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Spatial distribution and remodeling of elastic modulus of bone in micro-regime as prediction of early stage osteoporosis

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ABSTRACT

We assessed the local distribution of bone mechanical properties on a micro-nano-scale and its correlation to strain distribution. Left tibia samples were obtained from 5-month old female Sprague Dawley rats, including baseline control ($n=9$) and hindlimb suspended ($n=9$) groups. Elastic modulus was measured by nanoindentation at the dedicated locations. Three additional tibias from control rats were loaded axially to measure bone strain, with 6–10 N at 1 Hz on a Bose machine for strain measurements. In the control group, the difference of the elastic modulus between periosteum and endosteum was much higher at the anterior and posterior regions (2.6 GPa), where higher strain differences were observed (45 $\mu\varepsilon$). Minimal elastic modulus difference between periosteum and endosteum was observed at the medial region (0.2 GPa), where neutral axis of the strain distribution was oriented with lower strain difference (5 $\mu\varepsilon$). In the disuse group, however, the elastic modulus differences in the anterior posterior regions reduced to 1.2 GPa from 2.6 GPa in the control group, and increased in the medial region to 2.7 GPa from 0.2 GPa. It is suggested that the remodeling rate in a region of bone is possibly influenced by the strain gradient from periosteum to endosteum. Such pattern of moduli gradients was compromised in disuse osteopenia, suggesting that the remodeling in distribution of micro-nano-elastic moduli among different regions may serve as a predictor for early stage of osteoporosis.

1. Introduction

Bones, the load bearing organ, bear the weight of the human body and also of the loads applied on it during locomotion (Pekka, 2007). As a living tissue, the skeleton has the capacity to model and remodel itself (Burger and Klein-Nulend, 1999). It is demonstrated that loading increases the mechanical properties of bone, while the absence of loading causes loss of its mechanical strength (Burger and Klein-Nulend, 1999; Huang and Ogawa, 2013; Qin et al., 1998; Wolf, 1995). Bones of astronauts on long-term space flights, patients of prolonged bed rest or of paralysis and traumatic brain injury experience long states of functional disuse (Lau and Guo, 2011). Functional disuse under such conditions causes loss of overall bone strength, decrease in mineralization and a structural deterioration of bone architecture. It moreover leads to bone fragility and increased tendency of bone to fracture (Faibish et al., 2006). It is proposed that shear forces induced by bone fluid, due to the loading conditions, on the osteocytes leads to more bone formation (Burger and Klein-Nulend, 1999; Knothe Tate et al., 1998; Qin et al., 2010; Reich et al., 1990; Rubin et al., 1997; Weinbaum et al., 1994). The racquet holding arm of a tennis player being stronger than the contralateral is an example of this phenomenon (Taylor et al., 2009).

Work conducted in the past showed correlation between bone tissue strain and mechanical properties of bone (Carter, 1982; Frost, 1983; Lanyon, 1987). On a micro-scale also remodeling has shown to be dependent upon the loading the bone experiences. Meulen in 2006 showed that for cancellous bone in mice, the bone volume fraction, direct trabecular thickness and mineral apposition rate were higher for limbs exposed to periods of in vivo cyclical loading, when compared to their respective contralateral limbs which served as controls (van der Meulen et al., 2006). For cortical bone, it has also been shown that the regional microstructural properties are significantly influenced by the mechanical strains experienced. For example, using an avian ulnae model, there were significant differences between different bone regions in the cross sections, in which mean laminarity indices (*LI*) were varied between sub adult (40.0% ± 10.7%) and adult (50.9% ± 10.4%) bones, and their related bone strain (Skedros and Hunt, 2004). Regional variation in collagen orientation and volume, as well as in mineralization in cortical bone, were found to be dependent upon the regional variation in bone strain. Skedros et al. (2013) showed that both osteon size and the collagen

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Fig. 1. Indents (black dots) distribution in the cross section of bone at the anterior, posterior and medial regions of a rat tibia.

organization in the osteons are strongly influenced by the local strain magnitude (Skedros et al., 2013). Regional collagen fiber orientation and mineralization affect the regional bone mechanical properties. Manjubala et al. (2009) showed that with an increase in mineralization in embedded callus, there was also an increase in indentation modulus from 6 GPa at early stages to an average of 14 GPa at the later stages of mineralization (Manjubala et al., 2009). The possible effects of collagen fibers orientation on the mechanical properties of bones were shown to use bovine and equine samples (Martin and Boardman, 1993; Martin and Ishida, 1989; Skedros et al., 2006). Martin and Ishida in their study determined the importance of collagen fibers orientation on tensile strength of cortical bone, whereas the Martin and Boardman's (1993) study showed that the fibers orientation was the most significant predictor, using stepwise multiple regression, of cortical bone bending in bovine tibias. Skedros et al. (2006) showed that in strain-mode-specific testing fiber orientation had a significant effect on post-yield behavior and total energy in the testing.

Previous work has been conducted to measure micro-mechanical properties of the bone (Angker et al., 2005; Fan et al., 2002; Hengsberger et al., 2002; Hoffler et al., 2005). Donnelly et al. (2010) showed that bone nano-mechanical modulus was higher in the periosteal region and correlated it with the bone tissue composition using Raman Spectroscopy. But a complete picture of how these properties spatially differ in various regions of a transverse bone section, from the periosteal to the endosteal side of bone and its dependence on the local mechanical strain values have not been investigated. Hence the first objective of this study was to assess the effect of regional strain distribution on spatial distribution of micro-mechanical modulus in a control bone. Our hypothesis was that the micro-mechanical modulus distribution should be dependent upon the micro-strains distribution in the different regions of the bone. Areas of higher micro-strains should also have higher micro-mechanical moduli as compared to areas of bone, which experience lower micro-stains.

The second objective of the study was to assess remodeling in the spatial distribution of micro-mechanical moduli, due to functional disuse conditions. The hypothesis was that due to functional disuse conditions, the regional micro-mechanical moduli distribution would be significantly different from the control group for each investigated region.

To access the elastic moduli distribution in different regions, nanoindentation was used. Nanoindentation allows measurement of mechanical properties such as elastic moduli and hardness at a micro- and nano-scale resolution level (Angker et al., 2005; Ebenstein and Pruitt, 2004; Fan et al., 2002). Nanoindentation has been recently used in a lot of studies to measure bone mechanical properties (Pathak et al., 2011), lamellar level bone mechanical properties and sub-micron mechanical properties (Pathak et al., 2012; Silva et al., 2004).

2. Materials and methods

Bone samples from left tibia were obtained from 5-month old virgin female Sprague Dawley rats, including 1) baseline control ($n=9$), and 2) hindlimb suspended (HLS) (4 weeks, $n=9$) (Hu et al., 2012). The animal study was previously approved by the Stony Brook University IACUC.

2.1. Sample preparation

After a cross-sectional cut and clearing of the bone marrow using a water jet, 2 mm in longitudinal length segments at the midshaft were cut in the transverse plane using a diamond wheel saw (South Bay technology Inc., MODEL 650, Clemente, California, USA) under constant water cooling.

2.2. Embedding and polishing

The bone samples were stored in ethanol of subsequent higher concentrations of 70%, 80%, 90% and then 100% for 2 days each for complete drying. Subsequently, they were embedded in epoxy resin and let cure for a day. To expose the surface of the bone, the embedded samples were first polished with silicon carbide papers (Buehler-Carbimet, Illinois, USA) of grit numbers 320, 600, 1200, 2400, 4000 mounted on a Buehler grinder Power-pro 3000™ (Illinois, USA) in the respective sequential order. Finally the samples were polished by Polycrystalline Diamond Suspensions (Buehler MetaDi™ Supreme) of roughness 3 μm, 1 μm, 0.25 μm and finally by 0.05 μm in the given descending order.

2.3. Nanoindentation

The distribution of bone mechanical properties such as elastic modulus and hardness were measured on a micro-scale in the z or axial directions by Nanoindentation (Hysitron Triboindenter TI-950, Minneapolis, Minnesota) at precise