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The influence of water removal on the strength and toughness of cortical bone

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Abstract

Although the effects of dehydration on the mechanical behavior of cortical bone are known, the underlying mechanisms for such effects are not clear. We hypothesize that the interactions of water with the collagen and mineral phases each have a unique influence on mechanical behavior. To study this, strength, toughness, and stiffness were measured with three-point bend specimens made from the mid-diaphysis of human cadaveric femurs and divided into six test groups: control (hydrated), drying in a vacuum oven at room temperature (21 °C) for 30 min and at 21, 50, 70, or 110 °C for 4 h. The experimental data indicated that water loss significantly increased with each increase in drying condition. Bone strength increased with a 5% loss of water by weight, which was caused by drying at 21 °C for 4 h. With water loss exceeding 9%, caused by higher drying temperatures ($\geqslant$70 °C), strength actually decreased. Drying at 21 °C (irrespective of time in vacuum) significantly decreased bone toughness through a loss of plasticity. However, drying at 70 °C and above caused toughness to decrease through decreases in strength and fracture strain. Stiffness linearly increased with an increase in water loss. From an energy perspective, the water–mineral interaction is removed at higher temperatures than the water–collagen interaction. Therefore, we speculate that loss of water in the collagen phase decreases the toughness of bone, whereas loss of water associated with the mineral phase decreases both bone strength and toughness.

Keywords: Mechanical properties; Bone mineral; Collagen; Femur; Three-point bend

1. Introduction

Bone is a two-component composite material in which the mineral phase (mainly hydroxyapatite) confers the strength (Zioupos, 2001) and stiffness (Currey, 1988), and the organic matrix (mainly Type I collagen) primarily influences the toughness of bone (Wang et al., 2001, Zioupos, 2001, Zioupos et al., 1999). While mineral and collagen each contribute to the bone's competency, as do microarchitecture (e.g., porosity and trabecular connectivity), macrostructure (e.g., curvature of diaphysis and thickness of cortical shell), and in vivo microdamage (e.g., microcracks and diffuse cracks), their interaction with water is equally important to the mechanical behavior of bone. Thus, bone is also a fluid-imbibed material in which the distribution of water affects the mechanical properties of bone.

Early studies demonstrated that the stiffness, tensile strength, and hardness increases, whereas the strain at fracture and energy to fracture decreases, following the dehydration of bone tissues (Dempster and Liddicoat, 1952, Evans, 1973, Evans and Lebow, 1951, Sedlin and Hirsch, 1966, Smith and Walmsley, 1959, Yamada and Evans, 1970). Reduced energy to fracture has also been

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observed for dehydrated dentine (Jameson et al., 1993). In addition, as trabecular bone loses water, its buckling behavior changes from ductile to brittle (Townsend et al., 1975). Lastly, dehydration affects the viscoelasticity of bone: compared with wet bone, dry bone has less anelastic deformation (i.e., less recoverable strain from creep) (Currey, 1965), lower loss factor tan δ (Yamashita et al., 2001, Yamashita et al., 2002), and much higher relaxation rate (Sasaki and Enyo, 1995). Despite the documented effects of drying on bone properties, little is actually known about the underlying mechanism of such changes.

Water is not only present in the microscopic pores, which increase in number and size with age, but also exists within the extracellular matrix of bone tissues. The distribution of water in bone appears to change throughout life. It has been reported that water in bone tissues decreases with skeletal growth (Jonsson et al., 1985) and with progressive mineralization (Robinson, 1979, Robinson, 1975). The observation that mineral content increases with age, tapering at 60 years (Mueller et al., 1966, Timmins and Wall, 1977), implies that the amount of water in the tissue would likely be reduced in the elderly skeleton. Furthermore, non-enzymatic, glycation-induced collagen cross-links increase with age (Wang et al., 2002) and may decrease water's interaction with collagen as seen in connective tissues (Kopp et al., 1989). Understanding the role of water distribution in the mechanical behavior of bone may provide another insight into the susceptibility of bone to fracture in the elderly population.

It is presumable that the distribution of water within the tissue of bone—the amount of water bound to collagen, to mineral, and the mobile water in the vascular–lacunar–canalicular cavities—may certainly dictate the bone's mechanical behavior. To address this issue, the present study investigated the effect of water loss on the mechanical behavior of bone. Specifically, mechanical properties were obtained from three-point bend tests of cortical bone specimens that were dehydrated at varying temperatures. Based on the results, we put forth a model for how the distribution of water in the collagen matrix and the mineral phase affects the strength, toughness, and stiffness of bone.

2. Materials and methods

2.1. Specimen preparation

Six human cadaveric femurs (42–49 year old males) were collected from the Musculoskeletal Transplant Foundation (Edison, NJ). One cross-sectional segment ($\cong$35 mm in length) was dissected from the mid-diaphysis of each femur with a band saw. Using a circular diamond saw, six bone strips ($\cong$2.1 mm in thickness) were extracted along the longitudinal axis from the medial side of each cortex segment. With a bench top end-mill (Model 5000, Sherline, San Marcos, CA), we machined the bone strips into rectangular specimens (nominal dimensions of 30 mm × 4.2 mm × 2.1 mm). One bone specimen from each donor was included in each of the following test groups: control (no drying), drying in a vacuum oven at room temperature (21 °C) for an approximate time of 30 min, at 21, 50, 70, and 110 °C for 4 h. The highest temperature was chosen because it was below the temperature (160 °C) at which heat-induced collagen denaturation affects the mechanical properties of bone (Wang et al., 2001). Finally, the specimens were stored in gauze soaked with phosphate buffered saline at −20 °C prior to measurements and treatments.

2.2. Dehydration

After being thawed, each specimen was wiped free of surface water, weighed in air with an electronic balance (PB303-S, Mettler Toledo, Greifensee, Switzerland), and weighed again while submerged in water. Then, the specimens were dehydrated in a vacuum oven (Model 280A, Fischer Scientific, Pittsburgh, PA) with 25 ± 2 in of Hg and weighed in air immediately after drying and before mechanical testing. It was assured that the mass of bone specimens did not change (by more than 0.03%) while being measured nor during the time of testing. Water loss then was calculated as the difference between the mass of bone specimen before drying (W_{wet}) and the mass after drying (W_{dry}), normalized by W_{wet} and expressed as percent loss by weight. In addition, water loss was expressed as the percent loss by volume following Archimedes's principle,

$$\text{Water loss (\% by volume)} = 100 \times \frac{W_{wet} - W_{dry}}{W_{wet} - W_{sub}}, \quad (1)$$

where W_{sub} is the mass of wet bone when submerged in water.

2.3. Mechanical testing

Immediately after weighing, bone specimens were loaded at a cross-head speed of 5 mm/min in three-point bending using an EnduraTEC mechanical testing system (Elf 3300, Bose Corporation, Minnetonka, MN). The span across the support rollers for the three-point bending test was 16.5 mm. Mechanical properties were determined from each force (P) versus displacement (d) curve (Fig. 1). Thus, the modulus of elasticity (E) was determined by the slope of the linear portion ($\Delta P/\Delta d$) of the curve and the deflection equation of beams

$$E = \frac{\Delta P}{\Delta d}\frac{L^3}{48I}, \quad (2)$$