many lipid classes, it has been presumed that the SAPL is formed by adhesion and self-assembly through rheologic synovial fluid lipid deposition from synoviocytes or plasma dialysate. Combined with our prior work, we postulate that lipid droplet surface extrusion from the matrix suggests chondrocytes and synoviocytes may cooperatively attempt to reconstruct the SAPL through prototropic communication (viz. integrated proton transfer pathways through nanochannels, hydrogen bond networks, and titratable protonation sites), but that the effort becomes ineffective in osteoarthritis due to the lack of a suitable surface substrate.

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THE CONTRIBUTION OF PROTEOGLYCANS TO THE VISCOELASTICITY OF THE CANINE ANTERIOR CRUCIATE LIGAMENT

E.J. Comerford †, B. Geraghty ‡, R. Hamarashid ‡, A. Elsheikh ‡. † *Univ. of Liverpool, Neston, United Kingdom;* ‡ *Univ. of Liverpool, Liverpool, United Kingdom*

Purpose: Proteoglycans (PGs) are minor extracellular matrix proteins, which may play an important role in normal ligament mechanobiology. The contribution of PGs to the mechanobiology of complex ligaments such as the anterior cruciate ligament (ACL) has not been determined to date. Recently we have shown that PGs may have a pathological role in the canine ACL complex of the knee joint as a result of altered mechanical loads. Rupture of the ACL has profound implications for joint stability, and affected joints (in both human and other species such as the dog) develop progressive osteoarthritis. The aim of this study was to address the hypothesis that PGs play a vital role in ACL mechanobiology by determining the biomechanical behaviour at low strain rates with and without PGs.
Method:ACLs were removed from canine cadaveric knee joints (n=8 pairs) as bone (femur)-ligament-bone (tibia) (BLB) samples following institutional ethical approval. The BLB samples were mounted in an Instron 3366 uniaxial testing machine (Instron, Norwood, MA) using a custom made clamping mechanism surrounded by a Perspex chamber to allow full immersion of the sample. All samples were immersed in chondroitinase buffer for one hour prior to testing. 0.25iu/ml Chondroitinase ABC (Sigma, UK) was then added to the right BLB of each pair for 3 hours to remove the PGs. All samples were then subjected to cyclic loading up to 0.5MPa at increasing strain rates, namely 0.25, 0.5, 0.75, 1 and 10 %/min. Stress, strain and tangent modulus values were determined for each sample and the results analysed with an ANOVA (Friedman test) (GraphPad, Prism, US) (significance set at $p<0.05$).
Results: Our results showed small but statistically insignificant differences between the strain-rate dependent behaviour of specimens both with and without PGs. However, samples with PGs removed consistently exhibited lower values of tangent modulus at all stress levels At a stress of 0.1MPa, the average tangent modulus of all strain rates without ChABC treatment was 7.58±0.96 MPa and with ChABC treatment was 4.61±0.73 MPa which was statistically significant ($p<0.0001$).
Discussion:PGs are thought to contribute to tendon mechanobiology by allowing fibre sliding or by cross-linking collagen fibrils thereby contributing to load sharing within the tendon. We have shown that PG depleted ACLs are less stiff with low strain rates in stress strain curve. This is contrary to finding previously reported in the medial collateral ligament (MCL) where no differences were found with PG depletion. These findings suggest that PGs are vital to the normal mechanobiology of complex ligaments such as the ACL. Future work will examine these findings at a fascicular level as well as measuring which PGs are depleted with Western blotting.

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A COMPARISON OF THE EXTRACELLULAR MATRIX COMPOSITION OF TENDON/LIGAMENT TISSUE AND TISSUE ENGINEERED TENDON AND LIGAMENT CONSTRUCT

Y. Ashraf Kharaz, S. Tew, E. Canty-Laird, E. Comerford. *Univ. of liverpool, Neston, United Kingdom*

Purpose: Tendons and ligaments (T/L) play key roles in the musculoskeletal system, but are susceptible to traumatic or age-related rupture, which can lead to severe pain and immobility for the individual and increased susceptibility to degenerative joint diseases such as osteoarthritis. Tissue engineering offers an attractive therapeutic approach to treat T/L rupture but is hampered by our poor understanding of the defining characteristics of the two tissues. This study aimed to determine differences in extracellular matrix (ECM) macromolecules between native T/L tissue and tissue engineered T/L constructs.
Methods: The anterior cruciate ligament (ACL) and long digital extensor tendon (LDET) were harvested from cadaveric canine knee joints. Cells from ACL and LDET (n=3) were used to create tendon or ligament tissue constructs, which were grown in a fibrin-based 3D culture system. The ECM composition of native tissues (n=5) and engineered T/L constructs (n=3) were examined. Total collagen, DNA, sulphated glycosaminosglycans (sGAG) and elastin content were measured and normalised to dry weight of the tissues. A quantified histological comparison of the tissues was also performed. Finally, a proteomic comparison was also carried out, analysing protein extracted in 4M guanidine HCL. In-solution trypsin digested protein samples were analysed by liquid chromatography tandem mass spectrometry (LC-MS/MS). Data produced were searched using Mascot (Matrix science, UK), against canine protein sequence. The gene symbols for each identified protein were searched in UNIPROT database and converted to gene symbol of the corresponding human orthologue. Gene ontology (GO) and protein network analysis were undertaken using the bioinformatics tool; String.
Results: DNA content was significantly higher in both constructs than native ligaments (mean 8.6 μg/mg T/L construct, 2.9μg/mg ACL, $p=0.018$). Collagen content was 65% ACL, 70% LDET, 4.5% ACL construct and 2.5% LDET construct: this was statistically significant ($p<0.0001$). sGAG was 14.3μg/mg ACL, 8.3μg/mg LDET, 15.6μg/mg ligament construct and 11.3μg/mg tendon construct and was significantly higher between native T/L ($p=0.007$) and ACL construct compared to native tendon ($p=0.006$). Elastin content was significantly higher in ACL constructs (9.5%) and LDET construct (14.3%) compared to both native T/L (2.5% and 5%, $p<0.0001$). Histological analysis showed constructs were highly cellular and confirmed that they had lower collagen content than native tissue. Blood vessels were identified by elastin staining in T/L constructs, indicating that cells from each tissue could be reforming specific structures.
In the proteomic analysis, a total of 122 proteins were identified in native ligament, 105 in native tendon, 304 in ligament constructs and 481 in tendon constructs. In addition to many cellular proteins, T/L constructs also comprised numerous extracellular matrix proteins that were also found in the native tissues, including collagen I, III, VI and XII, decorin, bigylcan, fibronectin 1 and fibrillin 1. String analysis revealed clustering of ribosomal proteins in both T/L constructs. Interestingly, tendon constructs contained more cytoskeletal, ATP and heat shock associated proteins than the ligament constructs. The principal gene ontology processes were identified as ECM organisation in native ligament, collagen fibril organisation in native tendon. Principal ontologies associated with constructs involved protein translation and targeting.
Conclusion: The results of this study show that whilst the ligament and tendon constructs are composed of different proportions of ECM proteins, they share some similar characteristics with the native tissues. This might indicate that cells from each tissue retain a site specific "memory" which enables them to recapitulate specific ECM in 3D culture conditions. The rapid acquisition of sGAG and elastin content in the engineered constructs might indicate that these components mature faster than collagen matrix; as has been observed with chick tendon fibroblast construct.
Proteomics identified several specific ECM proteins such as asporin, tenomodulin in the tendon but not in ligament. Other proteins such as versican, SOD3 and proteoglycan 4 were identified in ligament but not in tendon. These may demonstrate a fundamental difference between tendon and ligament.

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MOLECULAR ANALYSIS OF ANTERIOR CRUCIATE LIGAMENT TEARS SHOWS TIME-DEPENDENT CAPACITY FOR REPAIR

M.F. Rai, R.H. Brophy, L.J. Sandell. *Washington Univ. Sch. of Med., St. Louis, MO, USA*

Purpose: Little is known about molecular changes in the injured human anterior cruciate ligament (ACL), particularly as it relates to time from injury. The purpose of this study was to test the hypothesis that gene expression in ACL tears varies by time from injury which may provide molecular insights into ACL repair at early (less than 3 mo.) and late (greater than 12 mo.) time points after initial injury.
Methods: Biopsies of ruptured human ACL tissues (N=24) were obtained intraoperatively at the time of clinically indicated ACL