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Effect of stress and temperature on the micromechanics of creep in highly irradiated bone and dentin

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ABSTRACT

Synchrotron X-ray diffraction is used to study *in situ* the evolution of phase strains during compressive creep deformation in bovine bone and dentin for a range of compressive stresses and irradiation rates, at ambient and body temperatures. In all cases, compressive strains in the collagen phase increase with increasing creep time (and concomitant irradiation), reflecting macroscopic deformation of the sample. By contrast, compressive elastic strains in the hydroxyapatite (HAP) phase, created upon initial application of compressive load on the sample, decrease with increasing time (and irradiation) for all conditions; this load shedding behavior is consistent with damage at the HAP–collagen interface due to the high irradiation doses (from ~100 to ~9,000 kGy). Both the HAP and fibril strain rates increase with applied compressive stress, temperature and irradiation rate, which is indicative of greater collagen molecular sliding at the HAP–collagen interface and greater intermolecular sliding (i.e., plastic deformation) within the collagen network. The temperature sensitivity confirms that testing at body temperature, rather than ambient temperature, is necessary to assess the *in vivo* behavior of bone and teeth. The characteristic pattern of HAP strain evolution with time differs quantitatively between bone and dentin, and may reflect their different structural organization.

1. Introduction

Hard bio-mineralized tissues such as bone and dentin have a complex, hierarchically organized structure. Although structurally different, in mammals both of these materials are composed of the same components: a mineral phase (calcium hydroxyapatite, HAP), a protein phase (mostly type-I collagen), and fluid phases (water, saliva, or blood) [1,2]. Through a unique assembly of these phases, bone and dentin exhibit a combination of high strength, high toughness, high wear-resistance (for teeth), and self-repair (for bone), enabling them to perform for years under physiological loads and at body temperature. There is strong evolutionary pressure to achieve optimized mechanical properties in these tissues, given that bones are the primary load-bearing structures of the body and that teeth enable feeding. The study of the mechanical properties of healthy bone and teeth is thus of interest, both fundamentally, and to provide a comparison with diseased or injured tissues. A thorough understanding of the mechanics of bone and teeth under long-term loading is also of interest for the success of bone and tooth synthetic implants or replacement materials.

A number of studies have examined the elastic [3–10] and fracture properties [11–20] of bone and dentin of humans, bovines, equines and elephants which are relevant to rapid and transient loads. Less attention has been devoted to the time-dependent behavior of these tissues, which behave visco-elastically under long-term stresses. Although not the most common loading condition for bone and dentin, long terms stresses do occur due to muscular action or during treatments of conditions such as scoliosis [21], the use of rib-spreaders during thoracic surgery [22], and the use of orthodontic braces [23]. Rimnac et al. [24] have studied the effects of stress, temperature and microstructure on the macroscopic creep strains in bovine femur, and demonstrated, using semi-empirical models, that the creep rate is positively associated with the volume fraction of secondary Haversian bone, temperature and stress. Jantarat et al. [25] have shown a positive correlation ($r^2 = 0.79$) between the macroscopic creep rate of dentin and stress (from human canines and incisors). However, to truly understand the time-dependent behavior of bone and dentin it is necessary to determine the response of their individual phases in addition to the macroscopic behavior.

Combined X-ray wide- and small-angle scattering (WAXS/SAXS) is a technique through which the mechanical coupling between the mineral and protein phases can be assessed at the nanoscale via average strains measured in these two phases. WAXS/SAXS using high-energy ($E = 70$ keV) synchrotron X-rays has been previously

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used to determine the strains, *in situ* under an applied stress, in the individual phases of metal-matrix composites [26–30], and biological composites like fallow deer antler [31], canine bone [32,33], bovine bone [34–39] and bovine dentin [40–44]. Recently, we used this technique to explore creep deformation in bovine bone, but this study was limited to a single temperature (37 °C) and a single compressive stress (−80 MPa) [36]. In this study, the magnitude of the elastic strain in the HAP phase increases linearly with increasing creep time due to load transfer from the visco-elastically deforming collagen phase. This behavior is also observed in inorganic composites where the more compliant creeping matrix sheds load to the stiffer reinforcement (whose strain thus increases) via a strong interface, as the bulk deformation of the sample increases [45]. Thus, the mechanism associated with creep in bone and dentin appears to follow classical composite mechanics. However, in a subsequent study on creep of bone and dentin carried out at a single temperature (37 °C) and stress (−95 MPa) using the same technique, we observed that, under much higher irradiation doses, the magnitude of the compressive strain (and thus stress) carried by the HAP phase decreased with creep time, while the compressive strain in the fibrils increased with time [35]. This load shedding from the reinforcement to the matrix phase was assigned to damage of the HAP–collagen interface degrading the transfer of load from the matrix to the HAP platelets during creep [35].

In this study, we examine, for the first time in a systematic manner, the effect of the applied stress and temperature, on the mechanisms of load transfer between HAP and collagen during creep deformation of bovine bone and dentin which were subjected to high radiation doses. With the use of the synchrotron WAXS/SAXS technique, we investigate the effect of creep stresses in the range of −40 to −110 MPa on bovine bone and dentin at two different temperatures, 27 °C and 37 °C and a wide range of radiation doses (580–9400 kGy).

2. Experimental procedures

2.1. Sample preparation

Fresh bovine femurs of a healthy 18-month old Black Angus cow were obtained from Aurora Packing Company Inc. (North Aurora, IL), within 1 hour of slaughter. The preparation procedures are described in Ref. [39] but are summarized here. The femur was cleaned of marrow and any attached ligaments using scalpels. With an autopsy saw, the length of the femur was divided into 3 cylinders, each ~2 cm in height. The cylinders were then stored in gauze soaked with phosphate buffered saline (PBS) and frozen at −20 °C until further cutting. The total time elapsed between slaughter of the animal and freezing of cut sections was ~5 hours. Prior to cutting, one cylinder was thawed to room temperature. Transverse sections of the cylinder, 5 mm in height, were cut using a low-speed diamond wafering saw perpendicular to the femur long-axis. These transverse sections were further cut to obtain samples with sizes of 5.45 (±0.01)×3.88 (±0.01)×2.86 (±0.01) mm^3. All cutting was done in deionized water to maintain the hydration state of the samples. Each sample was weighed on a precision balance three times, after blotting out the excess water from the surface. Their dimensions were measured three times with a point micrometer to calculate the volume. The density of the samples was calculated by taking a ratio of the average of the mass and volume measurements [46,47]. The samples were then stored in PBS and frozen at −20 °C until the time of the experiment.

Front incisors were extracted using dental pliers and scalpels from the lower mandible of another healthy 18-month old Black Angus cow obtained from Aurora Packing Company Inc., within an hour of death. The preparation of the samples, which has already been described in detail in Ref. [40], is summarized here. The extracted teeth were stored in a mixture of PBS, 1% antibacterial and antifungal solution and frozen at −20 °C. The total time elapsed between slaughter of the animal and freezing of extracted teeth was ~5 hours. Prior to cutting, the teeth were thawed to room temperature. Parallel cuts, 8 mm apart, were made with a low-speed diamond wafering saw perpendicular to the tooth-growth direction. From each tooth, 1–2 cylindrical root dentin samples were obtained, 7.5 mm in height and with the natural cross-section of the tooth. The cutting was done in deionized water to prevent drying of the dentin. To calculate the apparent density, Archimedes tests were performed in water. Further, to determine the accurate cross-sectional area of the dentin cylinders, laboratory micro-CT was used [41]. The samples were stored in PBS and frozen at −20 °C until the time of the experiment.

2.2. X-ray scattering experiments

Mechanical testing with concurrent X-ray diffraction was performed at beamline 1-ID-C at the Advanced Photon Source, Argonne National Laboratory (Argonne, IL 60432). Bone and dentin samples were thawed to room temperature before testing. An MTS-858 load frame applied compressive load along the femur long-axis of the bone samples, and along the tooth growth direction of the dentin samples. A hydration rig made of vinyl tubing was attached to the lower platen to maintain the samples hydrated and at the appropriate temperature. Tests were carried out at 27 °C (room temperature) and 37 °C (body temperature). A schematic of the setup is shown in Fig. 1, and is similar to the setup used in previous works [36,39].

The bone and dentin samples were placed in the sample hydration rig such that the X-ray beam passed through their vertical and horizontal centers, determined using absorption measurements. For each experiment, an initial scattering measurement was taken at zero applied stress on a single sample, after which the sample was loaded in less than 10 seconds up to the desired stress level and maintained at a constant value for the rest of the experiment, lasting for 2–4 hours. Tests were done in two segments—high stresses ranging between −90 and −108 MPa and slightly lower stresses, ranging between −40 and −75 MPa. All the stresses used here are significantly higher than the stresses experienced in a physiological situation, which are in the range of 10–20 MPa as measured on a human femur and tibia [48,49], but similar to our previous work on creep in bone and dentin [35,36,44] where the main goal was to identify deformation mechanisms in the limited synchrotron beam time available.

In the first experimental segment, bone samples were tested at stresses of −95, −100 or −108 MPa at 27 (samples A–C) and 37 °C (samples J–L). Dentin samples were tested at −90 or −100 MPa at 27 °C (samples M–O), and −90 or −95 MPa at 37 °C (samples P–R). The sample treatment conditions along with their code names are listed in Table 1. A parallel X-ray beam with 65 keV energy and 50×50 μm^2 cross-section was used for these experiments. The results from the tests on bone and dentin samples at −95 MPa at 37 °C (samples J and R) have been reported in our earlier work [43], and are used here for comparison with the other stresses. Wide-angle X-ray scattering (WAXS) patterns for the HAP phase were recorded with a MAR 345 detector (2,300×2,300 pixels, 150 μm^2/pixel) which was placed at a distance of 1,269 mm from the sample. Small-angle X-ray scattering (SAXS) patterns for the collagen were recorded with a PI-CCD detector (2,048×2,048 pixels, 80 μm^2/pixel), placed at a distance of 4,000 mm from the sample. The exposure times for the WAXS and SAXS measurements were 50 and 30 seconds respectively, resulting in a total absorbed radiation dose of 24 kGy per WAXS+SAXS measurements, as indicated in Table 1. A total of 33 measurements were performed at regular time intervals on each sample over a time span of 2–3 hours, resulting in an accumulated