



Effect of tooth bleaching agents on protein content and mechanical properties of dental enamel



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ABSTRACT

This study investigated the effect of two bleaching agents, 16% carbamide peroxide (CP) and 35% hydrogen peroxide (HP), on the mechanical properties and protein content of human enamel from freshly extracted teeth. The protein components of control and treated enamel were extracted and examined on sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS–PAGE). Marked reduction of the protein matrix and random fragmentation of the enamel proteins after bleaching treatments was found. The mechanical properties were analyzed with Vickers indentations to characterize fracture toughness, and nanoindentation to establish enamel hardness, elastic modulus and creep deformation. Results indicate that the hardness and elastic modulus of enamel were significantly reduced after treatment with CP and HP. After bleaching, the creep deformation at maximum load increased and the recovery upon unloading reduced. Crack lengths of CP and HP treated enamel were increased, while fracture toughness decreased. Additionally, the microstructures of fractured and indented samples were examined with field emission gun scanning electron microscopy (FEG–SEM) showing distinct differences in the fracture surface morphology between pre- and post-bleached enamel. In conclusion, tooth bleaching agents can produce detrimental effects on the mechanical properties of enamel, possibly as a consequence of damaging or denaturing of its protein components.

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1. Introduction

Dental enamel, as the hardest tissue in the body, acts as a protective covering of teeth and can withstand a wide range of functional and non-functional loads. Enamel is a hierarchical biocomposite made of 94–96 wt.% inorganic content, 1 wt.% organic matrix and 4–5% water [1]. The inorganic content is comprised of long thin hydroxyapatite crystals tightly packed together with a protein glue (hydrophobic enamelin) to form enamel rods [1]. These rods are encapsulated by a thin protein-rich organic sheath (primarily hydrophilic ameloblastin) around 0.8–1 μm thick [2]. Although the organic contents of enamel comprise less than 1%, they play a significant role in determining the mechanical behavior of enamel [3]. This organic content helps to define three-dimensional cleavage planes to deflect cracks which prevent fracture

from progressing through enamel and allows limited movement between the rods during stress [2,4].

The mechanical properties of enamel are dependent on its composition and its structural organization [5]. The hardness and modulus of elasticity of the rods have been reported to be much higher than that of the organic sheath [6]. The highest values of hardness and modulus of elasticity have been found at the occlusal surface and decrease toward the dentin-enamel junction (DEJ) as mineral density decreases and the organic matrix increases [7,8]. In addition, the fracture toughness of enamel ranges from 0.4 MPa m^{1/2} in the outer enamel to 2.37 MPa m^{1/2} in the inner region near the DEJ [9,10]. This increase is primarily due to an increase of the organic matrix and different rod orientations (decussation) which causes crack propagation resistance by the formation of protein bridges, crack deflection and microcracks [9].

Tooth bleaching has become a highly popular esthetic dental treatment as it is the easiest and the least destructive procedure for treatment of tooth discoloration. At present, there are three main tooth bleaching techniques being used: in-office bleaching, dentist supervised home bleaching and over the counter (OTC) bleaching products [11]. Contemporary bleaching techniques use

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hydrogen peroxide (HP) or its precursor carbamide peroxide (CP) as an active ingredient [11]. Bleaching peroxide penetrates through enamel and dentin eventually reaching the pulp in the course of which it undergoes chemical breakdown forming oxygen free radicals. These free radicals are highly unstable and capable of oxidizing and disintegrating a wide range of organic and inorganic materials including chromophores [12]. Despite their ability to disintegrate pigmented molecules in tooth structure, bleaching agents can also attack the organic and inorganic contents of tooth structure [13].

Morphological alterations of enamel surface after bleaching treatment can be seen as an increase in surface roughness and erosion [14,15]. Changes of the chemical composition of enamel and mineral loss has also been reported [15,16]. Micro- and nano-mechanical investigations of enamel subjected to bleaching agents have shown that HP significantly decreases the hardness and modulus of elasticity of enamel [17–19], as well as the fracture toughness and wear resistance [20,21]. These changes in the mechanical properties of enamel could be due to both, loss of mineral content as well as denaturation and degradation of the organic matrix by the oxidation reaction [18,19,21]. However, the mechanism(s) of action by which the bleaching peroxide induces detrimental effects on the mechanical properties of enamel has not been fully identified. Therefore, this study aims to analyze the influence of bleaching agents on the nanomechanical properties of enamel and enamel matrix protein/peptides along with the underlying mechanisms in an attempt to understand the structural alterations that accompany these changes. The nanomechanical properties of enamel were investigated before and after bleaching using a nanoindentation system. The fracture toughness and crack behavior of enamel were quantitated with Vicker's microhardness test then examined under field emission gun scanning electron microscopy (FEG-SEM). The effects of the bleaching agents on the enamel matrix content were analyzed on SDS–polyacrylamide gel electrophoresis (SDS–PAGE).

2. Materials and methods

2.1. Specimen preparation

Twenty-four fully erupted human third molars were obtained from healthy patients aged 18–40 according to protocols approved by the Sydney West Area Health Service (Ref. No. HREC/11/WMEAD/115). The extracted teeth were cleaned and embedded in a cold-curing epoxy resin (Epofix, Struers, Denmark). Upon curing the occlusal surface was ground flat with silicon carbide papers with grit sizes 240, 400, 600 and 1200 under continuous water irrigation to avoid overheating. Specimens were then polished by specimen polisher (RotoPol-22, Struers, Denmark) with 9 μm and 1 μm diamond polishing pastes then lastly with 0.04 μm colloidal silica. After each polishing cycle, specimens were ultrasonically cleaned (Unisonics, Australia) for 5 min and stored in Hank's balanced salt solution HBSS (Sigma–Aldrich, Germany) at room temperature between the bleaching treatments. Prior to indentation experiments, the specimens were examined under optical microscope to exclude excessively scratched and cracked surfaces. One half of the specimen was covered with nail varnish (control) and the other half was treated with one of the bleaching agents.

Two bleaching agents were used in this study: 16% carbamide peroxide (CP) home bleaching gel (Polanight, SDI Limited, Australia); and 35% hydrogen peroxide (HP) in-office bleaching agent (Polaoffice+, SDI Limited, Australia). The whitening treatments of enamel samples were performed at room temperature and according to the manufacturers' instructions as outlined below.

Briefly, for the CP group, 16% CP gel was applied on the tooth surface for 90 min. Samples were then rinsed thoroughly with water, dried and stored in HBSS. This process was repeated daily for 14 days. For the HP bleaching group, 35% HP was applied on the surface of specimen for 10 min, rinsed under running water and dried. The treatment was repeated 3–4 times in a single session. The mechanical tests were conducted within 24 h after application of bleaching agents.

2.2. Hardness and elastic modulus

The indentation experiment was conducted using an Ultra Micro Indentation System (UMIS-2000, CSIRO, Australia), equipped with a three-sided Berkovich indenter tip calibrated on a fused silica standard sample of known properties. Specimens ($n = 12$) were nanoindented using a maximum load of 10 mN. A total of 15 indents separated from each other by 20 μm were positioned on the occlusal surface of each specimen along its buccal aspect for both the bleached and non-bleached halves of each tooth sample. The UMIS system software (Ibis, Fisher–Cripps laboratories, Australia) was used for the subsequent calculation of the elastic modulus and hardness of the samples tested. The nanoindentation hardness (H) is the contact pressure of the indenter divided by the projected contact area (A) of the sample at maximum load (P_{max}) which can be estimated from [22]:

$$H = P_{max}/A \quad (1)$$

whereas elastic modulus of the specimen E_s can be calculated from [22]:

$$1/E_r = (1 - \nu_s^2)/E_s + (1 - \nu_i^2)/E_i \quad (2)$$

where E_r is the indentation elastic modulus (reduced modulus), E_i and ν_i are the elastic modulus and Poisson's ratio of the diamond indenter 1070 GPa and 0.07 respectively. The Poisson's ratio, ν_s , for enamel is 0.3 [23]. The data were analyzed according to Oliver and Pharr [22] and the hardness and elastic modulus values were averaged from the results of 15 indents.

2.3. Load dependent mechanical properties of enamel

To investigate the effect of tooth bleaching agents on subsurface enamel and its depth dependent mechanical properties, a range of loads 5, 10, 20 and 50 mN were applied to control and bleached enamel samples. Hardness and elastic modulus were obtained following the methods described above.

2.4. Creep behavior of enamel

The approach for determination of creep behavior of control and bleached enamel was the same as that used by He and Swain [24]. A 250 mN force was applied using the UMIS nanoindentation system with a 900 s holding time at maximum load. Another 900 s hold period was included during unloading at 2% of the peak load to analyze the creep recovery of enamel [24]. One-increment step during loading and unloading was used in order to reduce the viscoelastic effects that result from gradual loading and unloading [25]. The difference between the displacement during creep at maximum load and creep recovery at minimum load represents the amount of unrecovered deformation during a hold period at constant force. In addition, the relative recovery of the material in relation to the deformation was calculated as a percentage by the relationship:

$$\% \text{ Recovery} = h_2/h_1 \times 100 \quad (3)$$

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