Microcomputerised tomography evaluation of 10% carbamide peroxide applied to enamel

Neslihan Efeoglu*a, David Woodb, Candan Efeogluc

aDepartment of Restorative Dentistry, Leeds Dental Institute, University of Leeds, Worsley Building Level 6, Clarendon Way, Leeds LS2 9LU, UK
bDepartment of Oral Biology, Leeds Dental Institute, University of Leeds, Worsley Building Level 6, Clarendon Way, Leeds LS2 9LU, UK
cDepartment of Oral and Maxillofacial Surgery, Leeds Dental Institute, University of Leeds, Worsley Building Level 6, Clarendon Way, Leeds LS2 9LU, UK

Received 24 August 2004; received in revised form 28 November 2004; accepted 8 December 2004

Summary Objectives. There is still some controversy in the dental literature whether carbamide peroxide bleaching causes demineralization of teeth. One of the reasons for this controversy is that there is as yet no reliable, non-destructive in vitro metod for assessing mineral loss in bleached teeth. The objective of this study was to investigate the possible demineralization effect of 10% carbamide peroxide bleaching agent on enamel and dentine non-destructively.

Methods. µCT images were obtained of 12 human molar tooth sections. These sections had 10% carbamide peroxide applied for eight hours a day over a period of 15 days. Further tomographic images were obtained and the mineral content prior to and post bleaching assessed.

Results. A total of 144 regions were evaluated using the image processing language available in the work station. The application of 10% carbamide peroxide was found to cause demineralization of the enamel extended to a depth of 50 μm below the enamel surface (Paired t-test, p<0.05).

Conclusions. This study confirmed that µCT was indeed a highly suitable method for assessing mineral content of dental enamel after bleach application. It is recommended that application of bleaching agents should be carefully considered in patients susceptible to caries and tooth wear.

© 2005 Published by Elsevier Ltd.

Introduction

As a conservative method to lighten natural teeth night guard vital bleaching has gained worldwide popularity. The original and still material of choice for night guard bleaching is carbamide peroxide;
many of the systems available today use 10% carbamide peroxide as the active bleaching agent. The tooth whitening process involves the direct contact of the whitening product on the surface of the teeth for an extended period of time. However, this direct contact with enamel for prolonged times has increased concerns about the potential adverse effects of these agents on enamel. Previous studies have investigated the possible adverse effects of 10% carbamide peroxide agents on the physical and chemical properties of enamel with a variety of methods; SEM analysis, profilometry analysis, microhardness and fracture toughness tests, measuring the amount of calcium loss and infrared absorption spectroscopy.

Although there are many different techniques available to assess the mineral content of enamel, microhardness tests have been the choice of many researchers to evaluate the possible demineralization effect of the bleaching agents. Quantitative measures of mineral gain and loss are possible using microhardness methods; however, the method is destructive on enamel samples.

Microcomputerised tomography (μCT) is a new and developing technology that can be used to map the distribution of mineral in teeth non-destructively. μCT has been used extensively to investigate the mineral content of enamel and it has been reported as a useful tool for quantitative measurements in dental research. However, the use of μCT in investigating the effects of dental materials on mineral content of enamel and dentine has been limited to a number of laser application and etching studies.

Present knowledge of the effects of 10% carbamide peroxide agents on the mineral content of enamel is still controversial and more research examining the effects of bleaching agents on enamel tissues is required. μCT has not been used to evaluate the effects of bleaching agents on enamel previously. Therefore in this study, μCT was used to investigate the possible demineralization effect of 10% carbamide peroxide on enamel after cyclic bleaching in vitro.

Materials and methods

Preparation of the specimens

Six extracted sound human upper second molar teeth were stored in physiological solution at room temperature until required. 12 tooth rods each 2×3 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). Sections were taken in such a manner that all were from the buccal mid 1/3 of the anatomical crown. Specimens were brushed with a soft toothbrush (Oral-B no. 35, soft bristles; Oral B Laboratories, Belmont, USA) under running de-ionised water. All surfaces except the natural enamel surface were coated with nail varnish.

A μCT scanner (μ-CT 80, Scanco, Switzerland) was used to assess the mineral content of the tooth specimens both before and after carbamide peroxide application. After the first scan samples were transferred into a sterile cell culture well. Round sponge pieces with a slot in the middle were secured inside the wells. Before placing the specimens inside the slots, sponges were wetted with artificial saliva (Batch number 17336, Saliveze, Wyvern, UK) in order to mimic clinical conditions. This saliva contained calcium chloride, magnesium chloride, sodium chloride, potassium chloride, dibasic sodium diphosphate, sorbitol and carboxymethyl cellulose, as listed by the manufacturer.

The specimens were inserted leaving only the buccal enamel part exposed. 0.01 ml bleaching gel containing 10% carbamide peroxide (Regular, Ultradent, USA) was applied on the natural enamel surface with a 1 ml syringe (BD, Plastipak, Ireland). The pH of the gel was measured as 6.8, with a calibrated pH meter (Orion 920A, Thermo Electron, USA) applied on the natural enamel surfaces with a 1 ml syringe (BD, Plastipak, Ireland). The pH of the gel was measured as 6.8, with a calibrated pH meter (Orion 920A, Thermo Electron, USA) immediately after opening the gel. Specimens were kept in a humid environment at 37 °C for 8 h. Following this period, specimens were washed under running de-ionised water to remove the gel. Subsequently, specimens were immediately immersed in 2.5 ml of artificial saliva inside the wells and incubated in a humid environment at 37 °C for a further 16 h. The pH of the saliva substitute was 6.9. This bleaching cycle was repeated for 15 consecutive days.

Micro CT measurements and evaluations

Tooth specimens were scanned twice; before and after bleach application. The same scanning parameters were applied in both scans. The data sets from both scans were analysed allowing paired comparisons between the baseline data (first scan), and the data obtained after bleaching (second scan). The mineral content of the tooth rods at the surface and subsurface layers were quantified before and after bleaching. For this purpose the linear attenuation coefficients were converted to mineral concentration values assuming that the component absorbing the X-rays was calcium hydroxyapatite.
A custom sample holder was built to position the specimens in the sample holder of the µCT 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 thus preventing any cracks that might occur in a dry environment.

The entire thickness of the tooth rods were scanned at high resolution and the reconstructed image had a resolution of 2048×2048 pixels with an isotropic voxel size of 25 μm.

In the workstation, the grey values of an image are represented on a grey scale with values between −1000 to +1000. The more mineralized the tissue higher the grey value. The optimum threshold procedure was run and the threshold values for dentine and enamel were calculated as 296 and 580, respectively. Using the evaluation software available in the workstation of the scanner, six regions of interests (ROI) per tooth rod were chosen (Fig. 1).

On the work station, the threshold value for enamel was utilized in defining the ‘inner value’ during automatic contouring of ROI-1, ROI-2, ROI-3, ROI-4 and ROI-6. The outer value was chosen as 81 that corresponded to the nail varnish, air and the damp sponge therefore, excluding these from the evaluations. During the automatic contouring of the ROI, the number of iterations was chosen as ‘3×’ allowing good adaptation of the ROI borders to the enamel samples’ borders. ROI-5 was defined manually as it was easier to define the border between enamel and dentin manually (for practical reasons). The grey values that were higher than the threshold values of enamel and dentine were regarded as enamel and dentine, respectively.

Each ROI had a thickness of 50 μm. ROI-1,2,3,4 and 6 contained all of the voxels corresponding to enamel where as ROI-5 contained all of the voxels corresponding to dentine. In other words each ROI circumscribed all of the enamel or dentine available in two consecutive 25 μm thick slices. ROI-1 started from the surface enamel extending to 50 μm deep, followed by ROI-2, ROI-3 and ROI-4. ROI-5 extended from the dentinoenamel junction towards dentine and ROI-6 extended from the same point towards the surface.

Evaluations were carried out on each ROI both before and after bleach application. A total of 144 regions were evaluated using the image processing language available in the work station. Median values of grey values for each region were converted to g/cm³ assuming that the component absorbing the X-rays is calcium hydroxyapatite. For this purpose, an in house produced cylindrical hydroxyapatite block (Plasma Biotal Ltd, Buxton, UK) with 2.9 g/cm³ density, a diameter of 13 mm, and a height of 3 mm was scanned using the same scanning parameters. This data was then used to calculate the hydroxyapatite equivalent density.

Results
Mean values of the hydroxyapatite equivalent density of the ROIs are given in Table 1. Mineral content before and after application of 10% carbamide peroxide was compared (Paired t-test, p=0.05). There was a significant difference in the hydroxyapatite equivalent mineral content in the ROI-1 corresponding to the outer 50 μm of enamel (Paired t-test, p < 0.05). No significant difference was found for the other comparisons (Paired t-test, p > 0.05) (Table 2).

Discussion
According to the manufacturer’s recommendations patients should wear a nightguard filled with the bleaching agent overnight followed by a rest period during daytime. Therefore, in this study a cyclic model including periods of bleaching and
remineralization with artificial saliva was used to simulate physiological conditions during home bleaching procedures. It was assumed that a bleaching period of 8 h would reflect the clinical situation. The amount of carbamide peroxide was chosen to be sufficient to fully cover the surface of the enamel. The artificial saliva used had a neutral pH and contained electrolytes to mimic human saliva.

Although there are many different techniques available to assess the mineralization of enamel, quantitative measures of mineral gain and loss are possible only if direct chemical and radiographic techniques are used. Commonly used techniques like transversal microradiography (TMR) and cross sectioned microhardness methods can be used for determination of lesion depth; however, both methods are destructive on enamel whereas \( \mu \)CT can evaluate mineralized enamel samples non-destructively in three dimensions.

\( \mu \)CT has previously been used in dental research studies to map and to quantify the mineral content of enamel. However, the method used in this study to choose regions of interest (ROI) has not been reported before. For the present study, in the evaluation of the obtained images, selection of the whole horizontal enamel and dentine sections using the calculated thresholds with the 'iteration' command as ROI and the non-destructive nature of the \( \mu \)CT made it possible to evaluate the identical ROI of the same sample before and after bleaching. The custom made sample holder allowed exact positioning of the enamel samples for both pre- and post-bleaching scans.

In this study, nail varnish was applied to coat the cut section sides of the tooth specimens to prevent interaction of the bleaching gel with these surfaces. In a study by Iijima et al. the influence of nail varnish on the measurements of remineralization of enamel sections was investigated by microradiography. According to their results the effect of

Table 1  Mean values, std. deviations of the hydroxyapatite equivalent mineral content of the regions of interest (ROI).

<table>
<thead>
<tr>
<th>Region of interest (ROI)</th>
<th>Mean (g/cm³)</th>
<th>N</th>
<th>Std. deviation</th>
<th>Std. error mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>ROI 1</td>
<td>b 2.18</td>
<td>12</td>
<td>0.70180</td>
<td>0.20259</td>
</tr>
<tr>
<td></td>
<td>a 1.86</td>
<td>12</td>
<td>0.55995</td>
<td>0.16164</td>
</tr>
<tr>
<td>ROI 2</td>
<td>b 3.27</td>
<td>12</td>
<td>0.57694</td>
<td>0.16655</td>
</tr>
<tr>
<td></td>
<td>a 3.17</td>
<td>12</td>
<td>0.72124</td>
<td>0.20820</td>
</tr>
<tr>
<td>ROI 3</td>
<td>b 3.75</td>
<td>12</td>
<td>0.42238</td>
<td>0.12193</td>
</tr>
<tr>
<td></td>
<td>a 3.86</td>
<td>12</td>
<td>0.25291</td>
<td>0.07301</td>
</tr>
<tr>
<td>ROI 4</td>
<td>b 3.91</td>
<td>12</td>
<td>0.24763</td>
<td>0.07148</td>
</tr>
<tr>
<td></td>
<td>a 3.93</td>
<td>12</td>
<td>0.26258</td>
<td>0.07580</td>
</tr>
<tr>
<td>ROI 5</td>
<td>b 2.23</td>
<td>12</td>
<td>0.50657</td>
<td>0.14623</td>
</tr>
<tr>
<td></td>
<td>a 2.23</td>
<td>12</td>
<td>0.43138</td>
<td>0.12453</td>
</tr>
<tr>
<td>ROI 6</td>
<td>b 3.07</td>
<td>12</td>
<td>0.51976</td>
<td>0.15004</td>
</tr>
<tr>
<td></td>
<td>a 3.15</td>
<td>12</td>
<td>0.43874</td>
<td>0.12665</td>
</tr>
</tbody>
</table>

Letter ‘b’ indicates the values obtained before bleach application. Letter ‘a’ indicates the values obtained after bleach application.

\( \mu \)CT80 has approximately 15-90 or 10-74 \( \mu \)m isotropic voxel size and image matrix of 512 \( \times \) 512, 1024 \( \times \) 1024 or 2048 \( \times \) 2048 pixels. For the present study the reconstructed 3D image had the resolution of 2048 \( \times \) 2048 pixels with an isotropic voxel size of 25 \( \mu \)m. Other X-ray attenuation methods like TMR can only provide quantitative measurements of demineralized lesions of a thin planoparallel tooth section (typically 100 \( \mu \)m) so that a 1D (line) or 2D (area) array of mineral dissolution is obtained. \( \mu \)CT can also be compared to atomic force microscopy (AFM) which is a powerful tool for direct observation of surface processes with nanometer resolution. It has been reported as a suitable tool for high-resolution measurements \( (10^{-10} \text{ m}) \) of enamel crystals and surfaces to determine the early stages of mineral dissolution.

AFM has a higher resolution then the \( \mu \)CT, however the advantage of the \( \mu \)CT is its ability to evaluate both the surface and subsurface enamel layers in 3D.

Table 2  Paired \( t \)-test statistics (\( p=0.05 \)).

<table>
<thead>
<tr>
<th>Paired differences</th>
<th>Mean</th>
<th>Std. deviation</th>
<th>Std. error mean</th>
<th>Sig. (2-tailed)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pair 1</td>
<td>bROI-1-aROI-1</td>
<td>0.3173</td>
<td>0.36435</td>
<td>0.10518</td>
</tr>
<tr>
<td>Pair 2</td>
<td>bROI-2-aROI-2</td>
<td>0.1041</td>
<td>0.27410</td>
<td>0.07913</td>
</tr>
<tr>
<td>Pair 3</td>
<td>bROI-3-aROI-3</td>
<td>-0.1157</td>
<td>0.27427</td>
<td>0.07917</td>
</tr>
<tr>
<td>Pair 4</td>
<td>bROI-4-aROI-4</td>
<td>-0.0189</td>
<td>0.17328</td>
<td>0.05002</td>
</tr>
<tr>
<td>Pair 5</td>
<td>bROI-5-aROI-5</td>
<td>-0.0043</td>
<td>0.25492</td>
<td>0.07359</td>
</tr>
<tr>
<td>Pair 6</td>
<td>bROI-6-aROI-6</td>
<td>-0.0865</td>
<td>0.24956</td>
<td>0.07204</td>
</tr>
</tbody>
</table>

Letter ‘b’ indicates the values obtained before bleach application. Letter ‘a’ indicates the regions of interest (ROI) evaluated after bleach application. *indicates significant differences (\( p<0.05 \)).
nail varnish on the main parameters of microradiography, such as Ld (lesion depth) and Delta Z (mineral loss) was less than 5% of the mean values. In our study the possible influence of nail varnish, damp sponge and air on the measurements was excluded by using the ‘outer value’ threshold corresponding to these structures.

There is still some controversy in the dental literature whether carbamide peroxide bleaching causes demineralization of teeth. In the present study µCT evaluation of the same ROIs after 10% carbamide peroxide cyclic bleach application for 15 days revealed that there was significant mineral loss in the outer 50 µm of the enamel after bleaching. There was no damage in deeper enamel surfaces and in the dentino-enamel junction. There have not been any µCT studies in the literature that investigated the effects of bleaching agents on enamel and dentine however; Seghi and Dendry reported that bleaching enamel specimens with 10% carbamide peroxide for 12 h did not alter the surface microhardness values. Potocnik et al. also reported that 10% carbamide peroxide bleaching for 336 h did not significantly affect the microhardness of enamel. In a study by Lopes et al. 10% carbamide peroxide application to enamel for 3 h a day followed by immersion into artificial saliva for the rest of the day for 2 weeks showed no change in microhardness of the specimens. Inconsistency of these studies with our results might be due to the different methodology and the different exposure times used in this study.

Controversially Cimilli and Pameijer reported that application of 10% carbamide peroxide on enamel for 5 or 10 days for 6 h/day decreased the Vickers hardness at 110 µm below the enamel surface. Attin et al. measured the Knoop hardness of enamel after 10% carbamide peroxide application and stated that demineralization of enamel occurred on the superficial enamel to a depth of 1.08 µm below the enamel surface. Although the findings of the both studies showed significant mineral loss on the outer enamel layers, the extent of the demineralization greatly differed. Microhardness of enamel is linearly correlated with its mineral content however, the method is destructive on enamel samples therefore it is not possible to test the same volume for test and control. This may be the reason for inconsistency of these results compared with our study.

The baseline ROI-1 mineral density showed lower values than that of the other enamel layers (Table 1). It has been reported that the primary factor determining the development and reversibility of demineralization is the thermodynamic saturation level in the media surrounding the tooth mineral. Remineralization of the demineralized subsurface lesions requires calcium and phosphate, which are primarily supplied from saliva and plaque fluid. Since there is no information about the age of the patients and/or eruption state of the teeth used for this study, it can be argued that these low baseline values of mineral density may have made enamel more susceptible to demineralization. Between the bleach application intervals, enamel samples were immersed in artificial saliva with a neutral pH which contains calcium and phosphate therefore, it might be expected to have a remineralization effect. However, the baseline values obtained in this study were of the enamel samples prior to immersion into the artificial saliva therefore it was not possible to quantify the mineral gain from the artificial saliva.

The pH of the 10% carbamide peroxide gel used for this study was 6.9 therefore the demineralization seen in this study cannot be attributed to the low pH of the gel. On the other hand, some of the commercially available bleaching products include EDTA which is a liquid solution of the sodium salt of ethylenediamide tetraacetic acid with a pH of 7.3. It has been reported that EDTA has softening effect on dentine which may explain the demineralization effect of some of the bleaching agents. We have no knowledge about the EDTA or other calcium chelating content of the bleaching agent used in this study. Moreover, the demineralization effect presented could be the result of the possible alteration in the structure of the enamel caused by uncontrolled reaction of the peroxide radical.

In a study by McCracken and Haywood the amount of calcium loss from human enamel after exposure to a standard 10% carbamide peroxide bleaching solution was investigated. According to their results the amount of calcium loss from enamel treated with 10% carbamide peroxide for 6 h was significantly different than calcium loss from control teeth exposed to distilled water for the same amount of time. However, the amount of calcium loss from the teeth immersed in the cola beverage was not significantly different than the group treated with 10% carbamide peroxide. Consumption of these beverages is very common in patient population therefore, future studies with positive controls to examine the clinical significance of the results of our study are needed.

Surface demineralization seen in this study may increase susceptibility to tooth wear caused by abrasive factors like toothbrushing. Whether this demineralization can be remineralized in the longer term by saliva and/fluorides should be further
investigated comparing different brands and concentrations available.

Conclusion

This study suggests that the use of μCT with the proposed method of ROI selection and evaluation is indeed a reliable tool to investigate the effects of bleaching agents. The non-destructive method enables paired comparisons and avoids bias that may arise from comparing two different spots on a single tooth rod that may have different mineral concentrations. Within the limitations of this study in vitro application of 10% carbamide peroxide on enamel for two weeks caused demineralization of the enamel extending to a depth of 50 μm below the enamel surface. Further studies with treatment controls should be undertaken to verify this result. It is recommended that application of bleaching agents should be carefully considered in patients susceptible to caries and tooth wear.

Acknowledgements

This research has been supported by Department of Restorative Dentistry of University of Leeds. We would like to thank Dr Nigel Bubb for his intellectual and technical support in preparation of the hydroxyapatite discs and Mr Ian Smith for his technical support in preparation of the sample holders. The artificial saliva used in this study was donated by Wyvern Medical.

References


