Development of an In Vitro Bone-Tendon-Muscle Explant Culture Model

Brianne K. Connizzo[1], Hannah M. Zlotnick[1], John M. Drago[1], and Alan J. Grodzinsky[1]
[1]Massachusetts Institute of Technology, Cambridge, MA

Introduction: Degenerative rotator cuff injuries are among the most prevalent musculoskeletal disorders that affect the aging population, but unfortunately the etiology is relatively unknown. Explant culture has the ability to provide a controllable model system while maintaining the native extracellular matrix composition and structure for tenocyte health. The purpose of this pilot study was to investigate the efficacy of explant culture from mouse supraspinatus tendon as a model system for future studies on rotator cuff degeneration. To this end, we investigated the mid-foot portion of the flexor digitorum longus (FDL) tendon as an internal tendon-only explant, and the supraspinatus (SST) tendon along with the associated muscle and humerus as our rotator cuff explant.

Methods: Mouse FDL and SST were dissected free of soft tissue and placed free floating in tissue culture medium (Low glucose DMEM + 10% fetal bovine serum + 1% antibiotic solution) for up to 10 days in culture. Total protein and sulfated glycosaminoglycan synthesis were assessed via addition of ^{3}H and ^{35}S-labeled media, respectively, for 24 hours. Overall explant cell metabolism was assessed via incubation in media with Alamar blue for 3 hours. Explants were then washed, removed from culture and assayed for sulfated GAG content by the DMMB assay, DNA content by the PicoGreen assay, and total collagen by the hydroxyproline (OHP) assay.

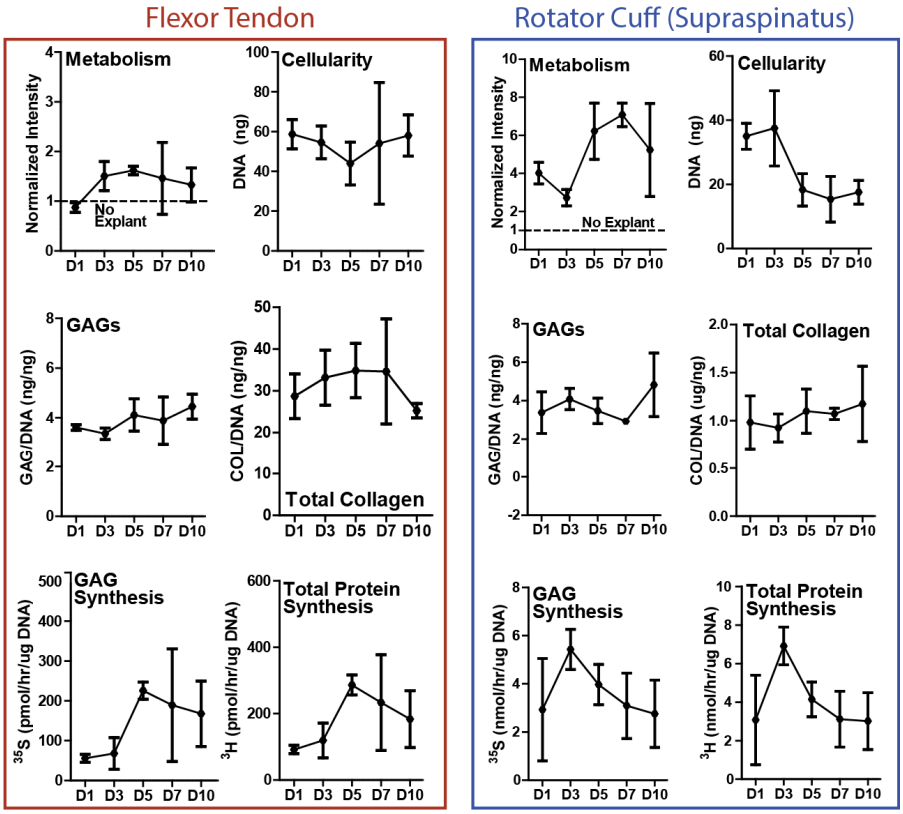

Figure 1. (left) Mouse flexor tendon explant metabolism, cellularity, GAG content, total collagen content, GAG synthesis and total protein synthesis. (right) Mouse rotator cuff explant metabolism and supraspinatus tendon cellularity, GAG content, total collagen content, GAG synthesis and total protein synthesis.

Results and Discussion: Flexor tendon explants exhibited a brief initial equilibration period to culture conditions but were then stable in almost every parameter up to 10 days in culture (Fig. 1, left), consistent with previous literature.[1–3] Total protein and GAG synthesis peaked around day 5, but was relatively stable thereafter. In the rotator cuff explants, all parameters reached a stable level following 3 days in culture, except for DNA content which decreased slightly over the course of culture suggesting increased cell death (Fig. 1, right). Further investigation is necessary to determine the mechanisms behind this decrease. We hypothesize that due to the addition of muscle and bone in the rotator cuff explant cultures, cell death in the adjacent tissues could cause release of metabolic mediators and cytokines that would in turn cause cell death and degradation of the supraspinatus tendon faster than the flexor (tendon only) explants. Ongoing studies are focused on these processes as well as detection of functional deficits as in previous studies of stress-deprived tendon culture.

Conclusions: Overall, this is the first known co-culture of bone-tendon-muscle explants and, while further characterization is necessary, these data provide promising evidence of the efficacy of this technique. If successful, this multi-tissue explant model would be a powerful clinically-relevant system to answer basic mechanobiology questions as well as a controllable potential therapeutic screening tool.

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References: [1] Devkota AC et al. *Med Eng Phys.* 2005. **27**:803–8. [2] Egerbacher M et al. *Clin Orthop.* 2008. 466:1562-8. [3] Gardner K et al. *J Orthop Res.* 2011. 29:587-7.