GENE EXPRESSION OF COLLAGEN I, COLLAGEN III, ALPHA-SMOOTH MUSCLE ACTIN AND MMP-1 IN A RABBIT PARTIAL ACL TRANSECTION MODEL

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Introduction: Extra-articular ligaments such as the medial collateral ligament (MCL) mount a well described healing response after injury. In contrast, the anterior cruciate ligament (ACL) seldom heals in vivo.  The anterior cruciate ligament remnants were isolated from knees and six with completely transected contralateral knees were sacrificed at each time point.  Six samples from each group (partial tear, transected and intact portion of the ligament, as compared to a sham control.  Gene expression of type I collagen (Coll. I), type III collagen (Coll. III), alpha smooth muscle actin (α-SMA) and matrix metalloproteinase 1 (MMP-1) was determined using semi-quantitative real-time PCR.

Materials and methods: Twenty four skeletally mature male New Zealand White rabbits were utilized.  Transection of the anteromedial bundle of the ACL was performed on the left knee of each animal, leaving the posterolateral bundle intact.  The contralateral knees were either sham operated (arthrotomy) or had a complete ACL transection (12 animals/group). Twelve animals were sacrificed at two weeks and six weeks post-operatively (six animals with sham operated contralateral knees and six with completely transected contralateral knees were sacrificed at each time point). Six samples from each group (partial tear, complete tear and sham) were analyzed in this report.  Anterior cruciate ligaments or ligament remnants were isolated from each animal immediately post sacrifice, snap-frozen in liquid nitrogen and then stored at -80°C. Tissue samples were homogenized and g of total RNA from each sample was reverse transcribed into cDNA and the levels of Coll. I, Coll. III, α-SMA and MMP-1 gene expression were quantified by real-time PCR and normalized to the expression of the housekeeping gene GAPDH.  Specific primers were designed according to published sequences for each target gene.

Results: All results are presented relative to the sham operated controls.  Collagen type I expression was elevated 1.7-fold in the partial transection (PT) at 2 weeks post operatively. Complete tear (CT) and intact (IT) segments did not show a statistically significant change in expression at 2 weeks.  At 6 weeks post-op, CT and PT showed a significant increase in type I collagen expression (5-fold and 3-fold respectively).  IT samples did not show a significant change in expression (Fig 1).

A 40% decrease in collagen III expression was seen at the 2-week time point in CT samples.  No significant change in expression was demonstrated in PT or IT specimens.  At 6 weeks, a significant increase in collagen III expression was seen in all groups: CT showed a 5.4-fold increase while PT and IT showed a 2.6-fold and 2.4-fold increase respectively (Fig. 2).

Discussion: Clinically, there are cases of partial or complete ACL injury which heal with a continuous strut between the femur and tibia, or fuse to the PCL. These ligament sections can maintain a grossly normal appearance in contrast to the discontinuous section of the injured ligament, which degenerates into an amorphous stump and involutes with increasing time post injury.  A partial ACL tear in a rabbit model was used to model the aforementioned clinical condition, and to analyze differences in matrix gene expression between the control, partially transected, completely transected and intact ligament sections.  A similar model was used previously to study the biomechanics and histology of the healing ACL.  By utilizing this model, we hope to elucidate the effect of mechanical stimulation on the maintenance of ligament homeostasis and response to injury.

Initially, we predicted a decrease in collagen gene expression after ACL injury, however an increase in the expression of collagen III was demonstrated in the complete transaction (CT) and partial transection (PT) specimens at 6 weeks post-op.  An increase in Collagen I expression was demonstrated at 6-weeks in CT and at both 2 and 6 weeks in PT.  This increase in collagen gene expression provides support for the theory that the ACL has the potential to heal, but the inability to form a stable clot or contact between the torn ends prevents the cells from successfully laying down a continuous matrix.

The increase in α-SMA expression, associated with myofibroblasts, seen in the 6-week CT and PT specimens is in agreement with a previous study in a rabbit MCL injury model which reported an increase in α-SMA-positive cells following ligament rupture.  This result suggests that myofibroblast activity may be partially responsible for the contraction of the transected ACL segments.  We have demonstrated a similarity between the intact ligaments and the intact segment of the partially transected ligaments at the level of gene expression for all the genes tested.  Additionally, the collagen and α-SMA gene expression of the partially transected segment was similar to that demonstrated in the completely transected ligaments.  The CT ligaments showed a more robust increase in gene expression than the PT samples.  This may reflect a limited response in the PT segments secondary to partial maintenance of ligament homeostasis.

References:
2. Hefti et al. JBJS, 1991
3. Murray MM et al. JBJS, 2000
4. Faryniarz et al. JOR 1996

There was no significant difference between groups in α-SMA expression at 2 weeks.  In contrast, at 6 weeks, CT shows a 7.5-fold increase and PT shows a 5-fold increase in α-SMA expression.  No significant difference was seen in the IT specimens (Fig. 3).  MMP-1 gene expression was significantly lower in PT (70%) and IT (80%) at 2 weeks, while no significant change was seen in CT.  At 6 weeks CT showed a 3-fold increase in MMP-1 expression while no significant change in expression was seen in PT or IT samples (Fig. 4).