding is the lower pH of this product (5.5) compared to the other agents evaluated. The measured pH value of Whiteness HP Maxx was close to the critical pH for enamel demineralization. Additionally, potentially remineralizing agents such as calcium and/or fluoride are not present in the composition. Demineralization can be attributed to low concentrations of calcium and phosphate and high concentrations of sodium and chloride ions present in the composition of the bleaching agent, a state that was favored by the low pH, which led to subsaturation of the bleaching gel in relation to enamel hydroxyapatite. In the present study, other high-concentration peroxide products for in-office use showed pH values that were closer to neutral or slightly alkaline. Klaric et al also reported that higher HP concentrations and higher acidity of the bleaching gel negatively affect enamel microhardness as well as the calcium and phosphate concentrations.[10] These findings were corroborated by Sa et al, who compared an acidic with an alkaline bleaching agent.[7] Even when using a bleaching agent with only a slightly acidic pH (6.6), Magalhães et al observed a decrease in enamel hardness after treatment.[6]

Due to mineral subsaturation on the enamel surface, which results in the loss of mineral content from the tooth substrate, manufacturers of bleaching agents with low pH values may incorporate fluoride or calcium to minimize demineralization. The addition of fluoride has been the most widely used strategy, since it also helps to reduce dentinal sensitivity from the bleaching treatment via mineral deposition on the tooth surface.[21] The combination of higher concentrations of sodium fluoride in the gel and a lower pH bleaching agent has resulted in fewer morphologic changes to the enamel by allowing for the maintenance of mineral content or even remineralization.[12,17,22] Furthermore, the presence of calcium in a bleaching agent, as either calcium chloride or calcium gluconate, has also shown positive effects on the maintenance of enamel microhardness due to the formation of deposits on the tooth substrate, which promote supersaturation of the enamel.[8,12,16,17]

Among the high-concentration HP agents evaluated, Opalescence Boost has fluoride in its composition and Whiteness HP Blue has calcium gluconate, which may have contributed to the higher enamel microhardness values in those groups. However, Pola Office+ features neither of those ions, containing only potassium nitrate, which plays a role in minimizing tooth sensitivity during bleaching.[21] This bleaching agent, however, has slightly alkaline pH values and requires shorter contact time between the bleach and the tooth surface—32 minutes per session as compared to other products (40 minutes or more). These factors perhaps could explain why the microhardness values measured in enamel bleached with Pola Office+ were similar to values in enamel treated with high-concentration HP bleaching products with remineralizing properties.

When the low-concentration HP bleaching products were compared, White Class caused a greater decrease in enamel hardness than Pola Day, a result that was probably due to the lower pH value recorded for the former agent. Although it was expected that both agents could have a similar effect on enamel microhardness because they both contain fluoride and recommend the same application time (30 minutes per day), this result was not observed. Furthermore, White Class also features calcium gluconate in its composition, which might have suggested that enamel hardness values would not be affected by the bleaching gel.[13,23] However, in the present study, although it seems that calcium gluconate may have contributed to higher enamel microhardness values in the high-concentration HP bleaching agent (Whiteness HP Blue), this effect was not observed in the lower concentration product. It is expected that calcium deposits are formed on the enamel surface when agents containing calcium gluconate are used, thus reducing the susceptibility to enamel erosion.[8,16] However, the beneficial effect of calcium gluconate on the enamel is still controversial, as demonstrated in the present study. Calcium gluconate is incompatible with strong oxidizing agents; therefore, the free oxygen and perhydroxyl present in the by-products released during bleaching could be inhibited by the calcium gluconate, thus preventing changes to the mineral content of the enamel.[3,20]

For all the bleaching agents evaluated, a significant decrease was observed in microhardness values at T4, which would correspond to the point before the third bleaching session for the high-concentration products or immediately before the 14th application of the low-concentration products. De Abreu et al observed decreases in microhardness over the course of bleaching treatments, differences that were significant at day 21 of treatment for products containing low concentrations (7.5% and 9.5%) and high concentrations (35% and 38%) of HP.[5] Some studies have shown that a reduction in enamel microhardness can be observed even after the first application of a high-concentration HP agent at chairside.[9,11] Borges et al, on the other hand, observed no change in enamel microhardness in response to the bleaching agents used in their study.[15] However, the authors only assessed higher HP concentrations (20%, 25%, 30%, and 35%) immediately after the first session (30 minutes posttreatment) and 1 week posttreatment, and the specimens had remained immersed in artificial saliva between measurements. Their results indicate that, in addition to the factor of time, the composition of the bleaching agents and their pH values can influence enamel microhardness. The findings from the present study demonstrate that treatment time (either through daily applications of low-concentration agents or weekly application sessions of high-concentration agents) influenced enamel microhardness values. Application time of the bleaching agent to the enamel appears to have a significant effect on mineral content, even between sessions, indicating that the enamel is permeable to the by-products of HP degradation and that the bleaching agent's action appears to be prolonged even at times when the agent is not in contact with the tooth surface.[9,10]

The use of remineralizing substances such as artificial saliva or fluoridated products after bleaching reportedly has resulted in recovery of initial microhardness values and possibly the calcium and phosphate content within the enamel.[5,8,10] In the present study, however, this effect was not observed; this might be due to the time in which the enamel blocks remained immersed in artificial saliva, which may have replenished the mineral content.

The findings from this study may aid the clinician in the process of choosing an appropriate bleaching agent that promotes minimal change to enamel microhardness values. Although all agents caused a decrease in enamel microhardness with time, regardless of the HP concentration or application approach, 3 high-concentration HP agents used at chairside (Whiteness HP Blue, Pola Office+, and Opalescence Boost) and 1 low-concentration agent for in-home use (Pola Day) provided the highest enamel microhardness values among the products tested, possibly due to their composition and higher pH values.

Conclusion

The composition, concentration, and application protocol for each bleaching agent tested in this study influenced enamel microhardness values. The microhardness values decreased over time, regardless of the agent used or the addition of calcium and/or fluoride.

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