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Nanoindentation study of human premolars subjected to bleaching agent

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Abstract

Bleaching of teeth is gaining popularity due to cosmetic reasons. However, the effect it has on teeth is still largely unknown. This paper seeks to evaluate the effect of a bleaching agent, 30% hydrogen peroxide, on the nanomechanical properties of dentin and enamel using the nanoindentation technique. The Young's modulus and hardness obtained from nanoindentation before and after bleaching were compared. Five newly extracted human premolars were used. Nanoindentation was first done on the sliced enamel and dentin regions to determine their mechanical properties. One batch of samples was kept in Hank's balanced salt solution as control while the other was bleached in 30% hydrogen peroxide for 24 h. The same number of nanoindentations was then done near the previously indented regions for both the control and bleached samples and the results compared. Using paired sample *t*-tests with $\alpha = 0.05$, it was found that there were no significant differences in both the Young's modulus and hardness of dentin and enamel kept in control. However, the mechanical properties of the bleached dentin were significantly decreased. For intertubular dentin, the mean hardness decreased by 29–55% and the mean Young's modulus decreased by 19–43%. For enamel, the mean hardness decreased by 13–32% while the mean Young's modulus decreased by 18–32%. The exact mechanism by which hydrogen peroxide affects the dentin and enamel has yet to be fully elucidated. However, it is observed to have an undermining effect on the nanomechanical properties of teeth.
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Keywords: Nanoindentation; Teeth; Bleaching agent; Young's modulus; Hardness; Mechanical properties

1. Introduction

Numerous methods have been introduced to improve the appearance of discolored teeth. One such method is the whitening or bleaching of teeth which is gaining popularity. Although researchers have examined the effects of bleaching agents on the mechanical properties such as hardness and Young's modulus of teeth, the exact mechanism with which these agents affect the teeth is still unknown (Goldstein and Kiremidjian-Schumacher, 1993). One particular concern is that bleaching may undermine the mechanical properties and weaken the teeth.

Nanoindentation is a widely used technique to determine the mechanical properties such as hardness and Young's modulus for thin films as well as micro and nanostructured materials (Alguero et al., 2001; Beegan et al., 2003; Miyahara et al., 1999; Stollberg et al., 2003). As nanoindentation indents a very small depth ($<1\,\mu m$), the mechanical properties that are obtained are assumed to be representative of the bulk material if it is homogeneous or of a very small discrete region where it is indenting if it is non-homogeneous. In recent years however, there is an increasing trend of using the nanoindenter to study the properties of hard biological

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tissues such as teeth (Angker et al., 2003; Balooch et al., 1998; Cuy et al., 2002; Finke et al., 2001; Habelitz et al., 2002; Kinney et al., 1996, 1999, 2003; Mahoney et al., 2000; Marshall et al., 2001). This technique is especially useful as it allows the determination and comparison of the Young's modulus and hardness at the various regions of both the enamel and dentin in the micron and even nanometer scale.

The aim of this paper is to evaluate the effect of 30% hydrogen peroxide on the nanomechanical properties of dentin and enamel using the nanoindentation technique. As hydrogen peroxide is described as one of the most effective bleaching agent to use (Hegedus et al., 1999), its effects on the mechanical properties of teeth must be studied in greater details.

The tooth mainly comprises the enamel, dentin, dentino-enamel junction and the pulp. Enamel is semi-translucent and is found in the crown of healthy teeth which appears yellowish-white (Berkovitz et al., 1977). This is the color of enamel being modified by the underlying dentin. The enamel on deciduous teeth appears much whiter as it is more opaque. Enamel is the hardest tissue found in the human body (Ten Cate, 1994). This property enables enamel to limit the amount of wear and withstand the heavy loads of mastication. Enamel has low tensile strength and is brittle. However, it has a high modulus of elasticity and together with the flexible support of the underlying dentin, the possibility of fracture is minimized. Mature enamel is highly mineralized (Balooch et al., 1998). It contains 96% inorganic material, 1% organic material and 3% water by weight and 89%, 2% and 9% by volume, respectively. The inorganic component is mainly calcium phosphate in the form of hydroxyapatite crystals. Other elements present are small amounts of carbonate, magnesium, potassium, sodium and fluoride. The exact composition not only varies between teeth but also within different parts of the same tooth and between the core and periphery of the same prism.

Dentin is pale yellow in color. It gives the crown its color as the enamel is semi-translucent. It is harder than bone and cementum but it is softer and less brittle than enamel. Compared to enamel, dentin has greater compressive and tensile strength. Dentin is made up of intertubular dentin with tubules that are surrounded by peritubular dentin (Fig. 1). The peritubular dentin is much harder than the intertubular dentin (Kinney et al., 1996). Due to the presence of tubules, dentin is readily permeable. Differences in permeability are reflected by the regional variations in tubule size and density. The composition of dentin by weight is approximately 70% inorganic material, 20% organic material and 10% water. The same components constitute 47%, 32% and 21% by volume, respectively. The main inorganic component is hydroxyapatite while the main organic component is Type I collagen. The non-mineral component is much higher thus making it less brittle as compared to enamel.

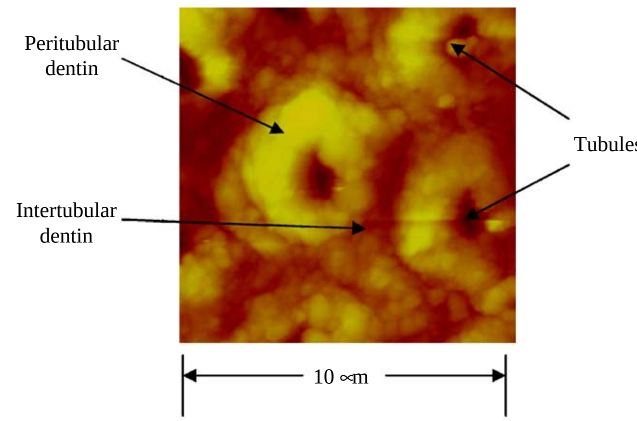

Fig 1. An AFM scan of dentin.

2. Materials and methods

2.1. Experimental samples

Five human premolars, freshly extracted for orthodontic reasons were used for this experiment. The teeth were initially cleaned of any soft tissue covering the root surface. They were then placed in Hank's balanced salt solution (Hyclone, 8 g/l sodium chloride, 0.05 g/l sodium phosphate diabasic, 0.40 g/l potassium chloride, 0.06 g/l potassium phosphate monobasic, 0.98 g/l magnesium sulphate, 0.14 calcium chloride, 1.00 g/l D-Glucose, 0.01 g/l Phenol Red-NA) until they were ready to be used. The root of each tooth was embedded in acrylic (Shofu, Kyoto, Japan) up to the cemento-enamel junction (CEJ) (Fig. 2a). The crown was then sectioned at around 4 mm apical to buccal cusp tip with the slicing plane parallel to the occlusal surface using a diamond saw (Minitom, Struers, Rodovre, Denmark) (Fig. 2b). A flat dentin surface on the remaining tooth was exposed. Care was taken not to section into the pulp, otherwise, the tooth was discarded.

The next step required is gradual polishing. The exposed surface was polished with 30 μm grade Imperial lapping film discs (3 M, St. Paul, MN, USA) for about 5 min and then by another 8 min using the 9 μm grade Imperial lapping film discs by hand. It was then followed by using 0.3 μm alumina powder (Buehler Ltd., Lake Bluff, IL, USA) for 30 min and finally 0.05 μm alumina powder for 40 min using a rotary polishing machine (Jean Wirtz, Dusseldorf, Germany) at 400 rpm for a smooth finish. The polished samples were then ultrasonicated for 5 min to remove any visible smear layer and debris.

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