

# Influence of Remineralizing Gels on Bleached Enamel Microhardness In Different Time Intervals

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## Clinical Relevance

Dentists should be aware of the reduction in enamel microhardness after bleaching procedures. The use of remineralizing gels after bleaching treatment can significantly enhance bleached enamel microhardness.

## SUMMARY

**This study evaluated the influence of bleaching gel pH, the effect of applying remineralizing gels after bleaching and the effect of artificial saliva on enamel microhardness. Seventy bovine inci-**

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**sors were divided into three groups: Group 1 (n=10) received no bleaching procedure (control); Group 2 was bleached with a 35% hydrogen peroxide neutral gel (n=30) and Group 3 was bleached with a 35% hydrogen peroxide acid gel (n=30). Each experimental group was subdivided into three groups (n=10) according to the post-bleaching treatment: storage in artificial saliva, application of a fluoride gel and application of a combination of calcium and fluoride gel. The specimens were stored in artificial saliva for 7, 15 and 30 days and enamel microhardness was evaluated. The Vickers microhardness data were analyzed by three-way RM ANOVA, which revealed a significant difference only for treatment factor.**

**The Tukey's test showed that the groups bleached followed by no additional treatment exhibited microhardness means significantly lower than the bleached groups treated with remineralizing gels. The Dunnet's test showed a significant difference only for the group bleached with acid gel without remineralizing treatment compared to the control group measured immediately after bleaching. It was concluded that acid bleaching gel significantly reduced enamel microhardness and that use of remineralizing gels after bleaching can significantly enhance the microhardness of bleached enamel.**

## INTRODUCTION

In recent years, the development of bleaching gels that use hydrogen peroxide in high concentrations (35% to 38%) have made in-office bleaching procedures easier. The advantage of this technique is the favorable immediate results achieved without a need for further patient cooperation.

The efficacy of bleaching agents is validated by *in vitro* and *in vivo* studies;<sup>1,2</sup> nevertheless, the adverse effects to dental tissues must be carefully evaluated in order to use them safely.<sup>1</sup> In fact, the effect of bleaching procedures on enamel is still controversial and needs to be elucidated.<sup>3</sup>

Although a reduction in enamel microhardness has been reported,<sup>4,7</sup> it must be assumed that this alteration reflects not only the bleaching procedure but also the pH of the formulation used.<sup>1</sup> In fact, some studies have found no significant differences in enamel microhardness after bleaching with hydrogen peroxide in high concentrations.<sup>8,9</sup>

Additionally, some *in vitro* studies also have found a loss of calcium from bleached enamel, although this alteration was not considered clinically significant.<sup>10-12</sup>

The possibility of remineralizing bleached enamel has been investigated, however, the results are conflicting. The addition of fluoride and calcium in the bleaching agent did not result in higher means of enamel microhardness.<sup>3</sup> In a study by Burgmaier and others, the authors did not observe any improvement in fluoride uptake in bleached enamel.<sup>13</sup> On the other hand, the use of fluoride following bleaching has been shown to restore microhardness and prevent mineral loss of bleached enamel.<sup>7,14</sup>

Calcium has been investigated in an attempt to enhance the remineralizing potential of fluoride in dentifrices.<sup>15-16</sup> Sullivan and others<sup>17</sup> observed that providing additional calcium improves the anti-caries efficacy of a fluoride dentifrice. Nevertheless, in order to act as a remineralizing agent, it is essential that calcium phosphate remains in soluble form. If calcium phos-

phate were to precipitate out of solution, it would function as an abrasive agent and not as a remineralizer. Adding glycerin to the composition acts to prevent calcium phosphate from forming a precipitate.<sup>18</sup>

In addition, attention must be paid to the fact that sodium fluoride and calcium phosphate react and form a stable, insoluble salt. Fluoride ions can also react with calcium to form calcium fluoride.<sup>17</sup> Therefore, the reaction of fluoride with calcium phosphate must be delayed until both are applied to enamel in order to improve remineralization efficacy.<sup>17</sup>

The current study evaluated the effect of bleaching procedures using two 35% hydrogen peroxide-based gels, both with a different pH; the effect of applying two remineralizing gels after bleaching and the effect of storage in artificial saliva on enamel microhardness. The null hypotheses investigated were: 1) the bleaching agent has no effect on enamel microhardness; 2) remineralizing gels applied after the bleaching procedures have no effect on enamel microhardness and 3) storage in artificial saliva has no effect on enamel microhardness.

## METHODS AND MATERIALS

### Sample Preparation

Seventy erupted and intact bovine incisors were extracted immediately after the animals had been sacrificed. The incisors were collected according to a protocol approved by the University Committee. Each tooth was transversally sectioned at the cemento-enamel junction and the roots were discarded. The pulp tissue was removed using endodontic instruments. The root canal openings were sealed with utility wax.

The teeth were positioned in a plastic mold and embedded using a self-curing acrylic resin. The enamel surfaces of the teeth were ground into a flat surface using SiC paper-grit #80 and polished using #600, #1200 and #2400-grit aluminum oxide abrasive papers and a 0.4  $\mu\text{m}$  alumina polishing suspension on a polishing machine (DP-10 Panambra, Panambra Industrial e Técnica SA, São Paulo, SP, Brazil).

The teeth were divided into three groups. Group 1 (control) received no bleaching treatment and was stored in artificial saliva. Group 2 was bleached with Total Bleach (Clean Line, Taubaté, SP, Brazil) composed of a 35% hydrogen peroxide gel; a mixture of components from two bottles with a neutral pH. Group 3 was treated with Red Peroxide (Fórmula & Ação, São Paulo, Brazil) containing 35% hydrogen peroxide gel and dispensed in a single bottle with an acid pH. Both bleaching agents were used for 30 minutes and, after each 10-minute period, the bleaching agents were changed according to the manufacturer's instructions.

The bleaching procedures were repeated twice, with a one-week interval and, during this period, the specimens were stored in 37% artificial saliva.

The specimens in Groups 2 and 3 (n=60) were divided into three subgroups (n=10) according to post-bleaching treatment: 1) storage in artificial saliva; 2) application of a 2% sodium fluoride gel (four minutes) and 3) application of equal amounts of 2% dihydrated calcium phosphate combined with 2% sodium fluoride gel (four minutes).

All specimens were stored in artificial saliva for 7, 15 and 30 days, after which the microhardness of enamel was evaluated.

The pH of the bleaching agents and remineralizing gels was measured using a pH Meter (Digimed DM-20, Digicrom Analítica Ltda, São Paulo, Brazil) with an electrode (Digimed DME-CV8) that was calibrated using pH 4.01 and 6.86 solutions prior to analysis of the gels. The basic composition of the materials and their pH is described in Table 1.

Microhardness Evaluation

The enamel microhardness determination was performed with a microhardness tester (FM-700, Future-Tech, Tokyo, Japan) fitted with a 50 kgf load, which was used to make indentations on the enamel surface. The loaded diamond was allowed to sink and rest on the enamel surface for 10 seconds and the Vickers hardness number was determined. Three indentations were performed on each specimen, with the distance of 100 µm between them, and they were averaged. Measurements were obtained after the last bleaching session and after 7, 15 and 30 days post-bleaching.

Statistical Analysis

The influence of the bleaching agent and post-bleaching treatment on enamel microhardness values was evaluated using three-way Repeated Measures ANOVA (time as the repeated variable) and Tukey's test. Comparison of the experimental conditions according

to the period with the control group was made using the Dunnet's test ( $\alpha=0.05$ ).

RESULTS

Table 2 shows mean microhardness values and standard deviations for the different conditions and the results of the Dunnet's test.

Application of the Dunnet's test showed a significant difference only in the group bleached with Red Peroxide without additional treatment compared to the control group, which was measured immediately after the bleaching treatment ( $p=0.045$ ).

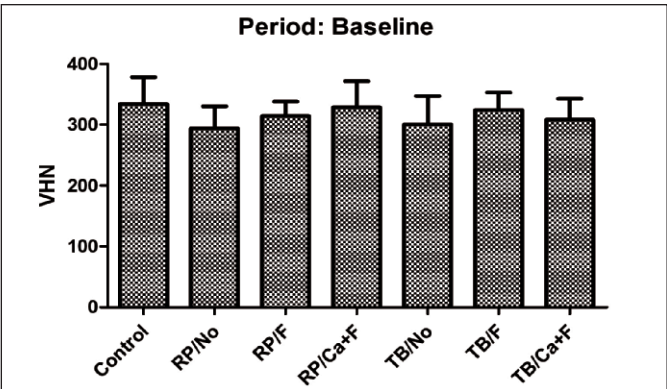


Figure 1: Microhardness values (VHN) and standard deviation of different conditions and treatment, according to the period of evaluation (baseline graph).

Control = No bleaching  
RP/No = Red peroxide/No treatment  
RP/F = Red peroxide/Fluoride  
RP/Ca+F = Red peroxide/Calcium + Fluoride  
TB/No = Total bleach/No treatment  
TB/F = Total bleach/Fluoride  
TB/Ca+F = Total bleach/Calcium + Fluoride  
(N=10)

Table 1: Bleaching Agents, Remineralizing Gels and Artificial Saliva Specifications			
Materials	Manufacturer	Basic Composition	pH
Red Peroxide	Fórmula & Ação, São Paulo, SP, Brazil	35% Hydrogen Peroxide, thickening agent	3.50
Total Bleach	Clean Line, Taubaté, SP, Brazil	35% Hydrogen Peroxide, thickening agent	6.60
2% NaF gel	Author's Laboratory at São José dos Campos School of Dentistry, SJC, SP, Brazil	2% NaF, glycerin, de-ionized water, thickening agent	7.97
2% Ca <sub>2</sub> PO <sub>3</sub> gel	Author's Laboratory at São José dos Campos School of Dentistry, SJC, SP, Brazil	2%Ca <sub>2</sub> PO <sub>3</sub> , glycerin, de-ionized water, thickening agent	8.06
Artificial Saliva	Byoformula, São José dos Campos, SP, Brazil	calcium chloride 0.166g; sodium benzoate 1g; CMC 10g; magnesium chloride 0.05g; potassium chloride 0.62g, sodium chloride 0.025g; sorbitol 42.74g, distilled water 944.53mL; dibasic potassium phosphate 0.8035g; monobasic potassium phosphate 0.326g	6.68

Table 2: Mean Values ( $\pm$  standard deviation) of the Different Conditions and Time Periods Tested and Results of Dunnet's Test

Time	Bleaching Gel	Treatment	Mean ( $\pm$ Standard Deviation) (VHN)	Comparison to Control*
Baseline	Control		334.17 $\pm$ 44.13	
Baseline	RP	No	294.13 $\pm$ 36.43	0.04*
Baseline	RP	F	314.61 $\pm$ 23.41	0.37
Baseline	RP	Ca + F	328.73 $\pm$ 43.23	0.75
Baseline	TB	No	300.76 $\pm$ 46.47	0.13
Baseline	TB	F	324.61 $\pm$ 28.70	0.64
Baseline	TB	Ca + F	308.60 $\pm$ 34.17	0.23
7 days	Control		304.92 $\pm$ 39.39	
7 days	RP	No	299.37 $\pm$ 49.23	0.74
7 days	RP	F	304.57 $\pm$ 23.07	0.85
7 days	RP	Ca + F	330.82 $\pm$ 53.15	0.99
7 days	TB	No	294.17 $\pm$ 38.77	0.61
7 days	TB	F	317.48 $\pm$ 29.36	0.97
7 days	TB	Ca + F	286.68 $\pm$ 16.14	0.41
15 days	Control		307.21 $\pm$ 39.98	
15 days	RP	No	276.04 $\pm$ 17.20	0.08
15 days	RP	F	299.13 $\pm$ 61.97	0.66
15 days	RP	Ca + F	299.28 $\pm$ 11.88	0.66
15 days	TB	No	291.12 $\pm$ 27.46	0.41
15 days	TB	F	312.96 $\pm$ 18.43	0.93
15 days	TB	Ca + F	310.22 $\pm$ 30.23	0.90
30 days	Control		298.85 $\pm$ 29.40	
30 days	RP	No	295.52 $\pm$ 16.14	0.76
30 days	RP	F	293.73 $\pm$ 15.26	0.71
30 days	RP	Ca + F	318.30 $\pm$ 19.30	0.99
30 days	TB	No	279.04 $\pm$ 23.18	0.19
30 days	TB	F	311.59 $\pm$ 24.24	0.99
30 days	TB	Ca + F	319.52 $\pm$ 47.01	0.99

\*significant difference (5%).

Table 3: Results of Tukey's Test for Treatment Factor

Post-bleaching Treatment	Mean (VHN)	Homogen Groups*
No Treatment	291.27	A
Fluoride	309.83	B
Calcium + Fluoride	312.17	B

\*Similar letters indicate no significant differences.

The three-way RM ANOVA revealed a significant difference only for treatment factor ( $p=0.000$ ). The Tukey's test was applied (Table 3), and it was observed that both bleached groups that received no remineralizing gel exhibited microhardness means significantly lower than the bleached groups treated with remineralizing gels.

Figures 1 through 4 show the microhardness values (VHN) and standard deviation of the different conditions and treatment according to the period of evaluation.

## DISCUSSION

The adverse effects of in-office bleaching treatment on enamel microhardness were previously investigated and controversial results were obtained. Significant alterations in bleached enamel microhardness were found by Lewinstein and others,<sup>7</sup> Pinto and others<sup>6</sup> and Attin and others,<sup>19-20</sup> but, in studies by Park and others<sup>8</sup> and Sulieman and others,<sup>1</sup> microhardness changes were not significant.

In the current study, although lower microhardness means were observed for both bleaching agents tested compared to the control group, there was a significant difference only with the acid bleaching gel.

The effects of bleaching on enamel microhardness are probably related to their pH,<sup>1</sup> as well as alteration of the organic matrix of enamel under the chemical action of hydrogen peroxide.

The strong oxidizing effect of hydrogen peroxide on the organic matrix of teeth plays a predominant role in the alterations observed after bleaching, which can probably be increased by low pH of the bleaching agent, causing subsequent alterations in the mineral composition, decreasing enamel and dentin microhardness.<sup>4,6,21-22</sup>

Demineralization and calcium loss are alterations that can occur in the inorganic composition of bleached hydroxyapatite.<sup>10-11,23-26</sup> Nevertheless, some authors consider these alterations reversible and probably without clinical relevance.<sup>5,11,23</sup>

The addition of fluoride to bleaching agents or its use after bleaching procedures has been investigated in an



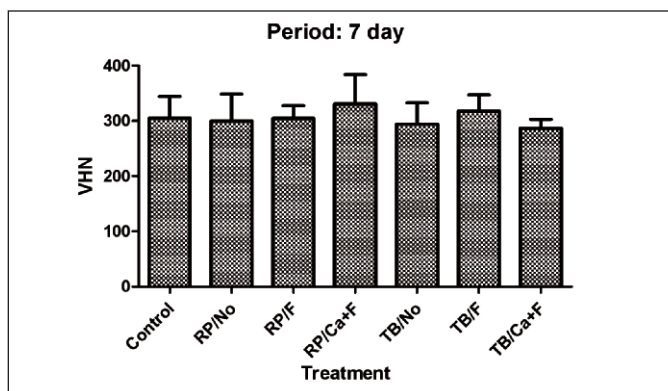


Figure 2: Microhardness values (VHN) and standard deviation of different conditions and treatment, according to the period of evaluation (7-day graph).

Control = No bleaching

RP/No = Red peroxide/No treatment

RP/F = Red peroxide/Fluoride

RP/Ca+F = Red peroxide/Calcium + Fluoride

TB/No = Total bleach/No treatment

TB/F = Total bleach/Fluoride

TB/Ca+F = Total bleach/Calcium + Fluoride

(N=10)

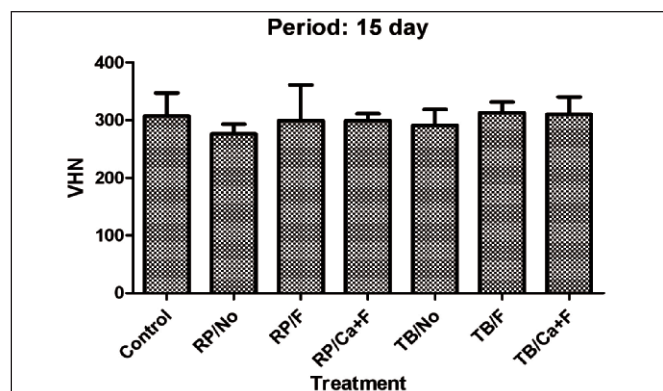


Figure 3: Microhardness values (VHN) and standard deviation of different conditions and treatment, according to the period of evaluation (15-day graph).

Control = No bleaching

RP/No = Red peroxide/No treatment

RP/F = Red peroxide/Fluoride

RP/Ca+F = Red peroxide/Calcium + Fluoride

TB/No = Total bleach/No treatment

TB/F = Total bleach/Fluoride

TB/Ca+F = Total bleach/Calcium + Fluoride

(N=10)

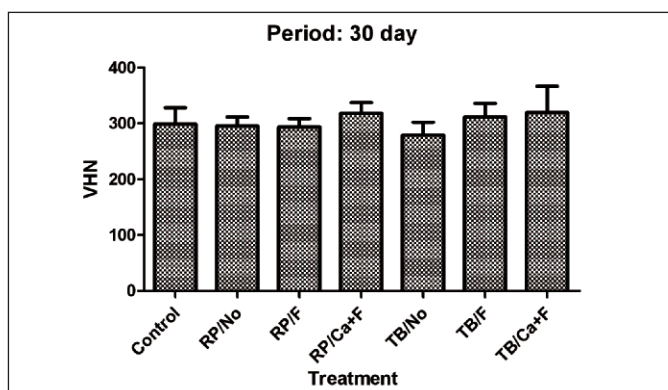


Figure 4: Microhardness values (VHN) and standard deviation of different conditions and treatment, according to the period of evaluation (30-day graph).

Control = No bleaching

RP/No = Red peroxide/No treatment

RP/F = Red peroxide/Fluoride

RP/Ca+F = Red peroxide/Calcium + Fluoride

TB/No = Total bleach/No treatment

TB/F = Total bleach/Fluoride

TB/Ca+F = Total bleach/Calcium + Fluoride

(N=10)

attempt to increase mineralization of bleached enamel.<sup>5,7</sup> Fluoride is incorporated into the tooth surface, forming a calcium fluoride layer that increases enamel hardness values.<sup>27</sup>

In the current study, the use of a fluoride gel after bleaching procedures increased enamel microhardness significantly when compared to groups that received no additional treatment after bleaching. Previous

studies also found that enamel mineral loss was significantly reduced when topical fluoride was applied following bleaching.<sup>7,14</sup>

Fluoride can also be incorporated into the bleaching gel. Attin and others<sup>20</sup> found that the addition of fluoride into the bleaching agent can support the rehardening of bleached enamel, with a shorter period needed for hardness to recover compared to gels without fluoride.<sup>20</sup> Nevertheless, an *in vivo* study determined that there were no significant differences between a 15% carbamide peroxide bleaching treatment with and without fluoride on enamel microhardness, probably due to the remineralizing effect of saliva.<sup>28</sup>

The remineralizing effect promoted by the association of sodium fluoride and calcium phosphate was also investigated in the current study. This association was previously tested in a dentifrice and provided a superior level of anticaries efficacy than did the dentifrice containing only sodium fluoride in the same concentration.<sup>15,29</sup> The combination of fluoride to calcium in dentifrices has increased the ionic activity of fluoride and calcium, resulting in improved remineralization of early carious lesions.<sup>30</sup>

It was also observed that the addition of small amounts of calcium to acid solutions can reduce the loss of enamel.<sup>31</sup> Thus, in an attempt to intensify the mineralization of bleached enamel, an association of calcium and fluoride in a dual component remineralizing gel was developed, although the literature studies are controversial. The incorporation of calcium into a 10% carbamide peroxide bleaching gel did not enhance the microhardness of enamel;<sup>3</sup> however, in a previous

study, the authors of the current study found that the addition of calcium and fluoride in 35% hydrogen peroxide enhanced the remineralization of surface and subsurface bleached enamel.<sup>32</sup>

The results of this study indicated a significant difference in the microhardness of bleached enamel treated with calcium-fluoride remineralizing gel compared to groups without additional treatment after bleaching. However, there was no significant difference between the two remineralizing gels used in this study (Table 3).

The low increase in remineralization obtained by the association of calcium to fluoride is probably due to the low solubility of calcium phosphate<sup>33</sup> in spite of the addition of glycerin into the gel to increase ion availability.<sup>18</sup> Different forms of more soluble calcium can be tested in future studies, aiming to increase enamel remineralization of bleached teeth.

Storage in artificial saliva was not observed to cause a significant difference in enamel microhardness during the evaluated periods. Rodrigues and others<sup>34</sup> also observed no alterations in intact enamel microhardness stored in artificial saliva. Although a remineralization effect of artificial saliva in bleached enamel has been reported,<sup>35</sup> this can be incomplete, leaving areas susceptible to further decalcification and plaque retention.<sup>7,36</sup> The use of a remineralizing agent with fluoride or with the association of calcium to fluoride can be an important factor to promote the remineralization of bleached enamel.

In addition to inhibition of the deleterious effects of bleaching agents on enamel mineral content, the benefits of using remineralizing agents in bleaching agents or after bleaching could include the reduction of enamel solubility and reduced sensitivity due to mineral deposition in enamel crystallites.<sup>37</sup>

The null hypothesis that the bleaching agent does not affect enamel microhardness was accepted for neutral gel and rejected for acid gel. In relation to post-bleaching remineralizing treatment, the null hypothesis was rejected, as there was an increase in bleached enamel microhardness when remineralizing gels were used and the null hypothesis regarding the effect of artificial saliva on enamel microhardness was accepted.

It should be noted that this is an *in vitro* study, and further studies need to be conducted to substantiate that acidic agents will cause a significant loss of microhardness with high concentrations of bleaching agents and determine whether sufficient fluoride and calcium phosphate is available intraorally to enhance bleached enamel microhardness in bleached teeth.

## CONCLUSIONS

According to the limitations of the current study, it was concluded that bleaching with an acidic agent resulted in a significant lowering of enamel microhardness compared to the control group; bleached enamel microhardness was enhanced with the use of remineralizing gels and artificial saliva did not affect enamel microhardness.

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