Mechanical, morphologic, and chemical effects of carbamide peroxide bleaching agents on human enamel in situ

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Objectives: To evaluate the morphologic, mechanical, and chemical effects of carbamide peroxide bleaching agents on human enamel in situ. Method and Materials: Enamel slabs from extracted human teeth were divided in two and mounted on contralateral sides of removable maxillary appliances fabricated for three participants. Soft vinyl trays were adapted intraorally over the arch; one side contained a bleaching agent, and the other served as a control. Vital bleaching was conducted in vivo three times with three different bleaching agents and with new enamel specimens each time. Tests of Knoop microhardness, scanning electron microscopy (SEM), and energy dispersive x-ray (EDX) were performed and analyzed by ANOVA. Results: No statistically significant differences were found between matched test and control specimens concerning microhardness values, morphology, or elemental content. Conclusions: Enamel surface showed no mechanical, morphologic, or chemical changes following bleaching in situ with three different carbamide peroxide agents. (Quintessence Int 2011;42:407–412)

Key words: EDX, microhardness, vital bleaching

Nightguard vital bleaching using carbamide peroxide could cause physical and chemical changes in the enamel, increasing susceptibility to fracture and caries. Saliva, with its high mineral content and neutralizing effect, may increase remineralization of the bleached enamel. The majority of previous studies examined enamel after bleaching treatments in vitro. However, in vivo parameters such as saliva, mechanical abrasion, oral habits, diet, and oral hygiene may affect the enamel surface properties. The few in vivo studies using different methods reported conflicting results.1–7 Energy dispersive x-ray (EDX) analysis to determine any chemical changes was not used in any of these studies. The purpose of our study was to evaluate in situ the mechanical effects and the morphologic and chemical changes of three commercially available 15% to 16% carbamide peroxide bleaching materials when used intraorally on the surface structure of human enamel specimens using the Knoop microhardness test, scanning electron microscopy (SEM), and EDX spectroscopy.

METHOD AND MATERIALS

The study was approved by the Ethics Committee at Hadassah University Hospital in Jerusalem, Israel.

Extracted human teeth were sectioned into 36 4 × 4-mm enamel slabs using a Buehler low-speed sectioning machine (Isomet) with a 127 × 0.4-mm diamond wafering blade (Buehler) under water irrigation.
The 18 slabs designated for microhardness testing were sequentially flattened with aluminum oxide disks mounted on the rotating plate of a polishing machine (Logitech) and polished with 1 μm and 6 μm diamond polishing suspension (Buehler); the remaining 18 were unaltered for SEM morphologic evaluation and EDX analysis. Each slab was then split in two: one fragment for the bleaching treatment and the other serving as a control, both from the same tooth, resulting in 72 enamel specimens. All slabs were sterilized.

Three healthy adults free of caries and periodontal disease participated in the study. Since the researchers were the participants, no signed informed consent was needed, as approved by the institutional ethics committee.

Intraoral removable vacuum-formed appliances (Proform) were fabricated using casts obtained for each participant. The dissected enamel specimens were fixed on the appliances with acrylic resin (Unifast Trad), four on each side. The two halves of each tooth were placed in matched contralateral sites (control and test). Three appliances were prepared for each participant for use with three carbamide peroxide–containing home vital bleaching agents (NiteWhite, 16% carbamide peroxide; Polanight, 16% carbamide peroxide; and Opalescence, 15% carbamide peroxide, Ultradent). Soft vinyl trays (Discus Dental) for the bleaching process were fabricated for the participants (Fig 1). Each agent was used for 10 consecutive nights, loaded on only one side of the soft tray each time.

After the clinical phase, 18 pairs of enamel specimens were tested with a DMH-2 microhardness tester (Matsuzawa Seiki) provided by a Knoop diamond indenter under a 100-g load applied for 10 seconds; these measurements were converted into Knoop hardness number (KHN) to compare between test and control matched specimens. The remaining 18 pairs were observed under a Quanta 200 SEM (FEI) at 500×, 1,000×, and 3,000× original magnification to detect morphologic alterations and were also examined by EDX spectroscopy using an x-ray detector system (EDAX) attached to the same low-vacuum SEM. The EDX system was operated at 15 kV accelerating voltage, with spot size of 5 nm and specimen tilt of 0.10. Measuring time was 30 s (live seconds) with resolution of 129.93 eV and dead time of 30%.

Element content in %wt and %at of calcium (Ca), phosphorus (P), carbon (C), oxygen (O), and fluoride (F) were measured as the relative amount of the total element.
content (100%). The Ca/P ratio was calculated. Changes in element content between bleached and unbleached enamel fragments of the same tooth were sought for each pair of specimens.

The microhardness values and changes in mineral content and in Ca/P ratio were statistically analyzed by analysis of variance (ANOVA).

RESULTS

The Knoop microhardness values varied from 95.3 to 307.4 on the bleached sides and 97.3 to 302.1 on the control sides. The distribution of the differences between test and control values was not statistically significant (P = .781), as determined by ANOVA (Fig 2). No interaction between the participants and bleaching materials was found. There was also no statistically significant difference between the test and control segments for either participants or bleaching agents.

A comparison between SEM images of bleached and unbleached paired enamel fragments revealed no alterations in surface enamel morphology in most cases. Where differences appeared, the bleached enamel was either smoother or more porous than the control with no favored trend (Fig 3).

EDX analysis measured the relative amounts of Ca, P, C, O, and F of the total element content (100%) in %wt and %at. The Ca/P ratio, was analyzed in tables and “spectrum” graphs (Fig 4). ANOVA analysis showed no significant differences between bleached and correspondent unbleached segments for Ca %wt (P = .335), Ca %at (P = .353), P %wt (P = .259), and P %at (P = .304). Similar results were shown for %at and %wt of C, O, and F. Nor were differences in the Ca/P ratio between test and control segments statistically significant (%wt P = .551; %at P = .545). The mean differences of %wt and %at of Ca and P between the bleached specimens and their corresponding controls showed differences between participants and among bleaching agents. All differences were positive; that is, higher content in the bleached specimens.

When Polanight was used, negative values were calculated, since Ca and P content was higher for the unbleached specimens. A difference in Ca/P ratio was found between the bleaching agents, with NiteWhite and Opalescence showing a higher ratio for the bleached specimens and Polanight a higher ratio for the unbleached specimens.
This study provides in vivo data on the widespread but not fully understood vital tooth-bleaching procedure. It was a unique experiment as it was conducted in situ using test and control fragments from the same tooth, examining three areas of possible damage to enamel: morphologic, mechanical, and chemical.

As measured by the Knoop microhardness test, 15% to 16% carbamide peroxide did not significantly affect the microhardness, suggesting no demineralization of the bleached enamel. The results confirm those of previous in vitro,8–14 in vivo, and in situ studies.1–3

The SEM comparison between enamel surface morphology of bleached and unbleached enamel fragments from the same tooth shows that 15% to 16% carbamide peroxide bleaching agents have no morphologic effects on the enamel surface. The majority of the previous studies using SEM images of enamel subjected carbamide peroxide to different in vitro models and are

**DISCUSSION**

Fig 4 EDX spectrum of two enamel segments from the same tooth. (a) Bleached (specimen AO14); (b) control (specimen AO24).
Therefore not comparable with the present study. Our results are in agreement with some earlier reports,\textsuperscript{15–17} but not with others that found surface alterations on bleached enamel.\textsuperscript{13,14,18–20}

EDX spectroscopy is an analytical technique used for chemical analysis. Every element has a unique response to the electron beam of the SEM and the x-rays generated by it can be used for elemental characterization. Any decrease of mineral content at the bleached enamel surface compared to the controls may suggest mineral loss. The ratio of Ca and P is also indicative of chemical changes, since a change can suggest that the mineral phase was altered or that a significant substitution of ions may have occurred.\textsuperscript{21} In the current study, element analysis revealed no statistically significant differences in the content of Ca, P, F, C, or O or of the Ca/P ratio between bleached enamel fragments and their paired unbleached fragments from the same tooth. We found no previous studies using EDX analysis to test chemical changes of bleached enamel.

The absence of mechanical, morphologic, and chemical changes of the enamel surface after vital bleaching, as demonstrated in this study, may be attributed to the protective effects of saliva, which provides dilution, buffering capacity, and a supply of Ca and P ions for tooth remineralization.\textsuperscript{22}

The differences found between participants regarding Ca and P may be the result of participants not following appropriate bleaching procedures in spite of the clear instructions provided or by different oral conditions due to physiologic and environmental factors. The variations between bleaching agents regarding amount of Ca, P, and the Ca/P ratio may be explained by different pH values and agent composition. None of these differences affected the statistical analysis.

The combined results of the microhardness test, SEM evaluation, and EDX analysis proves that at-home vital bleaching with 15% to 16% carbamide peroxide conducted in a real oral environment does not harm the enamel concerning hardness, morphologic structure, and chemical composition.

### CONCLUSION

According to the results of our study using the Knoop microhardness test, SEM evaluation, and EDX analysis of the enamel specimens, no mechanical, morphologic, or chemical changes were found following vital bleaching in situ with three carbamide peroxide agents.

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### REFERENCES