INTRODUCTION: The cellular and molecular processes involved in the course of skeletal tissue healing are known to be sensitive to mechanical factors [1, 2]. However, specific associations between these processes and candidate mechanical stimuli, such as shear and tensile strains [3, 4], have not yet been established, due in part to the paucity of experimental measurements of these stimuli in the region of a healing skeletal defect. Initial data on local (i.e., tissue-level) strains in mechanically stimulated bone defects have indicated that the distributions of shear and tensile strain are associated with patterns of bone and cartilage formation [5]. The goal of this study was to probe the potential link between tissue differentiation during bone healing and shear and tensile strains by examining a broad set of tissue fates in an in vivo model of mechanically generated pseudarthrosis. The objectives were: 1) to define the strain distributions that occur in the callus during the mechanical stimulation; 2) to determine the patterns of formation of bone, cartilage, fibrocartilage and fibrous tissue within the callus; and 3) to correlate the patterns of tissue type and strain.

METHODS: In vivo model. All animal care and experimental protocols were followed in accordance with NIH guidelines and were approved by our institution’s Animal Care and Use Committee. Retired, male breeder Sprague-Dawley rats (n=12) underwent production of a mid-diaphyseal, transverse, ~2mm-wide femoral osteotomy, stabilized with a custom-designed external fixator. Similar to a previously established model [2], mechanical stimulation of the osteotomy site, consisting of a cyclic bending motion (±15° at 1 Hz), was applied in the sagittal plane for 15 minutes daily, beginning on post-operative day (POD) 7, for five consecutive days followed by two days of rest each week. Femora with fixators attached were harvested at POD 7, 14, and 21. Strain Measurement. The mid-sagittal plane of the callus was exposed by removing the medial half of the callus. The exposed plane was speckled with black enamel paint, and the specimen was mounted to the same environment that influence bone healing is important for improving clinical outcomes of bone injuries and for identifying mechanisms of skeletal mechanotransduction in vivo. The results of this study indicate that the patterns of formation of bone, cartilage, fibrocartilage and fibrous tissues in a mechanically stimulated bone defect are strongly associated with the distributions of shear strain.

ACKNOWLEDGEMENTS: NIH AR053353 (EFM)


RESULTS: Similar spatial distributions of strains were observed across specimens and time-points, though peak values differed for the three strain types. The highest strains were concentrated at the periphery of the osteotomy gap and quickly decreased in the proximal, distal and medial directions (Figure 1A). Tissues within and surrounding the osteotomy gap at POD 7 consisted primarily of granulation and loose connective tissues. At POD 14, cartilage was observed at the gap periphery in a kidney-bean shape with underlying trabeculated woven bone along the periostemeum. At POD 21, abundant amounts of cartilage were found within the osteotomy gap and at the gap periphery, with endochondral ossification evident at the proximal and distal margins of the cartilage and with fibrous tissue located between the cartilage layers (Figure 1B).

Significant associations were found between the strain fields and the patterns of tissue formation for all specimens at all time-points (Figure 2). The occurrence of cartilage was positively associated with the magnitudes of all three types of strain, with the strongest associations observed for $E_{\text{oct}}$. For example, for every increase of 0.01 mm/mm of $E_{\text{oct}}$ at POD 7, cartilage was 349 times more likely to be found (i.e., a 349-fold change in odds ratio) in that region of the callus at POD 14 ($p<0.001$). For the same increase in $E_{\text{oct}}$ at POD 14, cartilage was 25.1 times more likely to be found in that region of the callus at POD 21.