

Human Metabolic Responses of the ACL Extensor Autografts to the ACL Remnant and the Synovium

Luke M. Annin, Kelsey R. Atteberry, Chance D. Strickler, Luke A. Baxter, Gillian M. Hark, Maria L. Hinkamp, Hailee C. Coleman, Richard Ma, James L. Cook, Aaron M. Stoker

Thompson Laboratory for Regenerative Orthopedics, University of Missouri, Columbia, MO

stokera@health.missouri.edu

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Introduction: Anterior cruciate ligament (ACL) reconstruction (ACLR) using extensor tendon autografts (GRAFT) is a common treatment for individuals after ACL injuries. Common extensor tendon autografts include the quadriceps tendon (QT) and patellar tendon (PT). While ACLR is generally successful, there are notable early morbidities that can develop after ACLR including arthrofibrosis and graft laxity. One mechanism for undesirable outcomes after ACLR can be due to the joint inflammatory and degradative environment after the injury and surgery. This could have a negative effect on intraarticular tissues including the ACL graft. An area of continued interest is also the perceived graft-specific differences in rates of post-operative morbidities after ACLR. As an example, higher rates of arthrofibrosis have been reported after QT autograft ACLR. Given the synovium is a major regulator of the joint homeostasis and its response to injury, we aimed to evaluate how the synovial tissue (SYN) recovered from patients undergoing ACLR would affect the metabolic responses of the extensor tendon autografts used for ACLR. Another potential source of local cellular signaling is the native ACL remnant given its proximity to the ACL tendon autograft. We hypothesized that GRAFT tissue co-cultured with the ACL remnant or SYN tissues would have higher pro-inflammatory and pro-degradative metabolic responses compared to GRAFT tissues cultured in isolation. Furthermore, given the higher incidences of arthrofibrosis after QT ACLRs, we hypothesize that QT GRAFT would exhibit a greater pro-inflammatory and pro-degradative metabolic responses when co-cultured with the ACL remnant and SYN tissues when compared to the patellar tendon (PT) GRAFT tissues.

Methods: Tissue harvest and culture: With IRB approval (IRB# 2009879) and informed patient consent, excess tendon autografts (QT and PT), ACL remnant, and SYN tissues were collected from patients undergoing ACLR surgery (n=45, 23.33 age, 21F). Tissue explants (4mm) were created from the excess tissues, and tissue explants (PT, QT, ACL, SYN) were cultured alone (MONO) or co-cultured with the ACL remnant (COA), SYN (COS), PT (COP), or QT (COQ) explants. The explants were cultured for 6 days in supplemented DMEM media then stored at -80°C for protein extraction. **Protein Extraction:** Tissues protein content was extracted using the T-PER Tissue-Protein Extraction Reagent (Fisher) with protease inhibitors using a mini-bead beater. The tissue extract was centrifuged and the supernatant stored at -80°C for analysis. **Tissue Protein Analysis** The protein content of the tissue extracts were determined using the BCA assay. Tissue extracts were analyzed for inflammation related (IL-1 β , TNF- α , IL-1RA, IL-4, IL-6, IL-8, MIP-1 α , MIP-1 β , MCP-1, MCP-3, Gro- α , RANTES, IFN- γ , Leptin, Adiponectin, Adipsin, CRP, Resistin), degradative enzyme related (MMP-1, MMP-2, MMP-3, MMP-7, MMP-8, MMP-9, MMP-13, TIMP-1, TIMP-2, TIMP-3, TIMP-4), and growth factor (VEGF, PDGF-AA, PDGF-AB/BB) proteins using commercially available Luminex assays. **Statistical analysis:** Differences between culture groups (mono vs. co-culture) within tissue types (QT, PT, ACL, SYN) and between QT and PT tissues within culture group (mono, COA, or COS) were determined by Mann-Whitney or Kruskal-Wallis test with Bonferroni correction depending on the number of groups in the analysis. Significance was set at P<0.05.

Results: Comparison of Graft Tissue in Monoculture vs. Co-Culture (Fig. 1): PT explants in the COA group had significantly higher MMP-3 and MCP-1 compared to the MONO group, and significantly higher MIP-1 β than the MONO and COS groups. QT tissues in the COS group had significantly higher IL-6 compared to the MONO group. **Effect of Graft Tissue on Joint ACL and Synovium (Fig. 1):** ACL tissues in the COQ group had significantly higher MMP-1, MMP-2, TIMP-1, and MCP-1 compared to the COP group, and the MONO group had significantly higher MMP-1, MMP-2, TIMP-1, and TIMP-2 compared to the COP group. SYN tissues in the COQ group had significantly higher MCP-1, MMP-1, MMP-2, MMP-3, MMP-8, and TIMP-1 compared to the COP group. SYN tissues in the MONO group had significantly higher MMP-1, MMP-2, MMP-8, MMP-9, TIMP-1, and TIMP-2 compared to the COP group, and significantly higher MMP-9 compared to the COQ group. **Graft-Specific Differences Between QT vs. PT (Fig. 1):** PT tissues in the COA group had significantly higher MMP-2, TIMP-2, MIP-1 β and PDGF-AA compared to QT tissues, and PT tissues in the COS group had significantly higher PDGF-AA compared to QT tissues.

Discussion: The data from this study indicates that human PT and QT autograft tissues have a different response when co-cultured with ACL remnants and SYN. PT tissues co-cultured with the ACL remnants exhibited a greater levels of specific inflammatory and degradative proteins response when compared to PT tissues alone and QT tissues co-cultured with the ACL remnant. Therefore, the presence of the ACL remnant during PT ACLR may have more of a localized impact than QT autografts. Interestingly, the data also indicates that the PT and QT tissues also have a differential effect on the SYN tissues. SYN tissues co-cultured with the PT resulted in lower concentrations of specific inflammatory and degradative proteins compared to SYN tissues co-cultured with the QT. Therefore, it is possible that factors released by the QT and PT placed during ACLR may contribute to changes in joint inflammatory signaling and degradative enzyme activity after ACL surgery. This may explain some clinical observations of higher incidences of arthrofibrosis after QT ACLR. Ongoing studies are aimed at determining how these differences in tissue response relate to patient outcomes after ACLR, and how these responses by the tissues during in vitro culture relate to patient demographics and time from injury to surgery. Understanding the patient-specific differences and the biological variances between PT and QT autografts tissues and its contribution to shaping the post-surgical ACLR joint environment may help develop patient-specific graft selection algorithms for individuals undergoing ACLR surgery.

Significance: The data from this study indicates that human PT and QT explants exhibit and exert a different biologic response in the presence of the joint synovium and the adjacent ACL remnant. These tissues may help shape a different intraarticular environment after ACL surgery. Determining if these differences between PT and QT autografts have an effect on patient outcomes after ACLR could help develop decision-making based on biologic activity of the graft tissue in graft selection algorithms that are patient specific.

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