

# Demineralization Effects of 2 Bleaching Procedures on Enamel Surfaces With and Without Post-treatment Fluoride Application

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## Clinical Relevance

The results of this study suggest that post-treatment fluoride application prevents mineral loss in bleached enamel surfaces.

## SUMMARY

**In this *in vitro* study, the demineralization effect of 2 different bleaching procedures on enamel surfaces with and without the post-treatment application of fluoride was determined.**

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Bovine enamel specimens (n= 180) were ground flat, polished and divided into 6 groups. Group A (n=30) specimens were bleached with Opalescence, 10% carbamide peroxide (Ultradent Products, Inc) for 8 hours daily for 2 weeks; Group B (n=30) specimens were treated with Whitestrips, 5.3% hydrogen peroxide (Procter & Gamble) for 1 hour daily for 2 weeks; Group C (n=30): the same as Group A, but after bleaching, a fluoride varnish was applied on the specimens and left for 1 hour (Duraphat, 2.26% F<sup>-</sup>); Group D (n=30): the same as Group B, followed by the same fluoride application as Group C; Group E (n=30): the specimens were covered with a glycerin gel as a control group; Group F (n=30): specimens were kept in Coca Cola 1 hour daily for 2 weeks. The mineral loss (vol%  $\mu$ m) and lesion depth ( $\mu$ m) were measured by microradiography. Data analysis was accomplished using the Kolmogorov-Smirnov, Kruskal-Wallis and Mann-Whitney U tests ( $p<0.05$ ) (SPSS 11.0).

The median mineral loss was statistically significantly higher in the non-fluoride groups (A: 271.20 vs C: 128.00 and B: 364.90 vs D: 151.10). The highest mineral loss was found in Group F (581.85 vol%  $\mu$ m) and was lowest in Group E (32.80 vol%  $\mu$ m). No statistically significant difference between groups was found for lesion depth.

## INTRODUCTION

Tooth discoloration creates a wide range of esthetic problems. The methods available to treat discolored teeth range from removal of surface stains, bleaching, tooth whitening techniques or operative techniques to camouflage the underlying discoloration, such as veneers and crowns. The use of a variety of bleaching techniques has attracted much interest from the profession, as these techniques are non-invasive. Current techniques may be classified as either professionally applied or patient applied.<sup>1</sup>

Vital tooth bleaching with 10% carbamide peroxide is a safe, well-accepted procedure for the treatment of surface and intrinsic tooth-staining.<sup>2</sup> A comparable new way of peroxide application is presented with Whitestrips, thin polyethylene strips of foil covered with a bleaching agent gel containing 5.3% hydrogen peroxide.

Some studies have shown that bleaching with 10% carbamide peroxide decreased the enamel calcium, phosphate and fluoride content.<sup>3-6</sup> In contrast, Crews and others<sup>7</sup> found that the amount of calcium and phosphate in enamel increased after bleaching with carbamide peroxide. Other studies have found a slight alteration in the enamel surface after bleaching.<sup>8-10</sup> However, there are also studies that did not find any aspects of destruction of the bleached enamel surface.<sup>11-13</sup>

It is widely accepted that topical fluorides promote remineralization and inhibit demineralization of dental hard tissues.<sup>14-15</sup> Attin and others<sup>16</sup> have shown that the loss of microhardness in bleached enamel could be outweighed by a remineralization period following the bleaching procedure. The positive effect of highly concentrated fluoride products related to caries prevention<sup>17-18</sup> and the inhibition of erosion<sup>19</sup> is well documented. However, there is insufficient scientific data on the influence of fluoride on bleached enamel. This study determined the enamel demineralization effect of 2 bleaching procedures with and without post-treatment fluoride application.

## METHODS AND MATERIALS

In this study, 180 freshly extracted bovine incisors were cleaned, immersed in 20 ml sterile distilled water in a volumetric container and irradiated with a cobalt-60 source, with a total dose of approximately 60 Gy.<sup>20</sup> Two enamel cylinders were prepared from each buccal surface with water-cooled trephine drills (Komet, Lemgo, Germany) and stored in distilled water. The specimens were embedded in acrylic resin (Technovit 4071) (Heraeus Kulzer, Wertheim, Germany), subsequently ground flat with water-cooled carborundum discs (500-4000 grit, Water Proof Silicon Carbide Paper) and polished with diamond spray (3 and 1 µm; DP-Spray P3).

Upon completion of the preparation, the samples were stored in isotonic 0.9% saline solution to avoid dehydration. Finally, all specimens were randomly assigned to 6 groups, cleaned with a soft toothbrush under tap water and air dried.

Half of the surface of each specimen was covered with a varnish (ChemFil, Dentsply DeTrey, Constance, Germany) to protect it from demineralization. The opposite experimental side remained uncovered in order to allow for exposure to the treatment material.

The 6 treatment procedures were:

Group A (n=30): Bleaching with Opalescence, 10% carbamide peroxide (CP) (Ultradent Products, Inc, South Jordan, UT, USA) at 37°C 8 hours a day for 2 weeks. The amount of gel applied resulted in a 1-mm thick layer.

Group B (n=30): Bleaching with Whitestrips, 5.3% hydrogen peroxide (HP) (Procter & Gamble, Cincinnati, OH, USA) at 37°C 1 hour daily for 2 weeks. The specimens were covered with the strips. After bleaching, the strips were carefully removed.

Group C (n=30): The same as Group A, but after bleaching, a fluoride varnish was applied for 1 hour (CPF) (Duraphat, Colgate-Palmolive, Piscataway, NJ, USA).

Group D (n=30): The same as Group B, followed by the same fluoride application as Group C (HPF). Then, the specimens were brushed using a soft toothbrush and water.

Group E (n=30): As a positive control group, the specimens were covered with a glycerin gel for 8 hours daily for 2 weeks.

Group F (n=30): As a negative control group, the specimens were kept in Coca Cola for 1 hour daily for 2 weeks.

At the end of each treatment period, the specimens were rinsed with tap water for 30 seconds to remove the active materials (CP, HP, fluoride, glycerin, and cola) and placed in artificial saliva at 37°C until the next day's treatment.

## Microradiography

After treatment, the specimens were analyzed using transversal microradiography (TMR). The specimens were cleaned with a soft toothbrush under running tap water to remove any remaining treatment material, cut in 200-µm thick sections perpendicular to the surface and subsequently polished with sandpaper (4000 grit). The sections and an aluminum step-wedge were exposed to a nickel-filtered copper (CuKα) x-ray source (PW 1830/40; Philips, Kassel, Germany) operating at 20 kV and 20 mA for 12 seconds. A high-resolution film was used (high speed holographic film, SO-253; Kodak, Stuttgart, Germany). The radiation source to film dis-

tance was 34 cm. Microradiographs were scanned with a digital image analyzing system (CCD Video camera Modul XC77E, Sony, Japan) that was connected to a universal microscope (Axiplan; Zeiss, Oberkochen, Germany) and a personal computer. The mineral distribution was evaluated in the central part of each lesion by its depth and loss of mineral. The lesion depth was determined as the distance from the surface to the point where the mineral content reached that of sound enamel. The loss of mineral was calculated by integrating the difference between mineral content in sound enamel and mineral content in demineralized enamel over lesion depth. Dedicated software (TMR for Windows, Release 1.24; Inspector Research Systems, Amsterdam, The Netherlands) was used to calculate the mineral content depth profiles from the image scans and the reference step wedge data. Furthermore, mineral loss ( $\Delta Z$ , vol%  $\mu\text{m}$ ) and lesion depth (L,  $\mu\text{m}$ ) were determined.

### Statistical Methods

Statistical analyses were performed with SPSS (SPSS 11.0 for Windows). The distributions were analyzed using the Kolmogorov-Smirnov test. Data analysis was accomplished using the non-parametric Kruskal-Wallis and Mann-Whitney U-tests. Mineral loss and lesion depth were compared among the 6 groups. The tests were performed at  $p < 0.05$  to test for significant differences among the 6 groups.

### RESULTS

Data regarding mineral loss and lesion depths were not normally distributed. Table 1 shows the median, 75<sup>th</sup> and 25<sup>th</sup> percentiles of the transversal microradiographic data.

Mineral loss, as assessed by microradiography, was significantly different between the groups ( $p < 0.05$ ). The mineral loss of Groups C and D was significantly lower compared to Groups A and B. The mineral loss of Group E was significantly lower compared to A and B and D and F. The mineral loss of Group F was significantly higher compared to all the other groups except for B (Figure 1). There were no statistically significant differences in mineral loss between B and F and C and E.

No statistically significant difference between groups was found for lesion depth.

### DISCUSSION

Although there are some differences between bovine and human enamel<sup>21</sup> (for example, decalcification occurs more quickly in bovine enamel), the authors used enamel specimens from bovine incisors, because their composition is more homogenous than human teeth, resulting in a more standardized experimental set up.<sup>22</sup>

Clearly, randomized controlled clinical studies are necessary to provide the ultimate proof of effectiveness, and *in vitro* studies are limited, as they can only simulate clinical conditions. However, prior to the expensive clinical trial stage, it seems useful to conduct *in vitro*

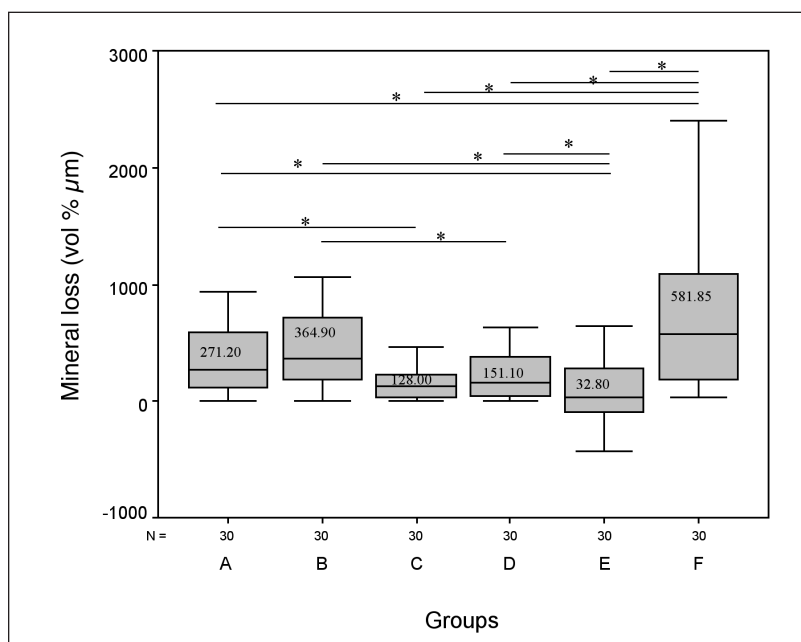


Figure 1. Median of mineral loss (Vol %  $\mu\text{m}$ ) in thin sections of enamel specimens *in vitro* (\* $p < 0.05$ ).

Table 1: Median (75<sup>th</sup> percentile; 25<sup>th</sup> percentile) of mineral loss and lesion depth between pre-treatment baseline measurement and immediate post-treatment

Median (75 <sup>th</sup> percentile; 25 <sup>th</sup> Percentile)	Mineral Loss vol% $\mu\text{m}$	Lesion Depth $\mu\text{m}$
Group A (Opalescence)	271.20 <sup>a,b,c</sup> (588.50; 113.10)	4.85 (15.00; 0.10)
Group B (Whitestrips)	364.90 <sup>d,e,f</sup> (717.30; 190.80)	1.65 (22.80; -4.00)
Group C (Opalescence + F)	128.00 <sup>a,d,g</sup> (232.60; 37.40)	2.70 (7.80; 0.00)
Group D (Whitestrips + F)	151.10 <sup>a,h,i</sup> (373.90; 48.80)	2.60 (17.20; -5.30)
Group E (Glycerin)	32.80 <sup>b,t,h,j</sup> (278.70; -88.80)	4.15 (12.20; -6.80)
Group F (Cola)	581.85 <sup>c,g,i,j</sup> (1094.90; 187.50)	13.70 (43.60; 1.30)

Values with the same letter (a,b,c,d,e,f,g,h,i,j) within columns were significantly different ( $p < 0.05$ ).



testing. In this study, a model comprising periods of treatment with bleaching agents and remineralization in artificial saliva was utilized, attempting to simulate physiological conditions during bleaching procedures. Further studies should prove how far intraoral conditions during remineralization of bleached dental hard tissue are reflected by the applied model.

The specimens were irradiated as described by Amaecha and others.<sup>20</sup> They determined that Gamma irradiation proved to be the most acceptable sterilization method for enamel, showing the fewest adverse effects. The artificial saliva used in this study is similar to human saliva with respect to organic and inorganic components.<sup>23-24</sup>

In order to simulate vital bleaching, treatment time and temperature were performed as recommended by the manufacturer of vital bleaching products for humans.

In this study, bleaching caused significant differences with respect to mineral loss on enamel surfaces but no significant differences for lesion depth. There is still some controversy in the dental literature as to whether carbamide peroxide bleaching causes demineralization of teeth.<sup>5,16,24</sup> Haywood and others<sup>25</sup> reported no change in surface morphology when 10% carbamide peroxide was used; whereas, others observed slight enamel surface modifications after use of the same agent.<sup>26-27</sup>

Few studies have demonstrated that no change in enamel hardness occurs after 10% carbamide peroxide treatment.<sup>5,13,27</sup> Results of profilometric analysis are also conflicting. McGuckin and others observed a slight increase in surface roughness,<sup>26</sup> whereas Hunsaker and others and Grgan and others reported no surface roughness.<sup>28-29</sup>

Controversially, Cimilli and Pameijer<sup>30</sup> reported that the application of 10% carbamide peroxide on enamel for 6 hours/day for 5 or 10 days decreased the Vickers hardness at 110 µm below the enamel surface. Attin and others<sup>16</sup> measured the Knoop hardness of enamel after the application of 10% carbamide peroxide. They observed a reduction in surface microhardness. However, the findings of both studies showed significant mineral loss on the outer enamel layers.

In this *in vitro* study, the specimens were bleached for 2 weeks. It may be speculated that, after this period, mineral loss was detected by microradiography. These results agreed with the results of studies that also detected alterations in enamel and dentin after treatment with 10% carbamide peroxide.<sup>3-4,10</sup>

A tooth may be regarded as a barely soluble salt (enamel and dentin) exposed to an aqueous solution (saliva). Ideally, there is a well-balanced equilibrium between de- and remineralization. Demineralization is initiated by protonation of phosphate in apatite of

enamel and/or dentin. Calcium will therefore not be bound to an adequate extent but rather will be lost.<sup>31</sup> On the other side, the presence of fluoride promotes the opposite reaction, catalyzing remineralization using the calcium of saliva and thus reverses the loss of substance before it can be detected microscopically. Furthermore, lesions that are already visible as white spots and have already spread into dentin can be rematerialized and healed with fluoride.<sup>32</sup>

It is accepted that topical fluorides promote remineralization and inhibit demineralization of dental hard tissues.<sup>14</sup> However, highly concentrated fluoride products are suspected of clogging the surfaces of incipient caries lesion, thereby impeding remineralization.<sup>33</sup> The concept that remineralization with fluoride can repair damage caused by demineralization is of crucial importance for preventive care management.

In this study, after bleaching, the specimens in Groups C and D were covered with a fluoride varnish during the first hour of the remineralization period.<sup>16</sup> Mineral loss was inhibited by the application of fluoride during the remineralization period. Mineral loss was increased in the absence of fluoride after using Opalescence and Whitestrips.

## CONCLUSIONS

In this study, the application of fluoride to bovine enamel results in a reduction in demineralization. On the basis of these results and within the limitations of this *in vitro* study, the results suggest that the use of a fluoride treatment following bleaching may be helpful in remineralizing the tooth surface. However, further studies are necessary to evaluate the clinical behavior of the application of fluoride after bleaching.

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