Introduction: There are over 200,000 anterior ligament (ACL) reconstructions performed each year in the United States. Tissue engineered ligaments have great potential to become a graft source that could be readily available and have no associated morbidity. Construction of a viable and biomechanically sound ligament requires a fundamental understanding of ACL fibroblast and matrix biology and cellular response to the local mechanical environment. We have demonstrated that ACL fibroblasts increase collagen production and decrease matrix degrading enzymes when subjected to tensile strain. The mechanism of mechanotransduction remains unknown. We hypothesize that ACL cell surface integrins, cytoskeletal structure and collagen alignment respond dynamically to strain when cultured in a 3-D gel matrix.

Materials and methods: Cells were obtained by explant culture or collagenase digestion from canine ACL tissue and cultured in medium M199 with 1% antibiotic antimycotic (AbAm) and 10% fetal calf serum (FCS), at 37°C, 100% humidity and 5% CO2. A liquid gel solution was formed by combining: 8ml Vitrogen collagen solution (2.9 mg/ml) (Cohesion Technologies) with 1 ml 10 M199, 1ml FCS, 100 µl AbAm and 100 µl 1N NaOH and 1x10^6 cells/ml. Linear 3D gels were created using 200µl of cell suspension in Flexercell Culture plates using a trough-loading system. Gels were untreated (control), tethered in the absence of external strain, or exposed to 2.5% cyclic strain at 6 cpm for 2 hrs/day for four days. Cell counts were performed using a Coulter Counter at the end of the experiment. Quantitation of cell surface integrins was performed on day 4, after termination of tensile strain application using monoclonal antibodies to human integrin subunit α1 and α5 (Immunotech) and β1 (Upstate Biotech.). Cells were washed 3X with FACS buffer, incubated with R-phycocerythrin – conjugated goat anti-mouse IgG at 4°C for 30 min, suspended in 0.2 ml FACS buffer and analyzed for fluorescence with a FACScan flow cytometer and CellQuest software (Becton Dickinson). Gel construct were embedded in paraffin, stained with Hematoxalin-Eosin (H&E) and examined under polarized light. A total of 5 experiments were performed.

Results: Integrin expression in cell constructs exposed to intrinsic (tethered) or extrinsic (strained) strain increased when compared with cells in free, untreated gels (control). While α1 was expressed in 50% of cells in all gels, there was no change in concentration of α1 receptors in response to strain. Expression of both α5 and β1 integrin subunits was increased significantly (p < 0.02) in fibroblasts in tethered and cyclically strained 3D collagen gels over control values (N= 5). (Figures 1 & 2) While there was no difference in β1integrin expression between strained and tethered constructs, there was a significant increase in α5 expression (p<0.05) in strained compared to tethered constructs.

Discussion: Anterior cruciate ligament fibroblast culture in 3D linear collagen gels permits the study of fibroblast biology in an environment where spatial orientation of cells may be more similar to an in vivo state. The alignment of cells along the collagen matrix in response to strain makes the histology of the constructs very similar to the in vivo state. This study confirms the presence of cell surface integrins in canine ACL fibroblasts previously reported in monolayer cultures exposed to cyclic tensile strain. The results also show a dynamic increase in expression α5 and β1 receptors in response to intrinsic and tensile strain. We have previously shown that ACL fibroblasts increase type I collagen RNA and type I collagen synthesis and decrease production of matrix metalloproteinases (MMP) in response to strain. It is possible that α5 and β1 receptors are responsible for mechanotransduction and subsequent alteration of DNA transcription in response to mechanical stimuli. Further work targeting these receptors and transduction pathways will help confirm their role in fibroblast mechanotransduction. Ultimately, the ability to control or manipulate these signal transduction pathways may enhance our ability to regulate fibroblast biosynthetic function and improve our ability to engineer implantable ACL grafts.

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References:
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