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Partial gravity unloading inhibits bone healing responses in a large animal model

Benjamin C. Gadomski [a], Kirk C. McGilvray [a], Jeremiah T. Easley [b], Ross H. Palmer [b], Brandon G. Santoni [c], Christian M. Puttlitz [a,*]

[a] Orthopaedic Research Laboratory, Department of Mechanical Engineering and School of Biomedical Engineering, Colorado State University, 1374 Campus Delivery, Fort Collins, CO, USA
[b] Surgical Research Laboratory, Department of Clinical Sciences, Colorado State University, Fort Collins, CO, USA
[c] Phillip Speigel Orthopaedic Research Laboratory, Foundation for Orthopedic Research and Education, Tampa, FL, USA

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ABSTRACT

The reduction in mechanical loading associated with space travel results in dramatic decreases in the bone mineral density (BMD) and mechanical strength of skeletal tissue resulting in increased fracture risk during spaceflight missions. Previous rodent studies have highlighted distinct bone healing differences in animals in gravitational environments versus those during spaceflight. While these data have demonstrated that microgravity has deleterious effects on fracture healing, the direct translation of these results to human skeletal repair remains problematic due to substantial differences between rodent and human bone. Thus, the objective of this study was to investigate the effects of partial gravitational unloading on long-bone fracture healing in a previously-developed large animal Haversian bone model. *In vivo* measurements demonstrated significantly higher orthopedic plate strains (i.e. load burden) in the Partial Unloading (PU) Group as compared to the Full Loading (FL) Group following the 28-day healing period due to inhibited healing in the reduced loading environment. DEXA BMD in the metatarsus of the PU Group decreased 17.6% ($p < 0.01$) at the time of the ostectomy surgery. Four-point bending stiffness of the PU Group was 4.4 times lower than that of the FL Group ($p < 0.01$), while μCT and histomorphometry demonstrated reduced periosteal callus area ($p < 0.05$), mineralizing surface ($p < 0.05$), mineral apposition rate ($p < 0.001$), bone formation rate ($p < 0.001$), and periosteal/endosteal osteoblast numbers ($p < 0.001/p < 0.01$, respectively) as well as increased periosteal osteoclast number ($p < 0.05$). These data provide strong evidence that the mechanical environment dramatically affects the fracture healing cascade, and likely has a negative impact on Haversian system healing during spaceflight.

1. Introduction

It is well known that microgravity and the associated inherent reduction in mechanical loading result in substantial loss of BMD and mechanical strength. These issues are particularly important when considering fracture risk during long-duration spaceflight missions. Specifically, the mean rate of BMD loss (in lower body bones) during spaceflight has been shown to be dramatically higher than experienced on Earth, averaging losses of 1–3% per month (Vico et al., 2000; Lang et al., 2004). Accordingly, simulations of alterations in mineralized tissue properties as a result of microgravity loading have predicted significantly elevated risk of fracture during long-duration (i.e. Mars) missions (Lang, 2006; Nelson et al., 2009).

What is not as well understood is how the mechanical unloading associated with spaceflight affects the fracture healing cascade. Fracture healing is a complex biological process with four distinct phases. The first step of the fracture healing cascade, occurring at the time of fracture, is the formation of a hematoma, preventing further blood accumulation at the fracture site (Kolar et al., 2011). Subsequently a soft callus forms as chondrocytes create new cartilage that bridges the two ends of the disjoined bone, providing initial mechanical competency (i.e. splinting) to the fracture (Einhorn, 1998). Finally osteoblasts replace the new cartilage with woven bone, which in turn is remodeled into a compact secondary osteonal bone structure (Schindeler et al., 2008). It has been postulated that this reparative process represents a recapitulation of development that involves complex mechanical and chemical factors (Ferguson et al., 1999; Ferguson

* Corresponding author. Tel.: +1 970 491 0956.
E-mail address: puttlitz@engr.colostate.edu (C.M. Puttlitz).

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et al., 1998). Further, it has been shown that the mechanical loading environment of the healing callus has a profound effect on the resultant cell differentiation and heterogeneous matrix phenotypes ("mechanobiology") (Choi et al., 2004; Le et al., 2001; Miclau et al., 2007; Thompson et al., 2002). *In vivo* experiments using fracture models have elucidated certain cellular and molecular scale events that are important in the repair process. However, alterations in the local mechanics induced via reduced gravitational loading in the repair process have not been rigorously described in bone tissues that have Haversian systems. Nonetheless it is clear that the mechanical environment, which transcends many length scales (from whole body to the subcellular), plays a key role in the subsequent fracture healing pathway and, ultimately, the nature and quality of the osseous repair. This certainly has significant implications when examining how microgravity, which impacts the relevant mechanical environment on all of these length scales, may affect bone healing.

Limited research has been performed to investigate mineralized tissue healing in microgravity environments (Kirchen et al., 1995; Durnova et al., 1991; Androjna et al., 2012; Kaplansky et al., 1991). Rodent studies have elucidated distinct histological and morphometric differences in animals that heal in gravitational environments versus animals that heal during spaceflight. Using a rat model, Kirchin et al. showed that bone healing was altered during spaceflight, resulting in suppression of chondrogenesis within the periosteal reaction and angiogenesis within the osteotomy gap (1995). Additionally, Durnova et al. reported decreased fracture callus size and consolidation strength resulting from inhibited osteoblast activity in rodents during a 14-day spaceflight experiment (1991). While these data demonstrate that microgravity has a deleterious effect on bone healing, the direct translation of these results to human bone healing is intractable due to the numerous differences between rodent and human bone microstructure and healing. Specifically, the basic microstructure of rodent bone can be observed as a primary lamellar structure lacking the osteonal (Haversian) systems characteristic of human bone (Egermann et al., 2008; Pearce et al., 2007). Further, it has been shown that the rate of bone healing is known to be inversely related to the species' ranking on the phylogenetic scale (den Boer et al., 1999). Consequently, the healing potential of rodent bone far exceeds that of adult human tissue (den Boer et al., 1999; Martini et al., 2001). Due to these distinct differences between species and the limited information regarding fracture healing in Haversian systems, the objective of this study was to investigate the effects of partial gravitational unloading on long-bone fracture healing in a previously developed large animal model using parameters derived from biomechanical, histomorphometric, and radiographic assessments.

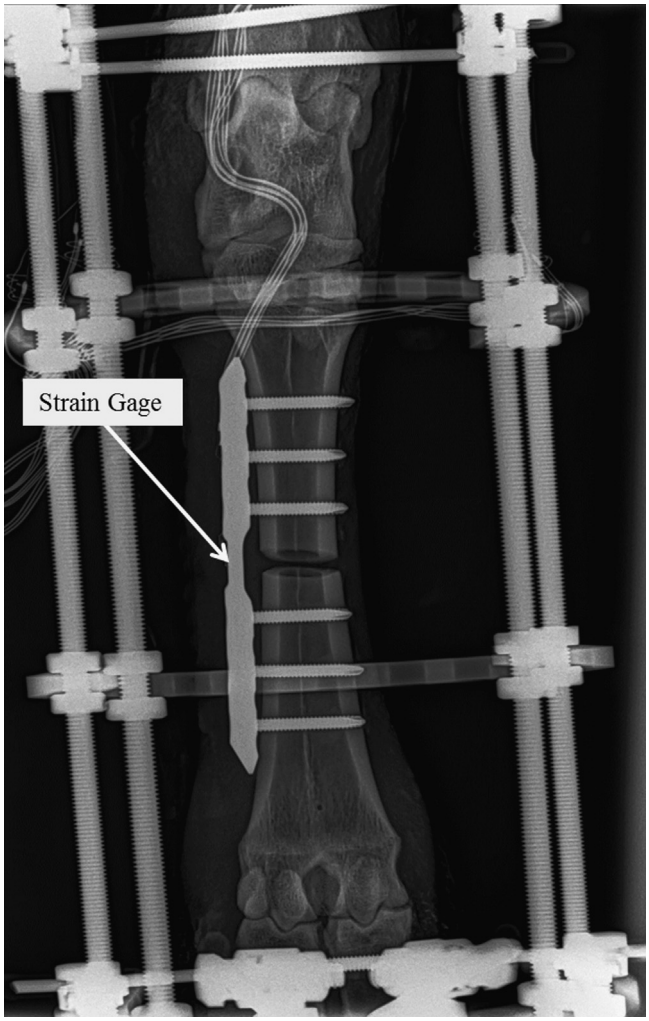

Fig. 1. A mid-metatarsal ostectomy was created and stabilized with an orthopedic locking plate instrumented with a rosette strain gage in order to monitor *in vivo* fracture healing and mechanical competence.

2. Methods

Skeletally mature Rambouillet Columbian ewes (age > 6 years) were used in this study. Animal use approval was granted by the Colorado State University Animal Care and Use Committee (Approval #11-2938A). Hindlimb metatarsal unloading was accomplished using the technique described by Gadomski et al. (2014). Briefly, a trans-biarticular external skeletal fixation device (IMEX, Longview, TX) was implanted on the right hindlimb of 5 skeletally mature female ewes such that the metatarsal bone was partially isolated from mechanical loading (PU Group, Fig. 1). Previous *in vivo* experiments demonstrated that this external fixation unloading system was able to reduce metatarsal bone loading by 75% (a relative gravity environment of 0.25*g*; Gadomski et al., 2014). The animals of the PU Group were exposed to partial unloading of the metatarsal bone via external fixation for a period of 3 weeks (21 days). An ostectomy procedure was performed at the 3-week time point by removing a 3.0 mm section of bone from the mid-diaphysis of the metatarsus. The ostectomy was stabilized via a laterally attached orthopedic locking plate (Synthes, Inc., Westchester, PA) instrumented with a rosette strain gage (Vishay Precision Group, Malvern, PA). This fracture method was deemed most appropriate for this study due to its high level of repeatability (i.e. measurability) and its ability to allow investigation of the healing cascade inside the fracture gap. Additionally this model allowed for the interrogation of the healing response of the body following an orthopedic surgical procedure in a partially unloaded environment, which will be clinically relevant in the future as spaceflight duration increases. An additional full loadbearing Earth gravity group (FL Group, $n=5$) was included in which an identical 3.0 mm ostectomy was created, plated, and secured via a fiberglass cast from the proximal tibia to the phalanges, allowing full loading to be transmitted through the metatarsal bone. Following the ostectomy procedure, both groups were subjected to a 4-week (28-day) healing period.

2.1. In vivo testing

Dual-energy x-ray absorptiometry (DEXA) scans (Discovery A, Hologic, Inc., Bedford, MA) were performed on the treated metatarsi of the PU Group at the time of the external fixation surgery, the ostectomy surgery, and every 2 weeks until sacrifice to obtain a clinical measure of BMD. DEXA scans were performed on the FL Group at the time of the ostectomy surgery and every 2 weeks until sacrifice. Longitudinal BMD values were normalized to the baseline BMD value for each animal for comparisons between groups (Gadomski et al., 2014).

In vivo mechanical competency of the healing fracture was evaluated biweekly via strain measurements of the orthopedic plate by testing each animal on a force sensing platform. Strain measurements from the metatarsal plate for single-limb ground reaction forces up to 200 N were recorded for the standing animals. The strains measured in the orthopedic plates were correlated to ground reaction forces between 50 N and 200 N, and the resultant construct stiffness slope magnitude ($\mu\varepsilon$/N) per testing day was calculated and normalized to the peak magnitude to characterize the change in load burden for each group over the course of the study. Utilizing the experimentally measured strains of the orthopedic fixation plate, and assuming that the longitudinal strains of the fracture site and fixation plate were