Effect of applied strain versus immobilization on tendon-to-bone healing in a rat model of ACL reconstruction

Robert H. Brophy1,2, David Kovacevic1, Carl Imhauser1, Mark Stasiak1, Kent Jackson1, Xeng Hua Deng1, Scott A. Rodeo1

1Sports Medicine, Hospital for Special Surgery, New York, NY; 2Department of Orthopaedic Surgery, Washington University School of Medicine, St. Louis, MO
brophyr@wudosis.wustl.edu

Introduction: Procedures such as anterior cruciate ligament (ACL) reconstruction require healing between a tendon graft and bone, which results from bone ingrowth into the tendon. Little is known about the effect of mechanical load on tendon-to-bone healing, which may have important implications for post-operative rehabilitation following ligament reconstruction. The purpose of this study was to compare the effect of cyclic strain to prolonged immobilization on tendon-to-bone healing using a novel approach to apply repetitive controlled strain to a healing tendon graft in an in vivo rat model to test the following hypotheses: (1) early graft motion causes inflammation that delays initial healing; (2) prolonged immobilization initiates the healing process but impedes progress to a mature tendon-bone interface.

Materials and Methods: 72 male Sprague Dawley rats (weight 250-350 grams) underwent ACL reconstruction using a flexor digitutum longus autograft. A custom-designed jig was used to drill co-linear tunnels in the femur and tibia, permitting placement of an external fixator across the knee that was parallel to the ACL graft. All other ligaments and tendons crossing the knee were sectioned to ensure that the ACL graft was loaded. The rats were randomly assigned to immobilization or daily loading for 14 or 28 days. Rats were able to ambulate in their cages with the external fixation bar locked to immobilize the knee. Each day, rats assigned to daily loading were secured to a novel in- vivo joint distraction device under anesthesia such that displacement of the femur and tibia was constrained to axial translation parallel to the graft tunnels, placing axial graft. Distraction was applied at 0.24 mm/sec up to 10% strain (based on the distance between the proximal and distal graft tunnels at the time of surgery) and then returned to neutral for a total of 50 cycles, and the resulting load-displacement curve was recorded. Preliminary cadaveric experiments verified that the ACL graft was loaded. At the end of the study period, the animals were sacrificed for biomechanical testing, micro-CT and histologic analysis.

Results: Daily load displacement: In rats that underwent daily loading, the load measured across the strained knees increased from 562 ± 265 g at baseline to a final value of 884 ± 413 g in the 14 day group, and from 414 ± 88 g at baseline to a final value of 820 ± 471 g after 28 days (p<0.05). For all groups, loads measured on and after day 10 were significantly greater than those measured on and before day 5 (p<0.05). Loads on and after day 12 were significantly greater than those measured on and before day 7 (p<0.05). The load measured across the strained knees at the time of sacrifice was 1250 ± 297 g in the 14 day immobilized group and 1336 ± 457 g in the 28 day immobilized group. The values in the immobilized groups were significantly higher than the daily loading group at both time points (p<0.001). There was no significant difference between the 14 day and 28 day groups in either the immobilized or loaded specimens. Final biomechanical testing is ongoing.

Micro CT: In the tibial aperture, the micro-CT demonstrated significantly lower bone volume (p=0.03) and total mineral content (p=0.05) in the strained rats at 4 weeks compared to 2 weeks. There was a trend towards thicker trabeculae in the immobilized rats compared to the strained rats at 2 weeks (p=0.08) and 4 weeks (p=0.06). At 4 weeks, the strained rats also demonstrated a trend towards greater trabecular spacing compared to the immobilized rats (p=0.08). In the tibial exit, there was also a strong trend towards less bone volume in the strained rats at 4 weeks compared to 2 weeks (p=0.06).

Histology: Concentrations of ED1 macrophages (phagocytic cells that accumulate in the first few days following surgery) at the tibial aperture trended lower in strained rats at 4 weeks (1.44 ±1.15) compared to 2 weeks (3.5±0.35) (p=0.07). Concentrations of ED2 macrophages (cells derived from the local tissue environment that accumulate later and have an anabolic role in tissue healing) and neutrophils demonstrated no significant differences between groups. At the tibial tunnel exit after two weeks, there were significantly more positively stained cells for Factor VIII (1.53±0.14) in the daily straining group compared to the immobilized group (0.48±0.21) (p=0.06). However, over the entire length of the tibial tunnel there was a trend towards less staining for Factor VIII in the immobilized rats at 4 weeks (0.62±0.49) compared to 2 weeks (1.54±0.22) (p=0.06). In the tibial tunnel,