INTRODUCTION  The rate and eventual success of fracture healing is dependent on many factors, including age, hormonal milieu, degree of trauma and particularly mechanical stability. Several investigators have demonstrated a beneficial effect of controlled cyclic mechanical strains on healing time in human patients. Although this therapy has demonstrated clinical potential, the precise relationship between the mechanical environment and bone formation dynamics is largely unknown. Previous work in our laboratory has developed, validated and characterized a rat model of fracture healing in which mechanical stimulation in bending is used to create a tensile to compressive mechanical environment in a healing fracture gap. This model can therefore be effectively used to study the influence of multiple strain patterns on bone healing in an individual specimen. This study addressed the specific hypothesis that bone formation occurs preferentially under certain mechanical conditions, and is part of a global research program investigating the relationship between local mechanical factors and biological response during fracture healing, as well as potential mechanisms involved in altering cellular processes during endochondral ossification.

MATERIALS AND METHODS  A total of 18 male, Sprague-Dawley rats underwent bilateral surgical placement of 4 percutaneous pins in the femoral diaphyses. A custom hinged external fixator stabilized by a locking plate was then attached to the pins prior to creation of a 2mm transverse ostetomy by an oscillating saw under constant irrigation. After one week post-operative, rats were anesthetized, the locking plate was removed from the fixators, and fractures in one leg were mechanically stimulated in bending in the AP direction using a custom computer-controlled stimulation device. The contra-lateral leg served as the unstimulated control. Stimulation occurred at 0.5Hz for 17 min. three times each week during weeks 2 and 3 post-operative, providing a total of 510 load cycles during each loading session. Rats were sacrificed after 1 (n=3), 2 (n=5), 3 (n=4), 4 (n=3) or 6 (n=3) weeks post-operative, and femurs were harvested and snap frozen in n-methyl butane pre-chilled in a dry ice/ethanol slurry. All pins and fixators were removed, and femurs were stored at −80°C until further analysis.

All femora were then scanned on a micro-computed tomography system, and 3-dimensional images were reconstructed at a mesh size of 50µm x 50µm x 50µm. Images were thresholded, and an analysis region was defined by the external borders of the fracture callus and the intact bone that defined the cut bone ends. The volume fraction of new bone in the gap was determined as the ratio of the number of mineralized voxels to the total number of voxels in the analysis region. Statistical analysis was performed on 2, 3, 4, and 6 week data using two-way ANOVA with treatment and time as factors and p<0.05 considered statistically significant.

The percent contiguity was defined as the ratio of the number of mineralized voxels to the total number of voxels in each column between the bone ends. The analysis region was further divided into anterior, posterior, medial and lateral quadrants using diagonals from opposite corners. The number of values in each quadrant that exceeded a given level of contiguity (i.e. 25%, 50%, 75%, 95% or 100%) were determined, and normalized to the total number of values in that quadrant. Statistical analysis was performed on 2, 3, 4, and 6 week data using multiple ANOVA with time, treatment and region as factors, and p<0.05 considered statistically significant. When significant differences were found, Tukey’s post-hoc was used for comparisons between groups.

RESULTS  Bone volume fraction results indicated that BV/TV continually increased over time (p<0.001). Although treatment was not found to be a significant factor (p=0.10), a trend toward decreased BV/TV in the mechanically stimulated leg at 3 weeks post-operative, or after 2 weeks of mechanical stimulation, was apparent (Figure 1). By 4 weeks post-operative, or after one week without mechanical stimulation, this difference was no longer evident. Contiguity analysis was performed as a measure of the extent of bridging across the fracture gap, which is related to the mechanical integrity of the healing fracture. The fraction of regions in each quadrant which attained a given level of contiguity increased significantly over time, which is consistent with BV/TV results. In the anterior quadrant, which was in a primarily tensile strain environment, the fraction of regions that were >25% contiguous was decreased in the mechanically stimulated leg after the stimulation period (Figure 2). By 6 weeks, however, this fraction exhibited a trend towards a slight increase in the stimulated leg compared to the unstimulated leg. In contrast, the fraction of regions that were >25% contiguous in the posterior quadrant, which was in a primarily compressive strain environment, was decreased in the stimulated leg by 3 weeks, and had not achieved the level of the unstimulated leg by 6 weeks (Figure 3). Statistical analyses revealed that treatment was a significant factor only when analyzing the fraction of regions that were >25% and >50% contiguous, although similar trends were evident at greater contiguity levels.

DISCUSSION AND CONCLUSIONS  Results demonstrated a trend towards decreased BV/TV and bone contiguity at 3 weeks, suggesting that imposed mechanical strains suppress mineralized tissue formation during the stimulation period. We believe that the mechanical stimulation may be stimulating a greater volume of chondrocytes and may be altering rates of chondrocyte differentiation. This discrepancy in BV/TV between stimulated and unstimulated fractures appeared to be resolved by 6 weeks post-operative, while contiguity data suggests that the distribution of newly formed bone may be influenced by strain character. These data suggest that a tensile mechanical environment (in the anterior quadrant) appeared to promote eventual bone bridging across the fracture gap, while compression did not demonstrate a similar effect. Although the current sample sizes may have prevented data from achieving statistical significance, these trends may suggest an interesting relationship between bone formation and the mechanical strain history. Continuing studies will investigate these effects at longer healing times, the effect of stimulation on non-mineralized tissue formation, particularly chondrogenesis, and will aim to determine the precise strain environment and its relationship with potential mechanisms of altered bone formation.


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