Evolution of Tissue Material Properties in a Mechanically Stimulated Bone Defect

INTRODUCTION:
Bone healing is known to be modulated by mechanical factors, but the specific identities and magnitudes of the local (i.e., tissue-level) mechanical stimuli that drive this process have not been established. Finite element (FE) analysis remains the most common method for estimating the distributions of candidate, local mechanical stimuli in a region of a healing bone defect or fracture, and these estimates have often been used to simulate the process of tissue differentiation during skeletal healing in response to mechanical loading. However, these approaches have relied on many assumptions regarding the mechanical properties of the various tissues that are present in the region, and the outcomes can be very sensitive to the assumed mechanical properties [1]. Although the mechanical behavior of many mature skeletal tissues has been well characterized, comparatively little information is available on the mechanical properties of healing tissues. The overall goal of this study was to quantify the changes in tissue elastic properties that occur within a mechanically stimulated bone defect over the course of healing. The specific objectives were: 1) to measure the moduli of the tissues in the defect region at two different timepoints; and 2) to compare the measured changes to those predicted by an established mechano-regulation algorithm [2].

METHODS:
Animal Surgery. A transverse osteotomy was created in the left femoral mid-diaphyses of retired breeder Sprague-Dawley rats and was stabilized with an external fixator according to an established protocol [3]. Samples from two timepoints were used in this study; one sacrificed after a seven-day latency period (n=2) and the other after one week of a bending stimulation regimen that followed the latency period (n=1). The bending stimulation consisted of a cyclic 30° (+15°/-15°) angular displacement applied at 1 Hz for 15 minutes daily for five consecutive days followed by two days of rest. The bending took place in the sagittal plane, about the center of the osteotomy, and was driven by a custom-designed servomotor linkage system. Nanoindentation. Each callus was processed into 200µm-thick sagittal sections according to a previously published protocol [4]. These sections were indented on a Hysitron Triboindenter (Minneapolis, MN) equipped with a 100µm conospherical tip. During indentation, a two-second loading ramp was followed by a five-second hold and a two-second unloading ramp. The maximum load varied from 10 µN to 200 µN with the higher loads used for stiffer tissues. The reduced moduli of the tissues were calculated with the Oliver-Pharr method [5]. These data were used to create a 3-D map of reduced modulus throughout each callus. Histology. Sections were stained with alizarin red and alcian blue. Finite Element Analysis. A FE mesh was created from micro-computed tomography images of one of the end-lateral callus sections. The images were segmented to create 3-D surfaces of the cortex and surrounding callus [6], and these surfaces were used to generate the 3-D mesh (I-A-FEMesh, The University of Iowa, Iowa City, IA). A reduced modulus was calculated for every node from the end-lateral indentation data using linear interpolation where possible and nearest neighbor elsewhere (MATLAB 7.8, The Mathworks, Inc., Natick, MA). A Young’s modulus was then calculated for each element by averaging the reduced moduli of the eight nodes and assuming a Poisson’s ratio of 0.167 [2]. Displacement boundary conditions were applied to simulate +15° and -15° of bending about the center of the osteotomy. Simulation of Tissue Differentiation. The Prendergast mechano-regulation algorithm [2] was used to predict the course of tissue differentiation within the callus over the one-week stimulation regimen. The material properties of the callus tissues were updated according to a rule of mixtures, as previously prescribed [2] with one exception: the Young’s moduli for granulation tissue, cartilage, and immature woven bone were estimated from reduced moduli determined by nanoindentation in this and previous [4] studies.

RESULTS:
The reduced moduli measured via nanoindentation varied from 0.16 MPa to 55 MPa. In the end-lateral calluses, the moduli ranged from 0.17 MPa to 1.21 MPa and tended to be highest and lowest in the central and periosteal regions of the osteotomy gap, respectively. The tissue in some of the periosteal regions was too compliant to test successfully due to the displacement limit of the nanoindenter; tissues in these regions were assigned a reduced modulus of 0.15 MPa in the FE model. The moduli on post-operative day 14 ranged from 0.16 MPa to 55 MPa (Figure 1.B,D). The highest values were also found in the center of the gap at this timepoint, and the largest increases in modulus from day 7 to day 14 were seen in the intracortical regions of the gap.

The mechano-regulation algorithm predicted Young’s moduli from 1.1 MPa to 3 GPa at day 14. Large regions of fibrous tissue were predicted to form in the osteotomy gap, with smaller regions of bone and cartilage in the center of the gap and along the periosteal surface of the cortical fragments (Figure 1.A,B). Histology from this study (not pictured) and previous work [3] showed similar patterns of bone and cartilage formation along the periosteal surface of the cortex, and some fibrous tissue formation in the gap. The histology also showed flat bony plates at the edges of the gap, partially capping the medullary canals, which is also consistent with the predictions. However, the sphere of bone that was predicted to form in the gap was not observed in any histological sections.

DISCUSSION:
Nanoindentation revealed a wide range of reduced moduli in the calluses at both timepoints. Many prior FE studies of mechano-regulation in bone healing have assumed uniform material properties within the osteotomy gap, but the present results show more than a seven-fold difference between the smallest and largest moduli. The sensitivity of predictions of local mechanical stimuli and of patterns of tissue differentiation to the relative values of material properties of the tissue [1] makes this a significant discovery.

The mechano-regulation algorithm correctly predicted some trends in the distributions of tissues and elastic moduli after one week of bending stimulation. However, the algorithm predicted some tissue formations that were not observed histologically and grossly over-predicted the Young’s moduli of the tissues in the defect region. In future simulations, the rule of mixtures should be modified to account for the relatively slow pace of mineralization such that the predicted evolution of material properties more accurately reflects the observed changes in properties during healing.