The Use of Atomic Force Microscopy in Determining the Stiffness and Adhesion Force of Human Dentin After Exposure to Bleaching Agents

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Abstract

Introduction: Oxidant bleaching agents may induce several alterations on mineralized teeth tissues. Our aim is to study, at the ultrastructural level, mechanical modifications induced on dentin after exposure to different bleaching agents. **Methods:** Nanoindentation performed with atomic force microscopy was used to measure changes in dentin stiffness as well as the adhesion force between the tip and the tissue both in intertubular and peritubular dentin. For each specimen, dentin localization, and bleaching agent, 100 independent nanoindentations were performed. Carbamide peroxide (30%) and hydrogen peroxide (35%) were used as bleaching agents. **Results:** A significant reduction of both stiffness and adhesion force was found for both carbamide and hydrogen peroxide in peritubular and intertubular dentin. **Conclusions:** The use of bleaching agents led to a significant reduction in dentin local (at the nanoscale) mechanical properties. *(J Endod 2009;35:1384–1386)*

Key Words

Atomic force microscopy, carbamide peroxide, dental bleaching, hydrogen peroxide, intertubular dentin, nanoindentation

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Commercial products commonly used as bleaching agents are based on hydrogen and carbamide peroxides (H_2O_2 and $CO(NH_2)_2 \cdot H_2O_2$, respectively). Hydrogen peroxides dissociates into free radicals, which perform their bleaching action through the oxidation of complex chromogenic molecules. On the other hand, carbamide peroxide decomposition gives rise to hydrogen peroxide that is, in the end, responsible for the final effect. However, superimposed onto the therapeutic effects, these oxidant agents may show deleterious effects that include alterations in the structure of teeth mineralized tissues (1–3); in any case, these alterations have not been found for low concentrations of the bleaching agent (eg, carbamide peroxide 10%, and hydrogen peroxide 3.5%) (4, 5). The dentin can be modified during internal bleaching in endodontically treated teeth; these alterations include modifications in Ca and P concentrations (6), increased roughness with acid etching appearance (7), alterations in the organic and inorganic phases of dentin (8), and hardness reduction (9, 10). These alterations are important because they may lead to weakening of the dental structure to biomechanical solicitations (10). There are only a few works that employ atomic force microscopy (AFM) to investigate alterations in teeth mineralized tissues (11–13) and are mainly focused on nanostructural modifications in enamel and intertubular dentin.

This work aims to investigate modifications at the nanostructural level in dentin, both peritubular and intertubular, induced by the application of the following high concentration bleaching agents: hydrogen peroxide 35% and carbamide peroxide 30%.

Materials and Methods

Ten sections containing both dentin and enamel were obtained from 10 first molars without carious lesions extracted by periodontal reasons and with an intact coronal structure transversely cut in 1-mm thickness slices. The section surface was polished by a set of abrasive discs of decreasing gradient in a polisher (SBT 900; South Bay Technology, Arlington, VA) with water refrigeration; afterwards, they were polished with diamond dust. Finally, samples were washed in an ultrasonic bath with distilled water for 20 minutes.

Samples were randomly distributed into two groups for the different treatments. Bleaching agents used were 30% carbamide peroxide (Vivastyle; Ivoclar/Vivadent, Schaan, Liechtenstein) and 35% hydrogen peroxide (PolaOffice; SDI, Victoria, Australia); they were applied using the following manufacturer recommendations: 3 applications of the bleaching agent for 30 minutes in the first case and 8 minutes in the second one. Each sample was treated with only one bleaching agent.

Nanoindentation experiments were performed in a NanoScope III Atomic Force Microscope (Digital Instruments, Santa Barbara, CA). A square pyramidal tip OTR8 (Veeco, Plainwiew, NY) with a spring constant 0.57 N/m (datum supplied by the manufacturer) and a tip half angle of 35° was used. Calibration of the tip sensitivity was performed in the same conditions as the experiments with a flat surface sample made of the same material as the tip and was calculated to be −5.721 nm/V after at least 10 repetitions (14). Sensitivity of the tip was used to correct the deflection of the tip caused by the vertical movement of the sample without penetration.

Samples for indentation experiments were sheets, around 1-mm thick, glued on a stiff surface. All experiments were performed with the instrument mounted on a vibration isolation system. Measurements were performed before and after the

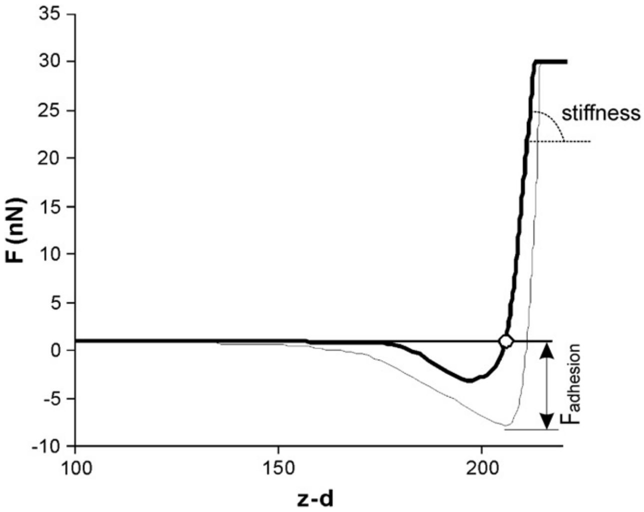

Figure 1. An example of a measured curve in an indentation AFM experiment; force versus the difference between the z displacement of the sample and the cantilever deflection d is shown. The thick line represents the indentation scan and the thin one the retraction one. Stiffness is calculated from the uploading curve and the adhesion force from the downloading one.

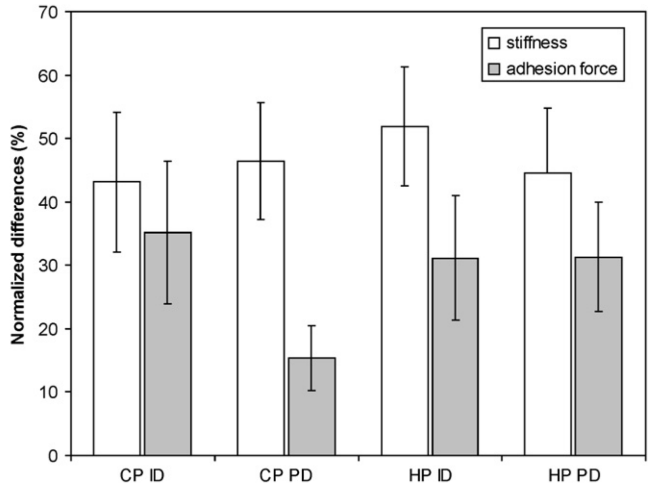

Figure 2. Normalized differences (percentage) for stiffness and adhesion force before and after bleaching treatments on peritubular and intertubular dentin. CP, carbamide peroxide; HP, hydrogen peroxide; ID, intertubular dentin; PD, peritubular dentin. White columns: before bleaching, gray columns: after bleaching.

treatment both in intertubular and peritubular dentin. Nanoindentation experiments allow one to get information about the local mechanical properties of the system. The result of one of these experiments is shown in Figure 1, where the force is represented against the difference between the displacement of the sample in the vertical axis, z, and the cantilever deflection, d. The variable z − d is the relative displacement between the tip and the sample surface. At low values of z − d, there is no contact between the tip and the surface (both the deflection of the cantilever and the measured force are near zero). The thick line (Fig. 1) is obtained during indentation, whereas the thin one corresponds to the retracting scan. The negative values of the force (attraction force between the tip and the surface because of van der Waals long-range interactions) are produced close to the contact point (15), which can be determined using the extrapolation shown in Figure 1 (ie, it is the intersection point between the experimental curve and the horizontal reference assumed for absence of contact). For greater values of relative displacement (h = z − d), short-range repulsive interactions take place between the sample and the tip as well as the indentation of the material, which overcome the previous attraction forces making the force *F* to increase. Ascribing the value of penetration zero to this point, the plot in Figure 1 can be transformed into a force-penetration graph. The adhesion force between the tip and the sample can be calculated from the minimum of the retracting curve. The stiffness *S* is then defined as the slope of this graph

$$S = \frac{\partial F}{\partial h}$$

In an elastic body, *S* can be related to the elastic modulus and the hardness (16), but for the purposes of this work, an analysis of the stiffness itself throughout the sample surface is sufficient. In fact, the stiffness can be calculated as

$$S = \frac{\partial F}{\partial (z - d)}$$

Indentation experiments were performed in every sample (after different treatments) in 100 points located on a 10 × 10 matrix with rows and columns separated 10 nm from each other. Thus, the stiffness is measured over a square of 1 × 1 μm. The nanoindentation area was selected after several imaging steps for every specimen. First, a region with homogeneously distributed dentin tubules (free of defect or impurities) was selected by using an optical microscopy (400×) coupled to the AFM. Afterwards, several images of increasing resolution were acquired (AFM) until a single dentin tubule in a 5 × 5 μm area was visualized. The nanoindentation areas (intentubular and peritubular dentin) were finally selected from this high-resolution image. Nanoindentation experiments provide the adhesion force and the stiffness for each sample as the mean value of 100 points. These measurements allow one to assess adhesion force and stiffness.

Results

The adhesion force and the stiffness were initially calculated for each sample as previously explained. Afterwards, bleaching treatments were applied (five with carbamide peroxide and five with hydrogen peroxide) and adhesion force and stiffness were immediately measured in new AFM experiments. The normalized difference (N_D, relative to the untreated value) between this magnitude before and after treatment were accordingly calculated in order to compare the effect of the treatment for the different specimens regardless of the absolute values of these magnitudes using the following equation:

$$N_D = \frac{S_b - S_a}{S_b}$$

S_a and S_b are the magnitudes (either adhesion force or stiffness) after and before the bleaching treatment, respectively. Accordingly to this, Figure 2 represents the mean value (five specimens) of the N_D for both stiffness and adhesion force. A significant reduction of approximately 50% is found for each bleaching agent (carbamide peroxide and hydrogen peroxide) irrespective of the application area (intertubular dentin and peritubular dentin) without differences among them. Similar trends are observed for the adhesion force, but the reduction in adhesion force is lower for carbamide peroxide applied on peritubular dentin.

Discussion

Two commercial bleaching products were selected in this work, which were applied by following the guidelines of the manufacturer. Both products, hydrogen peroxide and carbamide peroxide, were used in high concentrations as in clinical procedures, taking into account that peroxide carbamide 30% is equivalent to hydrogen peroxide 10% (17).

Several works have investigated the microhardness of mineralized dental tissues, mainly enamel, with contradictory results. In a recent review (18), it is argued that variability among results might be a consequence of the procedure followed to maintain teeth in between applications of the product. In our case, the application of bleaching agents was performed immediately after washing the previously applied agent. AFM experiments were performed after the last application. Besides, microhardness reductions have been described in dentin after contacting bleaching agents, both in low concentration, carbamide peroxide 10% (19), and high concentration, carbamide peroxide 30% (20), as well as significant changes in microroughness (21). Nevertheless, other authors have found no significant changes with hydrogen peroxide 35% for 2 hours (22) or hydrogen peroxide 35% for 30 minutes or 30% for 24 hours (23). The addition of clorhexidine as the antimicrobial agent during bleaching did not influence dentin microhardness (24).

Nanoindentation studies have also shown a reduction in dentin hardness (25, 26) as well as in the Young modulus after bleaching treatments (25). Only intertubular dentin was analyzed in these works by performing eight indentations per sample. We have investigated both intertubular and peritubular dentin, and, moreover, we have performed 100 (nano)indentations for each treatment and sample because of the nanometric character of our methodology. It is important to remark here that the error bars shown on each graph (Fig. 2) represent the standard deviation for the normalized difference for stiffness and adhesion force before and after bleaching treatments on peritubular and intertubular dentin for five specimens. That is to say, the comparison between 1,000 indentation curves (500 before and 500 after treatment) is included, which strengthens the reliability of our methodology as compared with standard indentation experiments.

Peritubular dentin is known to be compact and regular compared with the intertubular one, which is less regular and granular-like (27), with higher magnesium and whitlockite contents (28). Both peritubular and intertubular dentin consist of carbonated apatite, which differs in collagen and protein composition (29). We have identified a generalized loss of stiffness and adhesion force after applying any of the bleaching agents and for every dentin zone. Mechanical dentin modifications produced by bleaching agents could lead to biomechanical alterations, but there is no evidence to substantiate this (30). In the same way, clinical consequences are unknown (31). However, further studies are needed to investigate these alterations, which might give rise not only to clinical manifestations in the biomechanical properties of teeth but also in adhesion mechanisms of resins to dentin after bleaching treatments. Probably, new bleaching techniques (32) could improve both efficacy and safety.

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