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# Microdamage propagation in trabecular bone due to changes in loading mode

Xiang Wang, Glen L. Niebur\*

Tissue Mechanics Laboratory, Department of Aerospace and Mechanical Engineering, University of Notre Dame, Notre Dame, IN 46556, USA

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#### Abstract

Microdamage induced by falls or other abnormal loads that cause shear stress in trabecular bone could impair the mechanical properties of the proximal femur or spine. Existing microdamage may also increase the initiation and propagation of further microdamage during subsequent normal, on-axis, loading conditions, resulting in atraumatic or "spontaneous" fractures. Microdamage formation due to shear and compressive strains was studied in 14 on-axis cylindrical bovine tibial trabecular bone specimens. Microdamage was induced by a torsional overload followed by an on-axis compressive overload and quantified microscopically. Fluorescent agents were used to label microdamage and differentiate damage due to the two loading modes. Both the microcrack density and diffuse damage area caused by the torsional overload increased with increasing shear strain from the center to the edge of the specimen. However, the mean microcrack length was uniform across the specimen, suggesting that microcrack length is limited by microstructural features. The mean density of microcracks caused by compressive overloading was slightly higher near the center of the specimen, and the diffuse damage area was uniform across the specimen. Over 20% of the microcracks formed in the initial torsional overloading propagated during compression. Moreover the propagating microcracks were, on average, longer than microcracks formed by a single overload. As such, changes in loading mode can cause propagation of microcracks beyond the microstructural barriers that normally limit the length. Damage induced by in vivo off-axis loads such as falls may similarly propagate during subsequent normal loading, which could affect both remodeling activity and fracture susceptibility.

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#### 1. Introduction

More than 250,000 hip fractures were reported in 1996, and over 90% were associated with falls (Fuller, 2000). Falls may not lead to an immediate fracture (Fuller, 2000; Gregg et al., 2000), but may induce microdamage in both the cortical and trabecular bone in the proximal femur. Microdamage has a detrimental effect on the mechanical properties of bone, decreasing both the elastic modulus (Keaveny et al., 1994; Moore and Gibson, 2002) and the work to failure (Keaveny

et al., 1994) in trabecular bone specimens and in whole bones (Hoshaw et al., 1997). Changes in trabecular bone stiffness in even a small region can cause large differences in whole bone strength (Kopperdahl et al., 2000; Oden et al., 1999).

The stress states present in the trabecular bone of the proximal femur during a fall differ from those during activities of daily living. During normal loading, the principal stresses are predominantly aligned with the principal trabecular bone orientation, i.e., the stresses are "on-axis" (Lotz et al., 1995). During falls, the principal stresses are "off-axis" (Ford et al., 1996; Keyak et al., 2001; Lotz and Hayes, 1990). Moreover, existing microcracks propagate easily under changes in

<sup>\*</sup>Corresponding author. Tel.: +5746313327; fax: +5746318341. *E-mail address*: gniebur@nd.edu (G.L. Niebur).

the applied stress state (Wang and Niebur, 2004). As such, microdamage formed during a fall could contribute to bone fragility during future falls or to "spontaneous" fractures (Parker and Twemlow, 1997) if sufficient numbers of microcracks were to propagate during subsequent normal loading.

Microdamage formation in trabecular bone during on-axis compressive overloading (Moore and Gibson, 2002) and fatigue (Moore and Gibson, 2003) occurs when the apparent strain exceeds the yield strain. Microdamage development caused by shear strain has not been studied, but the yield strain is independent of modulus and density (Ford and Keaveny, 1996), as it is in compressive loading (Kopperdahl and Keaveny, 1998). However, in specimens that had accumulated microdamage from compressive loading, microcracks formed and propagated at strains below the shear yield strain during subsequent torsion (Wang and Niebur, 2004). This suggests that either pre-existing damage decreases the strain threshold for further damage, or there are fundamental differences in microdamage development in shear vs. compressive loading. Characterization of the microdamage development due to shear loading in undamaged trabecular bone and its propagation during subsequent compressive loading is needed to fully understand the causes and consequences of microdamage in trabecular bone during off-axis loading in trabecular bone.

The goal of this study was to investigate the effect of a sequence of shear and compressive apparent level strains on the mechanical property changes and microdamage formation in trabecular bone. Specifically, the aims were to: (1) determine the relative reduction in the axial and torsional stiffnesses of trabecular bone due to an on-axis torsional overloading followed by an on-axis compressive overloading; (2) quantify the microdamage development due to shear strains in trabecular bone; (3) quantify the development of new microdamage and propagation of pre-existing microdamage during a subsequent compressive load; and (4) compare the behavior to previous experiments where axial compression preceded torsional overloading.

#### 2. Methods

#### 2.1. Specimen preparation

Fourteen on-axis cylindrical specimens were prepared from eight bovine proximal tibiae. One or two specimens were obtained from the medial and lateral metaphysies of each tibia. The axis of the cylindrical specimen was aligned with the principal mechanical axis of the trabecular bone using micro-CT imaging (Wang et al., 2004). Briefly, the proximal tibia was sectioned into parallelepipeds that were scanned at 60 µm resolution

in a micro-CT scanner ( $\mu$ CT-80, Scanco Medical AG, Bassersdorf, Switzerland) while saturated in a solution of 40% ethanol and 60% buffered saline. The principal mechanical axes of the specimen were calculated using high-resolution finite element analysis (Van Rietbergen et al., 1996). Cylindrical specimens were then cored along the principal orientation using a diamond coring drill (Starlite Industries, Bryn Mawr, PA). On average, the principal fabric direction was aligned within 9.5° of the specimen axis. The mean diameter and length of the specimens were  $8.23\pm0.05\,\text{mm}$  (mean  $\pm$  std. dev.) and  $23.0\pm0.8\,\text{mm}$ , respectively.

The marrow was removed from the specimens using a submerged water jet in order to facilitate staining of microdamage with fluorescent dyes. Removing the marrow should not affect the mechanical properties at the strain rate used here (Carter and Hayes, 1977). The prepared specimens were wrapped in gauze saturated with saline and stored at  $-20\,^{\circ}\text{C}$  in airtight containers except during staining and mechanical testing.

#### 2.2. Damage staining

Microdamage was stained using a sequence of fluorochromes that allowed differentiation of damage due to each loading mode (O'Brien et al., 2003). Following specimen preparation, but prior to mechanical testing, the cylindrical specimens were stained in a solution of 0.5 mM alizarin complexone (ICN Biomedicals Inc., Aurora, OH) to label microdamage incurred in vivo or during specimen preparation. Damage due to torsional overloading was labeled by soaking in a solution of 0.5 mM xylenol orange (Acros Organics, NJ). After compressive testing, microdamage was labeled by soaking in a solution of 0.5 mM calcein (ICN Biomedicals Inc., Aurora, OH). Specimens were stained for 2 h in each step, followed by rinsing in deionized water.

#### 2.3. Mechanical testing

The specimens were loaded under strain control on an Instron model 8821s biaxial servo-hydraulic load frame (Instron Corp. Canton, MA). Specimens were embedded in brass endcaps (Keaveny et al., 1997), which were clamped into the load frame to limit end effects (Fig. 1). Strains were measured by a biaxial extensometer (model 3550, Epsilon Technology Corp., Jackson, WY) attached to the endcaps, with the effective gage length taken as the exposed plus half the embedded length of the specimen for axial loading (Keaveny et al., 1997) and the exposed length for torsional loading (Fenech and Keaveny, 1999). All tests were performed at room temperature with the exposed portion of the specimen wrapped in gauze saturated with buffered saline solution to maintain hydration.

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