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Interrelationships between electrical, mechanical and hydration properties of cortical bone

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ABSTRACT

Interrelationship between electrical and mechanical properties of cortical bone and the role of bone composition in this interrelationship are not comprehensively investigated to date. This study aimed to investigate associations of electrical properties (i.e., specific impedance, dielectric constant, and conductivity) with mechanical properties (i.e., toughness, strength and elastic modulus) of wet and sequentially dehydrated cortical bone. Bovine cortical bone samples (N = 24) were subjected to three-point bending test. A sequential heat treatment protocol ensued to tease out contributions of unbound water and bound water. Demineralization was performed to understand contributions of organic matrix and the mineral phase to the electrical properties of cortical bone. Raman-spectroscopy based water measurement was used to investigate involvement of collagen- and mineral-bound water in the electrical properties. Our results showed statistically significant correlations between electrical and mechanical properties of cortical bone. Toughness and ultimate strength were negatively correlated with impedance and positively correlated with conductivity and dielectric constant. The highest correlations between electrical and mechanical properties of cortical bone were typically found at the frequencies of 0.2, 0.5 and 1 MHz. The electrical properties of bone changed significantly as a result of sequential dehydration, indicating that unbound and bound water compartments are the key determinants of the electrical properties. Comparison of porosity matched bone samples with high and low amount of bound water showed that bound water compartments may have an independent role in determining electrical properties of cortical bone. Furthermore, the results indicated that collagen and mineral-bound water may contribute differentially to the electrical properties of a bone. In the overall, our results suggest that electrical properties of cortical bone may be used to assess bone toughness and strength, and also underline the necessity for developing techniques to measure these electrical properties in vivo.

1. Introduction

The fracture resistance of bone is associated with several factors including bone mass, bone morphology and architecture, degree of mineralization as well as the quality of bone's constituents (e.g., collagen, collagen crosslinks, water, non-collagenous proteins and crystallinity) (Currey, 1988; McCalden et al., 1993; Akkus et al., 2004; Vashishth, 2007; Unal and Akkus, 2015a, 2015b; Unal et al., 2016). Therefore, bone mineral density (BMD)-based diagnosis alone cannot assess bone fragility with high accuracy (Schuit et al., 2004; Kanis et al., 2005), calling for information on other measures that affect bone's fracture resistance.

The clinical interest in bone bioelectricity dates back to 1970s during when electrical stimulation was used in treating nonunions and congenital pseudoarthrosis (Isaacson and Bloebaum, 2010), although the first experimental measurement of electrical properties of bone was reported in 1937 (Osswald, 1937; Geddes and Baker, 1967). After discovery of bone piezoelectric properties in 1957 (Fukada and Yasuda, 1957), several investigators reported electrical properties of bone in 1960s–1970s (Cochran et al., 1968; Swanson and Lafferty, 1972; Behari et al., 1974; Liboff et al., 1975; Reinish and Nowick, 1976; Durand et al., 1978; Sansen et al., 1978). Considerable progress has been achieved in 1980s in understanding the association of electrical properties of bone with frequency, moisture/hydration levels and

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measurement directions (i.e., longitudinal and transverse) of bone (Chakkalakal et al., 1980; Kosterich et al., 1983, 1984; Reddy and Saha, 1984; Saha et al., 1984; Singh and Beharl, 1984; Singh and Saha, 1984; De Mercato and Garcia-Sanchez, 1988; Saha and Williams, 1989). More recently, Sierpowska et al. investigated the associations of electrical properties of trabecular bone with its microstructure (Sierpowska et al., 2006), composition (Sierpowska et al., 2007), and mechanical properties obtained from compressive mechanical testing of pre-damaged trabecular bone samples (Sierpowska et al., 2003, 2005). Although many earlier studies focused on the electrical properties of cortical bone as a function of moisture level and/or measurement direction (Marino et al., 1967; Reinish and Nowick, 1976; Chakkalakal et al., 1980; Kosterich et al., 1983, 1984; Reddy and Saha, 1984; Saha et al., 1984; Singh and Beharl, 1984; Singh and Saha, 1984; De Mercato and Garcia-Sanchez, 1988), as well as recently as a function of microstructure (Casas and Sevostianov, 2013), to the best of our knowledge, the interrelationship between electrical and mechanical properties of cortical bone has not been investigated comprehensively to date, and the factors affecting this interrelationship are poorly understood. The contributions of unbound water, bound water, organic matrix and mineral phases to electrical properties of cortical bone are still unclear. Since cortical bone is one of the main determinants of bone mechanical competence (Augat and Schorlemmer, 2006), this interrelationship is crucial for assessment of the diagnostic potential of electrical measurements. This understanding on the cortical bone would inevitably translate into improved understanding of the trabecular bone quality as well.

The effect of hydration on electrical properties of bone is known for the decades (Reinish and Nowick, 1976; Chakkalakal et al., 1980; Kosterich et al., 1983, 1984; Reddy and Saha, 1984; Saha et al., 1984; De Mercato and Garcia-Sanchez, 1988; Saha and Williams, 1989); however, it is still not clear whether this effect solely emerges from unbound water in bone's micropores and channels, or whether there is also contribution of bound water to the electrical properties. Recently, we developed a Raman spectroscopy based method (Unal et al., 2014; Unal and Akkus, 2015b) to assess associations of different bound water compartments (collagen- and mineral-bound water) in bone with bone's mechanical properties (Unal and Akkus, 2015a, 2015b). We showed that different bound water compartments in bone are associated with different mechanical properties indicating each bound water compartment has different influence on bone's fracture resistance (Unal and Akkus, 2015b, Flanagan et al., 2017). Therefore, investigation of association of collagen- and mineral bound water with electrical properties of cortical bone would be valuable. Such information may lead to new insight on the role of different water compartments on bone's electrical properties.

Accordingly, this study aimed to investigate associations of electrical properties (i.e., impedance, conductivity and dielectric constant) of cortical bone with mechanical properties, bone composition (i.e., water compartments, organic matrix and mineral), and porosity of cortical bone.

2. Methods

2.1. Experimental design

This study was planned in three experimental phases. In the first phase, correlations between electrical and mechanical properties of cortical bone were assessed. In the second phase, the contributions of unbound water, bound water, organic matrix, mineral and porosity to electrical properties of bone were investigated. In the third phase, associations of different bound water compartments (namely, mineral vs. collagen bound) with the electrical properties of cortical bone were assessed. In this way, interrelationships between electrical and mechanical properties of cortical bone, and the role of bone composition and porosity in this interrelationship were assessed.

2.2. Sample preparation

Five mature bovine femurs of freshly slaughtered animals were obtained from a local slaughterhouse. 4–5 specimens were extracted from each femur. Cortical bone samples were cut longitudinally from all four quadrants of the mid-diaphysis of the femurs using a low speed diamond blade saw (Buehler Ltd., Lake Bluff, IL). Samples were then progressively polished in the longitudinal direction (Buehler Ltd., Lake Bluff, IL) with 800, 1200 grade polishing paper and fine alumina powder (0.3 μm) to form the final shapes. Polishing and cutting debris were removed by sonication (Model 8890, Cole-Parmer, Vernon Hills, IL). The final dimensions of bone beams (N = 24) were 35 mm in length, 3 mm in width and 1.1 mm in thickness. Samples were stored wrapped in distilled water soaked Kimwipes and stored at −20 °C. Samples were thawed completely prior to the analyses. Each sample was used for all experiments.

2.3. Biomechanical testing

Cortical bone beams were fractured at the room temperature in three-point bending. The span-length across the support rollers was set at 25 mm (TestResources Servo-All-Electric™ 800 L, Shakopee, MN). One mm/second rate of vertical displacement was applied at the load point. The testing duration for each sample was ~ 1 min and hydration was maintained by dripping distilled water periodically on the sample during the test. The applied load and actuator displacement were recorded to construct load-displacement curves which were then converted to stress-strain curves to calculate the mechanical properties including toughness, elastic modulus and ultimate strength (Ünal et al., 2016). Toughness was reported as energy to fracture (the area under stress-strain curve). The elastic modulus was determined by the slope of the regression performed to the linear portion of stress-strain curve. The flexure formula was used to calculate the ultimate strength (Ünal et al., 2016, Unal and Akkus, 2015b).

2.4. Porosity measurement

Following the biomechanical testing, one of the fractured bone segments was immediately sectioned in a plane perpendicular to the longer axes of samples (7 × 3 × 1.1 mm) and ~ 1 mm away from the fracture surface. The porosity of bone segments was estimated by the ratio between the volume of liquid and total volume as described previously (Anovitz and Cole, 2015; Gao and Sevostianov, 2016). The volume of liquid was calculated by the ratio between the difference of wet mass and dry mass of specimens, and the density (ρ) of water. The porosity P (%) is:

$$P(\%) = V_W/V_T \times 100 = ((M_W - M_d)/\rho)((H \times L \times W))x100 \quad (1)$$

where Vw is the volume of unbound water and Vt is the total volume of bone sample, M_w is the wet mass and M_d is the oven-dried mass of bone sample, and ρ is density of water. H: height, L: length and W: width of the samples.

2.5. Sequential dehydration and Raman spectroscopy

Bone samples were probed by sequential heat treatment process (Unal et al., 2014; Unal and Akkus, 2015b) after biomechanical tests to tease out the contributions of unbound water, bound water, organic matrix and mineral to the electrical properties of cortical bone. First, bone samples were heated in a muffle furnace in air at a rate of ~ 15 °C per minute, held at 40 °C for 48 h to remove predominantly unbound water and possibly loosely bound water with minimal effect on tightly bound water (Unal et al., 2014). Next, bone samples were kept at 200 °C for ~ 30 min to evaporate tightly bound water (Marzec et al., 1996; Lozanoo et al., 2003; Mkukuma et al., 2005). Thereafter, bone samples were kept at 1000 °C for ~60 min and then cooled at the room