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Thirty-five percent carbamide peroxide application causes *in vitro* demineralization of enamel

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ABSTRACT

Objectives. The objective of this *in vitro* study was to investigate whether a high concentration 'in-office' bleaching agent affected the mineral content of enamel and dentin.
Methods. A commercially available 35% carbamide peroxide bleaching agent was applied for 2 h to sectioned teeth ($n = 11$). Specimens were then immersed in artificial saliva at 37 °C for a further 24 h to simulate the oral environment. Tomographic images of these sections were obtained (micro-CT 80, Scanco, Switzerland) prior to and post-bleach application. Eight three-dimensional regions of interest (ROI), starting from the enamel surface extending to the dentinoenamel junction, were selected for each section. The hydroxyapatite equivalent mineral concentrations (g/cm^3) of the ROIs were calculated. Any changes in mineral content as a consequence of the bleaching procedure were calculated in relation to each ROI.
Results. There was a significant reduction in the mineral content of enamel specimens post-bleach application extending to a depth of 250 μm (paired *t*-test, $p < 0.05$); this reduction in mineral content was greatest in the ROI's closest to the tooth surface. There was, however, no significant difference in the mineral content of dentin as a consequence of bleaching.
Significance. This *in vitro* study has shown that significant demineralization of enamel occurred following bleaching with 35% carbamide peroxide. The concept that 'in-office' bleaching is a non-destructive cosmetic procedure should be reconsidered.

1. Introduction

In-office power bleaching has been available to dentists for nearly a century [1]. Its current popularity is due to its claimed ability to produce immediate results. Despite the widespread use of in-office bleaching [2], there is still controversy in the literature as to whether these agents could adversely affect dental hard tissues.

Bleaching materials for in-office use contain high concentrations of hydrogen peroxide or carbamide peroxide; typically, 35% carbamide peroxide is used as the active bleaching agent. This high concentration of carbamide peroxide can be used either as a pre-treatment or in combination with at-home bleaching [1]. Morphological alteration of enamel following 35% carbamide peroxide bleaching has been shown by several researchers. Cavalli et al. [3] showed that 35% carbamide peroxide application increased the roughness of enamel surfaces. Oltu and Gurgan [4] reported a change of inorganic composition of enamel after 35% carbamide peroxide application. Bitter [5] stated that teeth that were bleached in vivo with 35% carbamide peroxide lost their aprismatic enamel layer and that the damage was not repaired after 90 days. Ernst et

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al. [6] showed that a high concentration of carbamide peroxide was detrimental to enamel surface integrity; however, the damage was less than that was seen after phosphoric acid etching. However, Gultz et al. [7] have reported that following treatment with 35% carbamide peroxide, no difference was observed in enamel surface morphology between treated and control specimens.

Previously reported work to investigate the demineralization effects of in-office bleaching agents on tooth structures has also been controversial. Suleiman et al. [8] reported no significant changes in hardness values for enamel and dentin after bleaching with 35% hydrogen peroxide whereas Attin et al. [9] and Lewinstein et al. [10] reported a significant reduction in Knoop hardness of enamel after 35% carbamide peroxide application.

Micro-computerized tomography (micro-CT) is a new and developing technology that can be used to map the distribution of mineral in teeth non-destructively [11]. The authors have previously described a new in vitro micro-CT method to show the demineralization effect of a 10% carbamide peroxide bleaching agent on enamel [12]. This method enabled quantification of the mineral content of tooth specimens in three dimensions. Comparisons were made to investigate the mineral content prior to and post-bleach application. Results showed micro-CT was indeed a reliable tool to investigate the effects of bleaching agents.

Present knowledge of the effects of in-office bleaching agents is still limited and controversial [3–10]. Therefore in this study the aim was, using micro-CT, to investigate the effects of in-office bleaching on enamel surface layers, subsurface layers and the dentinoenamel junction. It was hypothesized that 35% carbamide peroxide application would decrease the mineral content of enamel.

2. Materials and methods

2.1. *Preparation of the specimens*

Freshly extracted sound human upper second molar teeth were used for the study. The teeth were stored in physiological solution at room temperature until required. Eleven tooth rods each 2 mm × 3 mm in cross section and 4 mm in length were prepared under water-cooling with a reciprocating diamond wire saw (precision wire diamond saw, Well, Germany). All the sections were taken from the buccal mid-1/3 of the anatomical crown. Any debris was removed from the teeth by brushing with a soft toothbrush (Oral-B no. 35, soft bristles; Oral B Laboratories, Belmont, USA) under running deionised water. All surfaces except the natural enamel surface were coated with nail varnish.

2.2. *Micro-CT measurements and evaluations*

The mineral content of the tooth specimens both prior to and post-35% carbamide peroxide application was quantified using a micro-CT scanner (μCT 80, Scanco, Switzerland).

After the first scan, specimens were transferred to a sterile cell culture well. 0.01 ml bleaching gel containing 35% carbamide peroxide (Opalescence Quick, Ultradent, USA) was applied on the natural enamel surfaces with a 1ml syringe. To accelerate the activity the gel was heated under running hot water for 2 min. Immediately after opening the gel, the pH was measured with a calibrated pH meter (Orion 920A, Thermo Electron, USA) and found to be 6.7. A clinically realistic application time of 2 h was chosen as this corresponded to the maximum application time recommended by the manufacturer. During this time, specimens were kept in a humid environment at 37 °C. Specimens were then washed under running deionised water in order to remove the gel. Subsequently, 2.5 ml of artificial saliva (Batch number 17336, Saliveze, Wyvern, UK) was placed in the wells to simulate the oral environment. The saliva contained calcium chloride, magnesium chloride, sodium chloride, potassium chloride, dibasic sodium diphosphate, sorbitol and carboxymethyl cellulose (as listed by the manufacturer). Specimens were incubated in a humid environment at 37 °C for a further 24 h. The pH of the saliva substitute was 6.9.

Tooth specimens were scanned twice: before and after bleach application. The same scanning parameters were applied in both scans. The X-ray source was set at 45 kvP, and 177 μA. Integration time was 400 s. The entire thickness of the tooth rods were scanned at high resolution. The data collected were used to reconstruct images with a resolution of 2048 × 2048 pixels and with an isotropic voxel size of 25 μm.

A custom sample holder was used to position the specimens in the sample holder of the micro-CT scanner. During scanning, a damp sponge was placed in the sample holder and the holder was sealed with cling film to maintain a humid environment therefore preventing any cracks that might occur in a dry environment [12].

The ‘optimum threshold procedure’ was run and the threshold gray values for enamel and dentin were calculated as 296 and 580, respectively. The evaluation software available in the workstation of the scanner was used to define eight regions of interest (ROI) per tooth rod. The enamel surface and immediate subsurface were divided into two 25 μm thick regions of interest; ROI-1 started from the enamel surface extending to a depth of 25 μm followed by ROI-2, which extended a further 25 μm. Other ROIs had a thickness of 50 μm (Fig. 1). ROI-8 extended from the dentinoenamel junction (DEJ) towards enamel and ROI-7 extended towards the dentin. The threshold value for enamel was utilized in defining the ‘inner value’ during the automatic contouring of the ROI-1, ROI-2, ROI-3, ROI-4, ROI-5 and ROI-6. ROI-7 and ROI-8 were defined manually as for practical reasons it was easier to define the DEJ. The ‘outer value’ corresponded to the nail varnish, air and the damp sponge therefore excluding these from the evaluations. These were previously described in detail [12].

Evaluations were carried out on each ROI both prior to and post-bleach application. One hundred and seventy-six regions were evaluated using the image processing language available on the workstation. Gray level median values for each ROI were converted to g/cm^3 assuming that the component absorbing the X-rays was calcium hydroxyapatite. An in house produced cylindrical hydroxyapatite block (Plasma Biotal Ltd., Buxton, UK) with 2.9 g/cm^3 density was scanned with the specimens and the data was used to calculate the hydroxyapatite equivalent density (g/cm^3). In addition, the percentage of mineral loss in relation to each ROI was calculated.