ABSTRACT

INTRODUCTION: Anterior cruciate ligament (ACL) reconstruction surgeries using tendon grafts are performed in the US at a rate of nearly 350,000 per year. The current treatment using autograft and allograft is acceptable with limitations such as donor availability, biomechanical incompatibility, and immune rejection, leading investigators to develop strategies to engineer ligament tissue. Current methodologies for engineering ligament using scaffolds suffer similar limitations including immune rejection, degradation, non-physiological intra-articular mechanical properties and incomplete attachment to the bone tunnel. To more closely replicate native intra-articular ligament and promote integration with native bone within the bone tunnel, we have engineered a multi-phasic ligament with engineered bone at each end and a mechanically viable and biochemically relevant interface between the two tissues. Our scaffold-less method using bone marrow stromal cells (BMSCs) engineers an in vitro multi-phasic ligament model or bone-ligament-bone (BLB) construct that exhibits the structural and functional interface characteristics of young native tissue.

We have engineered BLBs from sheep BMSC and utilized these BLBs as grafts for ACL replacement in sheep. We have completed a 6-month recovery experiment and present data including collagen structure, vascularization and innervations, lubricin expression of cells within the intra-articular region and mechanical properties of the grafts.

METHODS: Engineered BLB constructs were fabricated utilizing sheep BMSCs as previously described. After one week of in vitro cultivation, the BLB constructs were used as grafts for ACL reconstruction in sheep. The BLB constructs were implanted for 6 months, after which, both knees were surgically removed and surrounding soft tissue was dissected leaving only the BLBs and contralateral (CL) native ACLs for morphological and mechanical characterization. Hematoxylin and Eosin (H&E) staining was used for general morphology observations and immunofluorescent staining was performed on longitudinal sections of BLB explants using specific antibodies that detect the presence of blood vessels and lubricin. Uniaxial tension testing was conducted to obtain the tensile stiffness of the in vivo BLB and CL ACL specimens using an MTS 810 servo hydraulic test system with a 25 kN load cell. ImageJ and Metamorph softwares were used for accurate strain determination via digital image correlation analysis.

RESULTS: H&E staining of 6 mo. BLB explants (Figure 1A) demonstrated organized collagen fibers that are similar to that of adult ACL (Figure 1D). Anti-CD31 and anti-NCAM antibody immunostaining showed the innervation and vascularization of the BLB at six months (Figures 1B and 1C) in vivo also resembled those of the adult ACL (Figures 1E and 1F). Immunohistological staining for lubricin on longitudinal sections of adult native sheep ACLs and BLB explants showed that lubricin was expressed by ligament cells in the native ACLs and also by the cells in our BLB explants. The lubricin antibody brightly stained all nuclei and was accumulated at higher amounts at the superficial areas beneath the surface than closer to the center of the ligament. The secondary antibody only stained nonspecifically the most superficial layer of the ligament (data not shown).

The tangent stiffness of BLB explants at six months (N=3) was measured (strain range: 0.10 – 0.35) along with the stiffness of the control ACL explants (CL ACL, N=3; strain range: 0.10 – 0.35). As shown in Figure 3, the tangent stiffness of the explants averaged 130.0±17.2 MPa and that of the adult CL ACL, 250.7±8.5 MPa. Geometric stiffness values were 359.0±150.6 N/mm for the BLB explants and 379.2±73.6 N/mm for the CL ACLs.

DISCUSSION: We have developed a multi-phasic engineered bone/ligament co-culture with a viable interface in vitro and the capacity to develop vascular and neural systems, complete ligamentization and maintain mechanical properties comparable to adult ACL. Lubricin, which is believed to play a role in the tribological (e.g. wear) and viscoelastic properties of soft tissues, decreases in concentration in the synovial fluid after ACL injury and may not return to pre injury levels after ACL reconstruction. The expression of lubricin in the BLB explants shown in the immunohistological staining indicates the ligamentization of in vivo BLB. The geometric stiffness of the engineered BLB constructs was 95% of that of the CL ACL indicating the BLBs were carrying physiological load levels in vivo. The engineered BLB constructs have great potential for ACL reconstruction.

REFERENCES:


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