The MRL/MpJ Exhibits Improved Biomechanical Outcomes with Increased Cellular Proliferation Following Full-Length, Full-Thickness Central Patellar Tendon Injury

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Introduction: Tendon and ligament injuries greatly affect the US economy, with 110 million patients presenting with an injury in 2008¹. Current treatments seek to restore normal activity by repairing or replacing the damaged tissue. Variability in long-term outcomes necessitates alternative methods to improve tendon repair. Tissue engineering is one strategy to augment the natural healing process. Investigating mechanisms involved in tissue regeneration may guide the tissue engineering design process. The Murphy Roths Large (MRL/MpJ) murine strain has shown regenerative healing potential in skin², heart³, cornea⁴, and articular cartilage⁵. It is hypothesized that a p21 deficiency, leading to increased cellular proliferation, may contribute to the improved healing response⁶. This study seeks to contrast the differences in the biomechanical response at 2, 5, and 8 weeks and cellular proliferation at 3, 7, and 14 days between the MRL/MpJ and C57/BL6 following a full-length, central patellar tendon (PT) injury.

Methods: All animal protocols were approved by UC IACUC. Experimental Design. Full-length, full-thickness central PT defects were created in 20 week-old MRL/MpJ and C57/BL6 animals. Animals were assigned to biomechanics at 2, 5, and 8 weeks (n=5 to 9) and histology/cellular proliferation at 3, 7, and 14 days (n=3) post surgery. Surgical Procedure. Longitudinal incisions were created along the PT borders, forceps were slid under the tendon to isolate it from the knee joint, and the central region was removed. A sham procedure in the contralateral limb was performed by creating longitudinal incisions along PT borders, but not removing the central third. Biomechanics. Medial and lateral struts were removed isolating the repair patella-PT-tibia unit, loaded into grips in a 37°C PBS bath, preconditioned to 0.02N for 25 cycles, and failed in uniaxial tension at 0.1%/second. Histology. Animals were given an IP injection of EdU (5-ethyl)-2'-deoxyuridine) at 3μg/g 24 hr prior to sacrifice to assess proliferating cells. Limbs were removed, trimmed, and fixed in 4% PFA, decalcified, submerged in 30% sucrose, and embedded in O.C.T. for frozen sectioning. Transverse sections (8μm thick) were made at six levels along the PT length and stained for DAPI and EdU. Proliferating Cells⁷. Grayscale images for EdU and DAPI were equally thresholded for all groups and converted to binary images. The DAPI image was overlaid on the EdU image to identify cells that were positive for EdU. Positive nuclei were counted using Fiji particle counter. Statistics. Biomechanical values, expressed as percentages of normal, were compared between sham and defect using a 3-way ANOVA with animal strain, time post surgery each significantly affected both ultimate load and linear stiffness for both strains. At 2 weeks, the MRL/MpJ achieved significantly greater percentages than C57/BL6, reaching 70% of normal ultimate load (p=0.04) and 72% of normal linear stiffness (p=0.01). Although no differences were found at 5 weeks, by 8 weeks, the MRL/MpJ reached 107% and 84% of normal ultimate load (p=0.01) and linear stiffness (p=0.009), respectively. Furthermore, the MRL/MpJ showed significant increases in structural properties from 5 to 8 weeks (p<0.05), while the C57/BL6 did not. With respect to the sham, the MRL/MpJ showed significantly improved properties at all time points, exceeding normal, uninjured values. Proliferating Cells (Fig.2). MRL/MpJ and C57/BL6 normal, uninjured tendon showed minimal proliferation (<0.5%). At Day 3, there was no difference between the MRL/MpJ and C57/BL6; however, at Day 7 and Day 14, the MRL/MpJ showed a 4.6% and 3.1% increase in cellular proliferation compared to the BL6/C57, respectively.

Discussion: In evaluating the MRL/MpJ’s healing capabilities with respect to a tendon injury, we have shown that this strain exhibits improved healing, reaching near normal percentages of ultimate load (107%) and linear stiffness (84%) at 8 weeks following injury. Further, the MRL/MpJ sham response resulted in improved properties, far exceeding normal. This may indicate the MRL/MpJ exhibits a heightened healing response to even small injuries compared to wild type healing. Natural tendon healing is insufficient, never reaching normal, biomechanical levels up to 8 weeks following injury⁸. The MRL/MpJ murine strain has been identified as a model of improved healing in a number of different injury models. Based on the positive biomechanical findings, we have begun to investigate the mechanisms regulating this improved healing phenotype, including mutations to the
p21/p53 cell cycle regulatory mechanism that results in p21 downregulation and increased cellular proliferation\(^6\). We are now assessing cellular proliferation early in the repair process at the onset of healing when cellular activity is heightened with initial results showing modest increases in proliferation for the MRL/MpJ with 4.6% at Day 7 and 3.1% at Day 14 compared to the C57/BL6. Increased cellular proliferation is a trademark of other regenerative model systems, such as the urodele, and may signify an immature cell population that is primed to differentiate and repair the injured tissue\(^9\), leading to improved mechanical outcomes at later time points.

**Significance:** This study demonstrates that the MRL/MpJ murine strain exhibits superior tendon healing compared to the wild type control which may be a result of mutations to cell cycle regulation mechanisms, leading to increased proliferation during early stages of healing. Future work will further investigate the molecular pathways involved in the MRL/MpJ healing process to elucidate therapeutic targets to improve future repair strategies.

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**References:**
Figure One: MRL/Mpj repair and sham show superior ultimate load and linear stiffness (as percentages of normal) compared to C57BL6 values at 2 and 8 weeks post surgery.
Figure Two: The MRL/MpJ showed increased cellular proliferation (represented as percentages of total cell number) at 7 and 14 days following injury. The images are representative images of the defect region for C57/BL6 and MRL/MpJ 7 days following injury (DAPI-Blue, EdU-Yellow). S: Strut, D: Defect.