Fresh vs Frozen Tissue Engineered 3D Bone-Ligament-Bone Constructs for Sheep ACL Repair

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Disclosures:

Introduction: The anterior cruciate ligament (ACL) is the most commonly torn ligament in the knee and healing of the damaged ACL is limited. Without surgical intervention there is potential for further injury and the risk of developing degenerative features found in the ACL-deficient knee. The current most popular options for ACL reconstruction are patellar or hamstring tendon autografts. The mismatched biomechanical properties of these current tendon grafts with those of native ACL tissue, however, have been implicated in the development of early onset osteoarthritis. To address these issues, our laboratory has fabricated tissue-engineered ligament constructs that demonstrate structural and functional properties similar to those of native ACL tissue after implantation. In addition, these tissue-engineered grafts achieve vascular and neural development that exceeds those of patellar tendon grafts [1]. Utilization of tissue-engineered grafts is limited, however, by the time necessary to produce the constructs. Additionally, the relatively short amount of time during which the construct has properties suitable for implantation while in vitro is also a limitation in their use. In this study, we investigated the efficacy of freezing our tissue-engineered constructs as a method of preservation versus our previous model of fresh constructs for ACL reconstruction. We hypothesized that frozen constructs would have similar outcomes compared to our fresh model thereby broadening the scope of tissue sources for our technology.

Methods: Bone marrow stromal cells (BMSCs) harvested from iliac crest marrow aspirations on ovine adult females were used to fabricate our engineered bone-ligament-bone (BLB) constructs using methods described previously [1,2] for anterior cruciate ligament (ACL) reconstruction. Constructs used to test frozen preservation were stored in culture media at -80C and thawed in a 37C bead bath prior to implantation. ACL reconstruction surgery was performed as previously described [1] with fresh and frozen constructs implanted on the same surgical day as a set (n=5) using BMSCs derived from the same animal source. After 6 months, both the experimental and contralateral knees were surgically removed. The intact knees were tested for joint laxity and then the BLB and contralateral ACL were isolated for uniaxial tensile testing and subsequently harvested for histology. All data are given as mean±standard deviation. All animal care and animal surgeries were in accordance with The Guide for Care and Use of Laboratory Animals (Public Health Service, 1996, NIH Publication No. 85-23); the experimental protocol was approved by the University Committee for the Use and Care of Animals.

Results: Following six months post-ACL reconstruction, fresh and frozen grafts showed similar morphological and biomechanical properties. H&E staining showed similar collagen fascicle formation and organization in frozen (Fig 1A) and fresh (Fig 1B) explanted tissue. Frozen BLBs had an average tangent modulus of 43±6 MPa (n=5) with the contralateral ACL modulus at 156±33 MPa (n=5) at a strain range of 0.04 - 0.075. Fresh BLBs showed an average tangent modulus of 35±13 MPa (n=3) with the contralateral ACL modulus at 159±33 MPa (n=3) at a strain range of 0.040 - 0.100 (Fig 2). There is not a significant difference between the two graft types (p>0.05). Two of the fresh samples were unable to be mechanically tested due to conditions unrelated to the experiment. Further histological analyses of the explanted fresh and frozen BLBs are to follow.

Figure 1. Explant Hematoxylin & Eosin Staining. H&E staining of longitudinal sections of BLB explants after 6 months of implantation as an ACL replacement from frozen (A) and fresh (B) BLB constructs. 20X magnification.
Discussion: Previous experiments performed in our lab demonstrate the effectiveness of our BLB approach using fresh tissue models [1,2]. The purpose of this study was to evaluate the outcome of BLBs that were frozen compared to that of fresh implants. Our results show that 6 months post-implantation, both graft models show similar mechanical and morphological outcomes demonstrating that freezing BLBs may be a potential option for preservation in using our methods for ACL reconstruction. These data indicate that our tissue-engineered ligament graft could potentially be stored frozen long-term after fabrication and used when needed. Additional histological analyses will be done to further characterize the fresh and frozen samples.

Significance: Traditional graft options for ACL reconstructions are biomechanically mismatched and lead to degenerative problems in the knee. Scaffold-less tissue engineered grafts provide a promising solution to this problem. This study shows a potential preservation method for these grafts allowing them to be more easily accessible when needed and expands the versatility of our approach.

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