stress state for each bundle by removing the twist and separating the bundles. The current ongoing study is to simulate this bundle separation procedure in a similar fashion in FE analysis to study the pre-tension on the ACL during ATT.

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CHAPTER 7

Conclusions and Future Directions

The research presented in this work has addressed several issues in the design and property characterization of a tissue engineered bone-ligament-bone construct for ACL reconstruction. For my dissertation, I developed a tissue-engineered graft that can restore proper biomechanics to the knee by fully and rapidly remodeling into native anatomy and function. The efficacy of the engineered graft was determined by examining its non-linear viscoelastic responses after 6- and 9-months *in vivo* as an ACL replacement and comparing these to a patellar tendon autograft prior to and after 9-months *in vivo*. With the experimental data obtained, I then developed constitutive models for the non-linear and viscoelastic responses of the native ACL, PT, and tissue engineered grafts. Finally, I characterized the native ACL anatomy and function and developed a finite element model of the ACL and knee joint. With the developed computational model, I simulated the native ACL response to an anterior tibial translation and compared the model results with the experimental results. The key findings of this research are summarized below.

- Native ACL is non-linear, viscoelastic, inhomogeneous and anisotropic. Characterization of native ACL is complicated by the twist in the tissue in anatomical positions and the existence of two bundles within the ACL.

- A non-linear viscoelastic model using non-linear (MacKintosh and neo-Hookean) springs and a linear viscous response, with an assumption of homogenous tissues, can capture the stress response of a native ACL and predict its strain rate dependent response well.

- The finite element model is predictive of the inhomogeneous response of the ACL during an anterior tibial translation test, including pinpointing the failure location.

- Native patellar tendon is homogeneous. After 9 months as an ACL replacement, patellar tendon grafts (PTG) remodel such that their viscoelastic response resembles that of native ACL. However, that remodeling also includes hypertrophic growth and sharp degradation of tissue level mechanical and structural properties. In contrast, tissue-engineered grafts from bone marrow stromal cells rapidly grow and remodel *in vivo* when used as ACL replacements and after 6-9 months *in vivo* have mechanical and structural properties that exceed those of the PTG and approach native ACL properties. The inhomogeneous, non-linear viscoelastic response of our tissue-engineered grafts also closely resembles that of native ACL.

The results presented here are very encouraging for enhancing tissue engineering derived grafts for ACL reconstruction. Some limitations associated with this tissue engineering approach and ongoing or future work that can address these limitations are as follows:

- The cells used to make these engineered BLBs are obtained from bone marrow stromal cells (BMSCs). It is known that BMSCs contain many cell types including mesenchymal stem cells, fibroblasts, osteoblasts, and fat cells [1, 2]. The mixture of different types of cells may result in inaccurate cell density determination. It is known that the extracellular matrix formation significantly correlates with the initial cell seeding density. Therefore, a cell type purification step may be needed prior to seeding these cells onto culture dishes to ensure that the seeding density is accurate.

- The daily activity level of sheep is relatively low. Even though the use of this model has many advantages as an ACL replacement model as described previously, the low activity level may slow down the rate of graft

183

remodeling. In future studies, rehabilitation programs will be developed to induce an activity level in sheep that resembles a human rehabilitation program after ACL surgery.

- Altered knee kinematics due to the ACL replacement affects other intra-articular tissue such as menisci, cartilage, and bone. It is known that the integrity of cartilage and menisci is highly correlated with the risk of OA [3, 4]. In the current ongoing study, we have collected the medial meniscus, cartilage, and MRI of the bone structures. These data will be used to study the sign of bone bruising and early signs of OA.

The present work provided new insights for the understanding of the biomechanical responses of native and engineered ligaments and tendons. However, some of the limitations associated with the current characterization do exist. These limitations and work needed to be addressed and are listed below:

- To this point, we have collected 2D strain contours of tissue during deformation. Because these tissues have a curved surface and are oriented in a 3D spatial configuration, out-of-plane deformations are also important to accurately characterize the biomechanical properties of these tissues. Therefore, future studies will expand the current 2D strain data acquisition to 3D to enable us to get more sufficient information.

- The parameters of the proposed microstructural constitutive model were obtained by fitting a combination of data collected from uniaxial tensile tests and stress relaxation tests from three different strain levels. The model incorporates the physiological structures of collagen and elastin. Therefore, the parameters are potentially measurable. To bridge the gap between the constitutive model and the physiological constitution of tissues, the ongoing study is to obtain these physical parameters from TEM and histological analysis of the tissue.

- The ACL geometry used in the current FEA was generated from physical measurements performed on the actual tissue. With some extrapolation of the measurements, the twisted, double-bundled ACL was generated using

the commercial non-uniform spline based modeling software, Rhinoceros®
4.0. In future studies, we will perform MRI analysis on each ACL bundle to
obtain a more accurate geometry. This study is in preparation.

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