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AFM-Based Nanomechanical Properties and Storage of Dentin and Enamel

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ABSTRACT

This study evaluated the affects of 2 different solutions, e.g. deionized water and Hanks' Balanced Salt Solution (HBSS), on nanohardness and elastic modulus of dentin and enamel from human third molars at storage times of 0, 1, 7, 14 and 28 days using a modified AFM (Triboscope). The pH values of the solutions were monitored throughout the test periods. Storing the specimens in deionized water resulted in a large decrease of mechanical properties, e.g. the reduced elastic modulus of dentin decreased from 24.0 ±1.5 GPa to 21.0 ±1.6, 8.6 ±1.1 and 5.2 ±1.1 GPa for storage times of 1, 7 and 14 days, respectively. Mechanical properties of dentin and enamel dropped by more than 12% after one day and more than 50% after a week when stored in deionized water. The observed changes in mechanical response were attributed to a superficial demineralization process during storage. In contrast, storing teeth in HBSS did not significantly alter the mechanical properties of dentin or enamel over the time studied.

INTRODUCTION

Nanoindention has become a common technique for the determination of local mechanical properties of structural features in biological hard tissues[1-4]. Nanoindentations usually do not exceed indentation depths of 500 nm. Although only a thin surface layer is examined, the mechanical properties obtained are assumed to be representative of the bulk material. Chemical changes in the surfaces of mineralized tissues resulting from storage solutions are, thus, important considerations for the accurate determination of mechanical properties. Tooth structure is composed of three calcified tissues: the outer enamel; the bulk of the tooth, dentin; and cementum. Enamel is the most highly calcified tissue (85 vol.% mineral) and therefore the hardest tissue in the human body [4]. Its unique microstructure consists of keyhole-like rods, which are aligned parallel and run from the dentino-enamel junction towards the surface of the tooth (Figure 2). Each rod consists of carbonated apatite fibers of 30 to 80 nm diameter and up to several millimeters length. These fibers, which are covered with a thin protein layer, are aligned parallel in the center of the rod and flare laterally towards the outer rod. The oriented microstructure results in anisotropy of the mechanical properties [3].

Dentin is a complex hydrated biological composite structure containing approximately 45 vol.% mineral in the form of carbonated apatite, 35 vol.% organic components (mostly type I collagen) and 20 vol.% fluids. Its distinct microstructure is characterized by tubules of 1 to 2 μm diameter (Figure 1) that were the paths of the odontoblasts during tooth formation and run from the dentino-enamel junction towards the pulp [4,5]. A 1 µm thick layer of higher mineralization, the peritubular dentin, surrounds each tubule.

Tooth samples prepared for mechanical testing are often stored in an aqueous solution to maintain hydration. In particular for nanomechanical testing storing of samples can become a critical issue since chemical reactions such as etching and dissolution may affect the chemical composition of the surface, which might alter the mechanical response during nanoindentations. Therefore, we evaluated changes in the nanomechanical properties of dental tissues during storage. This study reports the impact of storing dentin and enamel from human third molars for 0, 1, 7 and 14 days in deionized water or HBSS on nanohardness and elastic modulus.

MATERIALS AND METHODS

Human third molars with documented history were extracted according to protocols approved by the UCSF committee on human research, sterilized by gamma radiation and stored (less than 4 months) in deionized water at 4° C until prepared. Three teeth were sectioned longitudinally, ground down to 4000 grit and subsequently polished with water based diamond suspensions to 0.25 µm particle size. Finally, samples were cleaned ultrasonically in deionized water for 10 s. Mechanical properties of the specimens were studied immediately after final surface preparation by AFM-based nanoindentations. Specimens were subsequently immersed in the corresponding storage solutions of 30 ml volume. Deionized water, and HBSS (400 mg/l KCl, 60 mg/l KH_2PO_4, 8000 mg/l NaCl, 1000 mg/l Glucose, 90 mg/l $Na_2HPO_47H_2O$, 350 mg/l $NaHCO_3$, 140 mg/l $CaCl_2$, 100 mg/l $MgSO_47H_2O$, 100 mg/l $MgCl_26H_2O$) were used for storage. Specimens were studied by atomic force microscopy (AFM), (Nanoscope III, Digital Instruments, Santa Barbara, CA) with the standard head replaced by a Triboscope indenter system (Hysitron Inc., Minneapolis, MN), as described elsewhere [6]. A cube corner diamond indenter with a tip radius of about 20 nm was used for indentation and imaging. AFM imaging facilitated selection of indentation sites. Fused silica was used for calibration of and to define the tip area function for indentation depths over a range of 50 to 600 nm. Indentation loads of 1500 and 750 µN were applied on freshly polished enamel and dentin surfaces, respectively. Loads were adjusted in order to maintain indentation depths between 300 and 400 nm. A minimum of 12 indentations was performed under hydrated conditions on enamel and dentin. The indentation load-displacement data were analyzed to determine the hardness, H, and the reduced elastic modulus, E, according to the method of Oliver and Pharr [7] and calculated according to:

$$\mathrm{H} = \frac{P_{\max}}{A}$$

and

$$\mathrm{E} = \frac{S\sqrt{\pi}}{2\beta\sqrt{A}}$$

with P_{max} = maximum load, A = projected area of contact, S = elastic contact stiffness (S = dP/dh), β = constant dependent on geometry of indenter.

RESULTS AND DISCUSSION

Figure 1 shows an AFM image of nanoindentations on human dentin after final surface polishing. Using AFM in combination with nanoindentation ensured that indentations were placed exclusively on the intertubular dentin and not on the harder and stiffer peritubular dentin. Figure 2 is an AFM-image of nanoindentations on human enamel after final surface polishing. The characteristic prism or rod-like structure of enamel can be observed. Enamel was studied without +respect to the rod orientation. However, indentations were placed preferentially on the intrarod enamel, in order to avoid the protein-rich and softer interrod enamel [3].

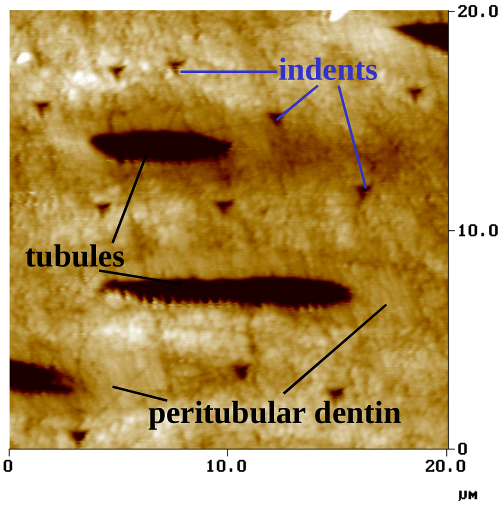

Figure 1. AFM-image of nano-indentations on human intertubular dentin.

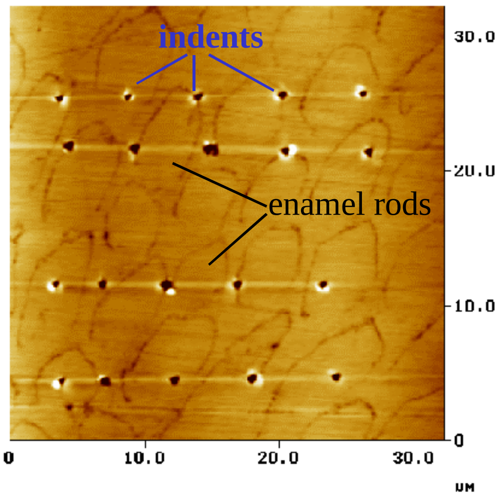

Figure 2. AFM-image of nano-indentations on human enamel

Figures 3 to 6 show the mechanical properties of enamel and dentin versus storage time in deionized water and HBSS. Nanohardness of freshly polished dentin and enamel specimens varied between 0.9 and 1.2 GPa and 3.0 and 3.9 GPa, respectively. The elastic modulus of dentin was observed in the range from 19 to 26 GPa, while enamel exhibited values between 69 and 88 GPa.

In all samples a rapid decrease in hardness and elastic modulus was observed when stored in deionized water. Within one day of storage hardness decreased by about 25% and after 1 and 2 weeks by 60 and 70%, respectively. The impact of storage in water on the elastic modulus was slightly less. At 1 day an average reduction of about 12% was observed. At 7 and 14 days, however, decreases on the order 50 % and 70% were measured, respectively. In contrast, storing teeth in HBSS for two weeks had no significant influence on the mechanical properties of the specimens, as shown by ANOVA. The variations in properties at different storage times are attributed to local variations in mineral content and in the case of enamel to the anisotropy of the material[3].

A demineralization process at the surface is assumed to be responsible for changes in the apparent mechanical response of dentin and enamel during storage in deionized water. The deionized water had a measured pH of 6.5 and thus has a slight tendency to dissolve the calcium-

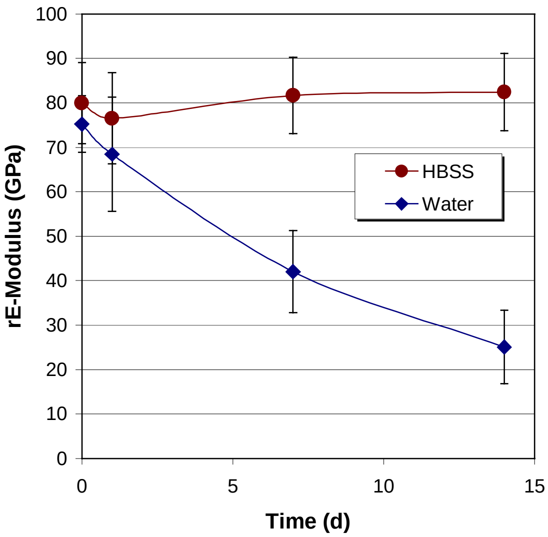

Figure 3. Reduced elastic modulus of enamel stored in water and HBSS

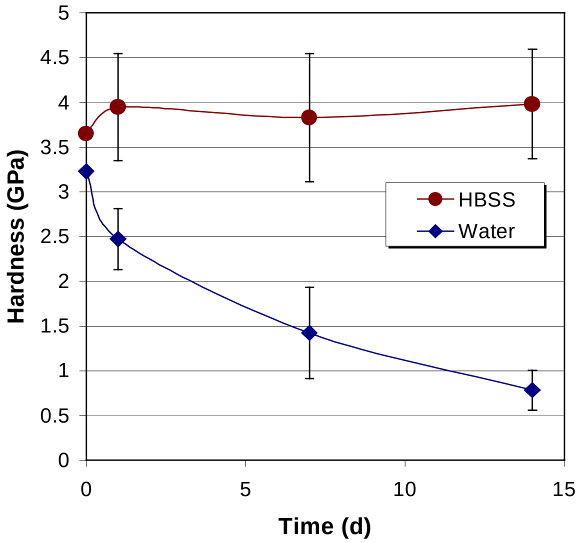

Figure 4. Nanohardness of enamel stored in water and HBSS

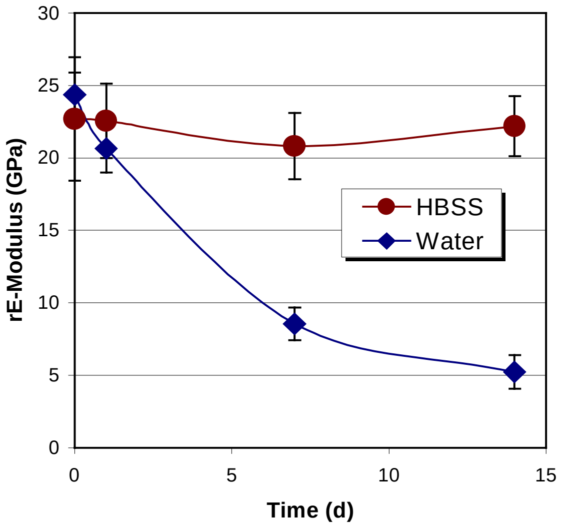

Figure 5. Reduced elastic modulus of dentin stored in water and HBSS

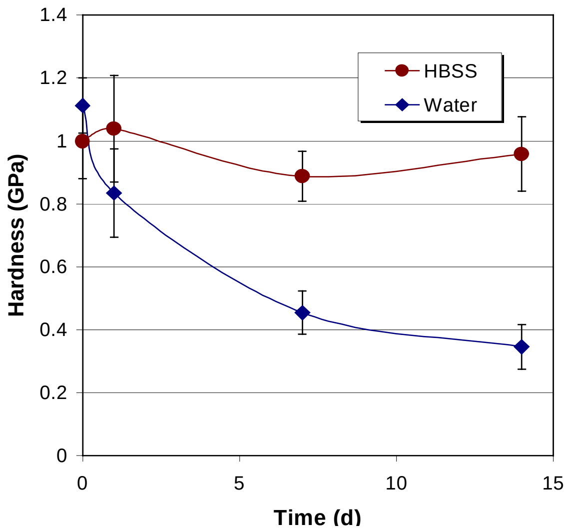

Figure 6. Nanohardness of dentin stored in water and HBSS

phosphate mineral phase of enamel and dentin by acidic etching. Moreover, deionized water lacks calcium and phosphate ions. Therefore the chemical potential for dissolution of the mineral phase of dentin and enamel is high. After two weeks of storage the pH increased to 7.6, which is attributed to an increased concentration of alkaline ions, e. g. Ca^{2+}, $Mg^{2+}$and Na^{+} in solution. HBSS is slightly basic (pH = 8.0) and thus cannot dissolve the mineral phases by acidic attack. Its ionic concentrations of Ca^{2+}, Mg^{2+}, Na^{+}, PO_4^{3+} and Cl^{-} are high with respect to the chemical potential of dental mineral phases stored in a solution. Therefore, the chemical potential of HBSS to dissolve the calcium phosphate phases in teeth is low and demineralization of the dental tissues is prevented. However, a slight decrease of the pH during storage to 7.6 was observed, indicating that minor chemical changes of the tissue surfaces are also introduced by storage in HBSS.

CONCLUSIONS

As shown here for the case of deionized water, storage can tremendously alter the mechanical properties of a thin surface layer of mineralized tissues. This effect might be a reason for discrepancies of values of nanomechanical properties of teeth reported in the literature [2,8,9]. If mechanical testing cannot be performed immediately after final surface preparation, storage in HBSS is recommended.

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