The Effect Of Granulocyte Colony Stimulating Factor (G-CSF) On Rat Rotator Cuff Healing Following Acute Injury And Repair: Biomechanics, Histology, And MRI.

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Disclosures:

Introduction: Failure rates of healing after rotator cuff (RTC) repair are unacceptably high. Tissue engineering strategies employing mesenchymal stem cells show promise but carry the disadvantages associated with the time/resources required for isolation and expansion. It is hypothesized that cytokine-mediated mobilization of stem cells into the peripheral blood following cuff repair may result in increased homing of the mobilized cells to the site of the granulocyte-colony stimulating factor (G-CSF) administration following repair of the supraspinatus tendon in a rat model.

Methods: Eighty mature, female Sprague-Dawley (SD) rats received a full thickness supraspinatus surgical defect, which were acutely repaired. Animals were randomized to treatment (100µg/kg subcutaneous injection of G-CSF once daily for 5 days) or control (saline injection) groups. Additionally, animals that only received an acute supraspinatus defect without surgical repair or biologic treatment (defect only) were used as negative controls. Animals were euthanized following 12 or 19 post-operative days. Shoulders were dissected en bloc and imaged with T2, T1, and Proton Density (PD)-weighted sequences using a small-animal 3T microMRI (Fig 1) (MR Solutions Ltd, Guildford, UK). MRI images were reviewed by a blinded musculoskeletal radiologist to assess discontinuity in tendon fiber signal. Specimens from n=5 animals per group per time point were dissected for histology, fixed in 10% neutral buffered formalin, and processed for H&E and Safranin-O staining. H&E-stained sections were analyzed via white light microscopy and polarized light microscopy. The modified Bonar score described by Fearon, et al (Ref 1) was utilized to assess tendon structure. This scoring system including grading of cell morphology (rounded vs. aligned), cellularity, vascularity and collagen orientation with higher grades indicating worse tendon structure. In addition to characterization of the tendon, the degree of metachromasia at the tendon-bone interface was qualitatively assessed on Safranin-O/Fast Green-stained sections. Qualitative analysis of marrow cellularity and bone remodeling proximal to the insertion were also performed under white light microscopy. Specimens from n=8 animals per group per time point were frozen until biomechanical testing. Biomechanical testing was performed on a materials testing frame (MTS Insight 5, MTS Systems Corp, Eden Prairie, MN, USA) submersed in saline (pH = 7.4) in a temperature-controlled environmental chamber (39.1°C). Cross-sectional area (CSA) was calculated from three repetitive measurements with a laser-based system. Specimens were subjected to stress relaxation testing where they were preloaded to 0.1N, preconditioned with 10 cycles between 0.1N and 0.3N, and then held at 0.1N for 300s. Immediately thereafter, tendons were elongated with a 5% strain at 50%/s for 1200s. After the tendon was allowed to fully relax, the tendon was preloaded, preconditioned, and loaded to failure at a rate of 0.3%/s. Load and displacement data was collected via MTS software and analyzed via processing software (Matlab, MathWorks, Natick, MA, USA). Differences in all assessed variables were analyzed using one-way ANOVA with a modified Bonferroni post-hoc test of multiple comparisons (α=95%).

Results: MRI analysis demonstrated discontinuity in tendon fibers in 100% of defect-only animals at both 12 and 19 days, demonstrating that the rat supraspinatus tendon remains retracted if not repaired. Fiber discontinuity was observed in 50% of saline-treated animals and 100% of G-CSF-treated animals at 12 days (P=.045), and in 16.6% of saline-treated animals and 66.6% of G-CSF-treated animals at 19 days (P=.078). Defect-only (non-repaired) animals showed no improvement in any mechanical parameter between 12 and 19 days. Saline-treated animals had significant increases in ultimate stress (0.81 MPa ± 0.33 to 1.45 MPa ± 0.7, P = 0.033), yield stress (0.63 MPa ± 0.21 to 1.14±0.62, P=0.048), Young’s modulus (1.96 MPa ± 0.9 to 4.14 MPa ± 2.29, P = 0.001), peak stress (0.090 MPa ± 0.05 to 0.17 ± 0.078, P = 0.026), and equilibrium stress (0.029 MPa ± 0.016 to 0.071 MPa ± 0.036, P = 0.010) between 12 and 19 days (Fig2A-D). G-CSF-treated animals showed increases only in Young’s modulus (1.96 MPa ± 0.9 to 4.14 MPa ± 2.29, P = 0.035) but no other variable between 12 and 19 days. There were no significant differences in mechanical parameters between Saline and G-CSF-treated animals directly. Neither Saline nor G-CSF-treated animals had better biomechanical properties or different CSA compared to the defect-only, no-repair animals at 12-days. At 19-days, saline-treated animals showed improvement in all mechanical parameters compared to defect-only animals, but G-CSF-treated animals were not significantly different to defect-only animals in ultimate stress (G-CSF: 1.24 MPa ± .74; Defect: 0.649 MPa ± 0.31, P = .057) and yield stress (G-CSF: 0.91 MPa ± .5; Defect: 0.51 MPa ± 0.2, P = .075). All comparisons to healthy, nonsurgical tendons were significant. G-CSF-treated animals (Fig 3, right column) demonstrated increased cellularity within the humeral head, with higher numbers of osteoblastic-lining cells compared to Controls (Fig 3, left column). More new bone formation and bony remodeling was observed in proximity to the tendon-bone insertion in G-CSF-treated animals. G-CSF-treated animals displayed more intense
metachromasia at the tendon-bone insertion at 12 days, but Control animals demonstrated more intense insertional metachromasia at 19 days (Fig 3). Similarly, tendons from the G-CSF-treated animals had a lower aggregate histologic score 12 days than the Control animals (Control: 8.25, G-CSF: 7.5), and a higher aggregate score than the Control animals at 19 days (Control: 6, G-CSF: 6.875). The comparison of histologic grades was not statistically-significant at either endpoint.

**Discussion:** G-CSF-treated animals displayed both reduced recovery of tendon mechanical properties following cuff repair, and a higher incidence of abnormal MRI findings, compared to controls. Despite a lack of recovery of tendon mechanical properties, G-CSF administration stimulated increased marrowcellularity, new bone formation and bony remodeling proximal to the insertion. These animals also demonstrated more intense insertional metachromasia compared to controls, which has been associated with improved mechanical strength. Interestingly, no significant biomechanical difference was noted between either saline-treated or G-CSF-treated and “defect-only no repair” animals at 12 days. While saline-treated animals started to recover their mechanical properties at 19-days, G-CSF-treated animals showed less improvement, suggesting a delayed window of remodeling due to G-CSF treatment. A longer post-operative time point may be warranted to fully evaluate the role of G-CSF in RTC repair. The incorporation of locally administered stem cell homing factors to exploit the increased cellularity caused by G-CSF treatment may also prove to be efficacious.

**Significance:** Subcutaneously administered granulocyte colony stimulating factor (G-CSF) aimed to increase stem cell mobilization increased marrowcellularity and induced bony remodeling but thwarted recovery of tendon mechanical properties in a rat supraspinatus injury model.

**Acknowledgments:** N/A


![Fig 1 : MRI of the healthy rat shoulder (PD = Proton Density)](image)
Figure 2: Cross sectional Area (CSA) and Biomechanical Parameters

Figure 3: Safranin-O/Fast Green-stained sections of saline- and G-CSF-treated animals at 12 and 19 days post-operatively.

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