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NON-LINEAR VISCOELASTIC MECHANICS OF NATIVE AND ENGINEERED LIGAMENTS AND TENDONS

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ABSTRACT

Patellar tendon (PT) autografts and allografts are the most common methods currently used to replace a torn anterior cruciate ligament (ACL). The PT is not only much stiffer than the ACL it replaces it also exhibits qualitatively and quantitatively different non-linear viscoelastic behavior from those of the ACL. These mis-matched biomechanics may be contributing to the high incidence of early onset osteoarthritis suffered by patients who have had ACL surgeries. Thus there is a need for an ACL graft that can reproduce normal ligament biomechanics and knee function. This talk examines the inhomogeneous, non-linear viscoelastic response of native ACL and of a tissue engineered ACL graft designed to rapidly grow and remodel in vivo to restore the proper biomechanical properties of native ligament. The results using this graft as an ACL replacement are compared against those using a PT autograft for the ACL replacement. Uniaxial loading reveals that after nine months as an ACL replacement, the tissue-engineered graft develops a strain contour pattern closely resembling that of native ACL whereas the PT graft fails to similarly remodel in vivo.

METHODS

Bone Marrow Stem Cell Isolation and Expansion: Bone marrow stromal cells (BMSCs) were isolated and expanded according to a previous protocol developed in our lab with some slight modifications [1]. Briefly, the marrow from both tibias and femurs are harvested. The marrow is minced then vortexed and pelleted using centrifugation at 1500 rpm for 5 minutes at 25ºC. The supernatant is removed and the cells resuspended in 10 ml either bone or ligament growth medium (GM) in 100 mm diameter tissue culture dishes. The dishes are incubated at 37ºC, 95% humidity, and 5% CO_2. After 48 h, the non-adherent cells are removed by feeding the plates with fresh GM. The adherent BMSC are cultured to 80% confluence, at which time cells are enzymatically removed from the 100 mm plate using a 0.25% trypsin-EDTA solution (Gibco), passaged and plated onto construct dishes.

Preparation of Self-Organized Bone Constructs: Bone constructs are engineered following a previously developed protocol [2,3]. After incubation, the GM is aspirated and 2 x 10^5 cells, suspended in 16 ml bone GM, are seeded onto 60 mm cell culture plates. The medium is changed every 2–3 days. When the cells become confluent, after approximately 5 days, bone differentiation medium (DM) is substituted for GM to indoce construct formation. After approximately 2 days, a bone monolayer forms on each dish. The monolayers are transferred from the culture dishes to Sylgard coated dishes. Two minutien pins are placed on the monolayers approximately 20 mm apart in the dish, and within another 2-3 days the monolayer self-assembles into a 20 mm long cylinder between the minutien pins. The DM is changed every 2–3 days until the bones are used to form BLB constructs.

BLB Construct Formation: Cell suspension containing 2 x 10^5 cells/ml of ligament GM are plated in 100 mm culture dishes. The dishes are then placed in a 37°C 5% CO_2 incubator and the GM changed every 2–3 days. Approximately three days later, a ligament monolayer forms and ligament DM is replaced with GM. The monolayers are transferred to Sylgard coated dishes. Engineered bones (fabricated as described above) 20 mm in length are pinned, using two minutien pins, on top of the ligament monolayer, and in-line axially so that the inner ends are 30 mm apart to fabricate a 70 mm long BLB as the ligament monolayer self-assembles into a cylindrical construct around the bone ends. Individual BLB constructs have a diameter of 0.6 to 0.8 mm. Within one week prior to implantation six of these constructs are pinned together laterally at their bone ends to form a larger BLB that is rolled into a cylinder of 4 mm diameter. This large BLB fuses together laterally prior to implantation, is of sufficient size for implantation as a sheep ACL replacement and does not develop a necrotic core during this period of time in vitro.

Surgical Procedures: Sheep were obtained from the Michigan Livestock Exchange, various farms in the area or

intra−university transfer. All animals were acclimated to our Sheep Research Facility at the University of Michigan for one week prior to any procedure. Sheep were allowed to free range in the pasture until used for surgical implantation. The animals were given access to food and water ad libitum. The BLB constructs were implanted into the ACL site as a replacement tissue and the animals were allowed to recover for nine months before explantation. All surgical procedures were performed in an aseptic environment with anesthesia induced by i.v. injections of Ketamine and Diazepam and sustained with inhalation of halothane gas. After any surgical procedure, the animals were singly housed in secluded pens for two weeks and then released back into the herd until the date of explantation. 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.

Uniaxial Response: Uniaxial loading of the knee joint was applied using an MTS 810 servohydraulic test system. We examined the strain contour fields of native ACLs and of BLB and PT explants using two high speed Photron Fastcam SA1.1 cameras, software (Photron Fastcam Viewer 3, Correlated Solutions Vic-2D 2010) and hardware (Dell Precision T1500) for 2D digital image correlation (DIC). Tangent moduli and geometric stiffness values were determined using the average strain in the ligament and the average cross-sectional area.

RESULTS AND DISCUSSION

The characteristic strain contours of the left knee's native ACL are closely mirrored by the BLB explant from the right knee as seen in Figure 1. These data demonstrate that the BLB graft had developed the functional gradient of native ACL. The PT explant is also from a right knee. Its strain contour plot is approximately uniform therefore it has not developed a functional gradient after 9 months in vivo as an ACL replacement.

The tangent moduli and geomtric stiffnesses of BLB explants at 9 months (N=7), PT graft explants at 9 months (N=4) (strain range: 0.10 – 0.35), contra-lateral (CL) ACL (N=11; strain range: 0.10 – 0.35), BLB constructs in vitro and native PT prior to implantation appear in Figure 2. The tangent modulus of the BLB explants averaged 57.0±44.7 MPa, that of the PT graft explants, 45.0±39.3 MPa, and that of the adult CL ACL, 106.1±28.5 MPa. In terms of geometric stiffnesses, the values found were 122.8±101.0 N/mm for the BLB explants, 91.0±66.6 N/mm for the PT graft explants and 214.8±90.4 N/mm for the CL ACL. Prior to implantation the BLB constructs had a tangent modulus of 1.4±0.14 MPa (N=3; strain range: 0.2 – 0.3). The modulus of native PT prior to implantation was 170.0 MPa. These data indicate that after 9 months in vivo as an ACL replacement the BLB constructs increased in modulus by a factor of over 90 to attain 54% of the tangent modulus and 57% of the geometric stiffness of the adult CL ACL. In contrast the tangent moduli of PT grafts decreased in vivo to 26% of its in vivo value. The mechanical properties of the BLB explants after 9 months in vivo exceeded those of the PT grafts. The tissue-engineered BLB graft appears to have remodeled more fully towards native ACL phenotype at 9 months in vivo compared to the PT graft.

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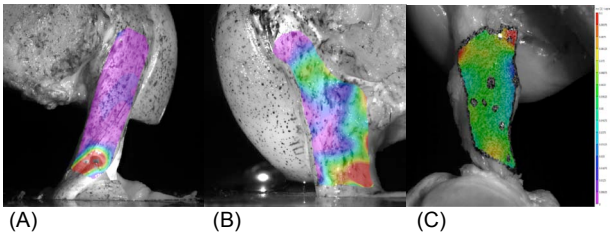

(A) (B) (C)

Figure 1: Strain contour plots of the native ACL (A) (left knee), a BLB explant (B) (right knee) and a PT explant (C) (right knee). The characteristic inhomogeneous strain contours of the native ACL are closely mirrored by the BLB explant whereas the PT explant demonstrates an approximately homogeneous strain field. In all cases red denotes the highest strain level of 0.10 and purple is lowest.

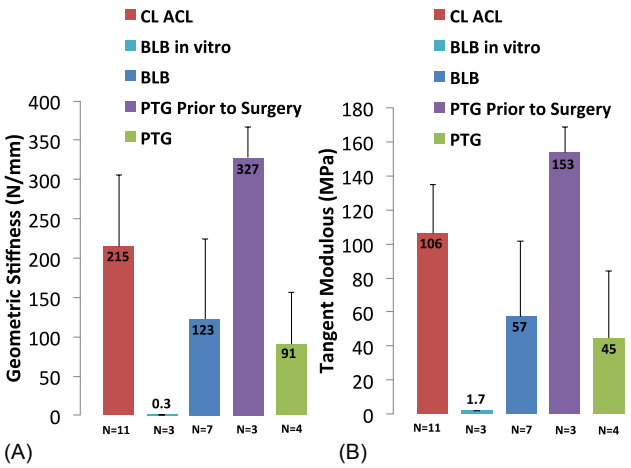

Figure 2. Geometric stiffness and modulus comparisons of BLB (BLB) and PT graft (PTG) explants at 9 months vs. contralateral ACLs (CL ACL) and PTs (CL PT) of adult sheep and in vitro values for the BLB constructs (BLB in vitro). (A) Geometric stiffness of the linear portion of the stress-strain response curves over a strain range of 0.10 – 0.35 and (B) corresponding tangent modulus.