EVALUATION OF HAVERSIAN BONE FRACTURE HEALING IN SIMULATED MICROGRAVITY

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INTRODUCTION AND MOTIVATION: The inherent reduction in mechanical loading associated with microgravity has been shown to result in dramatic decreases in the bone mineral density (BMD) and mechanical strength of skeletal tissue. Importantly, there is a concomitant increase in fracture risk during long-duration spaceflight missions. Rodent studies have highlighted distinct differences in animals that heal in gravitational environments versus those that heal during spaceflight[1]. While these data have demonstrated that microgravity has a deleterious effect on osteogenic healing, the direct translation of these results to human skeletal repair remains problematic due to the substantial differences between rodent and human bone (most notably the lack of Haversian systems in rodent bone)[2,3]. Thus, the objective of this study was to investigate the effects of microgravity loading on long-bone fracture healing in a previously-developed Haversian bone model of simulated microgravity[4].

MATERIALS AND METHODS: A trans-biarticular hybrid fixator was applied to the right hindlimb of five skeletally mature sheep for 21 days (Microgravity Group, n=5) in order to mechanically unload the metatarsal bone. After 21 days of simulated microgravity, a 3.0 mm transverse mid-diaphyseal ostectomy was performed and stabilized with an orthopaedic locking plate instrumented with a rosette strain gage. An additional Earth gravity (Control, n=5) group was included in which an identical 3.0mm ostectomy was created, plated, and casted, allowing full loading to be transmitted through the metatarsal bone. Both groups were euthanized after a 28-day healing period. *In vivo* fracture healing was assessed by evaluating the strain transmitted through the fracture fixation plate over the course of the healing period. DEXA BMD measurements were performed on the treated metatarsi at the time of the external fixation surgery, ostectomy/bone fixation surgery, and every other subsequent week until sacrifice. Post-sacrifice, mechanical competency was evaluated via four-point bending tests, and trabecular microarchitecture was quantified with micro-computed tomography (μCT). Bone formation parameters and cellular activity were quantified via static and dynamic histomorphometry. Statistical comparisons between groups were performed via a Student's t-test or one-way repeated measures ANOVA (for DEXA analysis) with the Student-Newman-Keuls *post hoc* test (α=0.05).

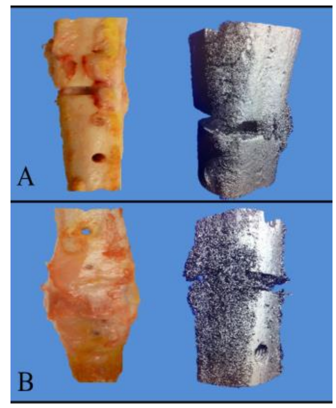

Figure 1: Representative (left) gross necropsy images of the mid-diaphyseal fracture callus and (right) respective μCT three-dimensional reconstructions demonstrated reduced mineralization and callus formation for the (A) Microgravity Group as compared to the (B) Control Group.

RESULTS: *In vivo* strain measurements demonstrated significantly higher orthopaedic plate strains in the Microgravity Group as compared to the Control Group following the 28-day healing period due to inhibited healing in the microgravity environment. DEXA BMD in the treated metatarsus of the Microgravity Group decreased 17.6% ($p<0.01$) at the time of the ostectomy/bone fixation surgery. BMD of the Microgravity Group decreased an additional 5.4% during the 28-day healing period. Four-point bending stiffness of the Microgravity Group was 4.4 times lower than that of the Control Group. Additionally, μCT and histomorphometry demonstrated reduced bone volume and formation in the Microgravity Group as compared to the Control Group (Figure 1, Table 1).

CONCLUSIONS

The data reported herein provide strong evidence that the mechanical loading environment dramatically affects the fracture healing cascade and resultant mineralized tissue mechanical competency. Given the results reported herein, it is imperative to study and develop techniques and technologies that counteract the deleterious effects of microgravity unloading on mineralized tissue healing.

Experimental Parameter	Microgravity	Control
4-Point Bending Stiffness	48.95 (47.81)	213.25 (36.20)
Periosteal Callus Bone Volume [mm^3]	71.27 (23.26)	637.14 (177.50)
Periosteal Callus Bone Area [mm^2]	2.05 (1.56)	19.29 (8.31)
Mineralizing Surface [%]	0.42 (0.08)	0.53 (0.02)
Mineral Apposition Rate [μm/day]	2.78 (0.65)	4.47 (0.33)
Bone Formation Rate [μm/day]	1.17 (0.41)	2.39 (0.28)
Periosteal Osteoblast Number [mm$^{-1]}$	14.37 (2.01)	26.89 (5.68)
Periosteal Osteoclast Number [mm^{-1}]	0.37 (0.32)	0.01 (0.05)
Endosteal Osteoblast Number [mm^{-1}]	11.66 (2.44)	25.83 (7.39)

Table 1: Mean (standard deviation) mechanical, histological, and micro-CT parameters for the Microgravity and Control Groups. These data represent statistically significant differences between groups ($p < 0.05$) for all parameters.

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