In-vitro treatment with Raloxifene Affects Damage Tolerance of Human Cortical Bone

Mary Arnhart[1], Laura Pyrak-Nolte[2], John Howarter[3], Rachel Surowiec[4], Matthew Allen[5], Joseph M. Wallace[4], Thomas Siegmund[1]

[1]Department of Mechanical Engineering, Purdue University, W. Lafayette, IN 47907 [2]Department of Physics & Astronomy, Purdue University, W. Lafayette, IN 47907 [3]Department of Materials Engineering/Env. and Ecological Engineering, Purdue University, W. Lafayette, IN 47907 [4]Department of Biomedical Engineering, IUPUI, Indianapolis, IN 46202 [5]Department of Anatomy, Cell Biology & Physiology, IUPUI, Indianapolis, IN 46202
marnhart@purdue.edu

Disclosures: No disclosures

INTRODUCTION: There is an ongoing need for new approaches to reduce fracture risk in the skeletal system and to improve the quality of life within the aging population. There is prior evidence that in-vitro treatment of human cortical bone with the compound Raloxifene, RAL, can increase bone toughness but not strength [1]. The observed increase in toughness was attributed to increased bone hydration in RAL-treated bone. Experiments in [1] were, however, performed at specimen dimensions (thickness < 1.5 mm) substantially smaller than the maximum cortical wall thickness of a typical human femur [2]. Given that there is a fundamental size effect on strength and toughness [4,5], the question remains on if/how RAL treatment affects the biomechanical behavior of bone for specimen dimensions approximately equal to the cortical thickness. While strength and toughness were considered as properties in [1], here, the additional consideration of tissue damage [4] is introduced. We hypothesize that damage mechanics can be used to characterize biochemical treatment in cortical bone in response to a mechanical load.

MATERIALS and METHODS: Cortical bone beams were extracted from the femur of a 75-year-old male donor. Bone beams obtained through the Indiana University Donor program were machined to nominal dimensions of 4.0 x 4.0 x 24 mm^3. All specimens underwent micro-computed tomography (μCT, 16.85 μm) imaging. Cortical porosity, or voids within the cortex, was determined to be in the range between 1.5-16% of the beam volume. For the results documented in this abstract, a total of six cortical bone beams were used; additional experiments are ongoing. Specimens were grouped into control (VEH) and treatment (RAL) groups. RAL group specimens were exposed to a 2 μM RAL solution in phosphate buffered saline (PBS), while VEH group specimens were exposed to PBS only. The treatment extended for 14 days at 37ºC with a solution change every two days. After treatment, specimens were stored frozen in PBS. Prior to the mechanical loading experiment, the side of the beams intended for tension was polished by a sequence of MicroCut Discs 1200-grit, 4000-grit, and a 0.05 μm diamond suspension on polishing cloth to eliminate surface defects. Specimens were subjected to a three-point bend loading protocol (span L=16 mm) under a constant applied displacement rate such that the strain rate is of the order of 0.001 [1/s]. During loading, specimens were submerged PBS. Optical observations of the specimen and its deformation, as well as the detection of stress whitening, were enabled by an optically transparent window in the bath container. A CCD camera was used to record the specimen deformation. Images were processed by a Weka-segmentation approach (ImageJ) to determine the beam deflection d. Flexural stresses were determined from the actual beam cross section (depth w, height d), the test span L and the measured force F as $\sigma = (FLh)/(8I)$, with $I = (wh^3)/12$. Strength σ_{max} is obtained from the maximum force F_{max}. Strain is $\varepsilon = (6hd)/L^2$. Toughness T is obtained as the area under the stress-strain curve. A scanning electron microscope (SEM) in backscatter mode was utilized to observe the fracture surface. The maximum damage $\widehat{D}$ sustained by a specimen is calculated as $\widehat{D} = 1 - \sigma_{max}/\sigma_u$, where σ_u is obtained by the extrapolation of the initial, undamaged elastic response up to the deformation at failure (Fig. 1).

RESULTS: Bone beams exhibited some degree of nonlinear deformation past damage initiation, followed by abrupt failure (Fig. 1 is a representative stress-strain curve). Stress whitening was observed in all specimens as a visual indicator of nonlinearity in deformation (Fig. 1 - insert). Table 1 summarizes the data. Strength σ_{max} was found to not distinguish well between treatment groups ($p=0.35$) even if corrected for porosity ($p=0.35$). Toughness provides more notable separation between groups with specimens of the RAL group tending to have higher toughness than those of the VEH group ($p=0.20$). RAL group specimens were found to be more damage tolerant than the VEH group specimens ($p=0.13$), with D larger for RAL than VEH. On the fracture surface, Fig. 2, the initially stable damage evolution is visible as a domain (extension up to 1000 μm from the tensile face) with notable osteon pull-out, while the subsequent complete fracture occurs abruptly and results in a flat fracture surface in both VEH and RAL specimens.

DISCUSSION: This study demonstrates the application of a damage mechanics concept to the investigation of the biomechanical integrity of cortical bone under mechanical loading. Preliminary results indicate that the damage mechanics measure can distinguish between treatment and control group. RAL treatment was found to increase bone toughness and damage tolerance. The findings on a toughness increase with RAL treatment are consistent with the those in [2] on smaller size specimens. The findings on a damage tolerance with RAL treatment increase are consistent with in vivo treatment studies in [6]. Experiments expanding the sample number are ongoing.

SIGNIFICANCE/CLINICAL RELEVANCE: This work demonstrates the application of damage mechanics concepts in bone biomechanics as an alternative to measures of strength and toughness commonly used. Preliminary data indicate that in-vitro RAL treatment favorably alters the damage tolerance of cortical human bone if the specimen size approaches the cortical wall thickness.

REFERENCES: REFERENCES: [1] Gallant et al. Bone. 2014. [2] Treece at al. Med Image Anal. 2010. [3] Kim et al., Int J Fract. 2013. [4] Bazant. Int J Solids Struct. 2000. [5] Lemaitre, A course in damage mechanics. 2012. [6] Allen et al. Bone. 2006.
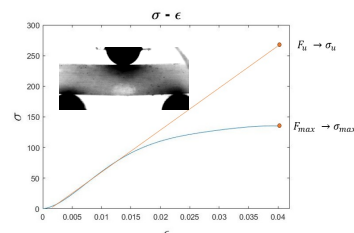
IMAGES AND TABLES:

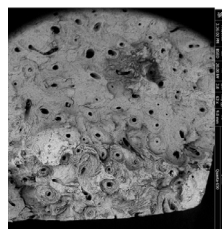*Figure 1: Stress vs. strain curve for one of RAL treated specimens*

Figure 2: Partial view of fracture surface

Table 1: Specimen results: flexural strength, toughness, and damage

Name	σ_{max} (MPa)	Corrected σ_{max} (MPa)	Toughness (MPa)	Damage
VEH 1	184.8211	191.6884	3.0472	0.1319
VEH 6	167.6618	170.0346	3.5146	0.1434
VEH 9	166.1550	172.2469	2.9259	0.1455
RAL 3	164.7762	198.4425	4.1944	0.2298
RAL 8	182.3212	187.9584	3.8651	0.2192
RAL 10	159.1504	163.3253	2.7738	0.1138