INTRODUCTION:
The anterior cruciate ligament (ACL) is the most frequently injured ligament in the knee, with over 100,000 reconstruction procedures being performed a year in the United States. Patellar tendon grafts have been commonly used to reconstruct the ACL with the tendon graft to bone fixation site being the mechanically weakest region. To improve graft fixation, our approach is to pre-induce the mineralization of tendon grafts and promote graft osteointegration. Simulated body fluid (SBF) soaking is a low temperature processing technique that has commonly been used to form calcium phosphate (CaP) coatings on substrates. It is hypothesized that this process can be used to promote the nucleation of CaP onto collagen fibrils of the tendon graft.

To test this hypothesis, the objective of this study is to apply the SBF method for the induction of CaP formation on tendon grafts. Immersion in a modified simulated body fluid with elevated calcium and phosphate concentration may improve osteointegration and maintain mechanical strength. Different pre-treatments on the tendon samples will also be tested in order to increase tissue porosity by promoting collagen fibril degradation. It is anticipated that increasing the porosity of the tendons prior to immersion SBF will allow for greater infiltration of SBF into the tendon matrix and increase its mechanical properties.

METHODS:

Tissue Harvesting: Patellar tendons were obtained from neonatal bovine knee joints and frozen at -80°C prior to use. Tendon Pre-treatments: Trypsin treated tendons were prepared by immersing tendons in distilled water for 1 hour at 37°C while stirring, which was replaced with 0.05% trypsin in media for 1 hour. Samples were then transferred to serum containing media for 24 hours to halt the action of trypsin. Acetic acid treated tendons were immersed for 20 minutes in 0.5M acetic acid in distilled water at room temperature while stirring. All samples were rinsed thoroughly with water and frozen at -80°C.

Calcium Phosphate Coating: Tendons were coated following serial treatments in SBF A and SBF B, each with 5x the normal concentration of calcium and phosphate ions compared to physiological fluid. For SBF A, 1.0L of water was maintained at 37°C. NaCl, MgCl2-6H2O, and CaCl2 were added while stirring. The pH of the solution was brought down below 4 through bubbling with CO2 gas before the Na2HPO4·2H2O and NaHCO3 were added. The CO2 was left bubbling in the solution until the pH fell below 6.2. The CO2 source was then removed and the tendon samples were immersed for 24 hours. The samples were removed and agitated in distilled water to remove excess calcium phosphate and lyophilized. The SBF B solution is made following the SBF A procedure except that the ion concentrations of calcium and phosphate were increased 5-fold.

Control and SBF Acetic Acid Treatment: Tendons were immersed for 20 minutes in 0.5M acetic acid in distilled water at room temperature while stirring. Acetic acid treatment was performed a year in the United States. Patellar tendon grafts have been commonly used to reconstruct the ACL with the tendon graft to bone fixation site being the mechanically weakest region. To improve graft fixation, our approach is to pre-induce the mineralization of tendon grafts and promote graft osteointegration. Simulated body fluid (SBF) soaking is a low temperature processing technique that has commonly been used to form calcium phosphate (CaP) coatings on substrates. It is hypothesized that this process can be used to promote the nucleation of CaP onto collagen fibrils of the tendon graft.

RESULTS:

Tendon Matrix: Changes in tissue porosity due to the different pretreatments and coating processes are shown using ESEM (Fig. 1, control-top, coated-middle). Both acetic acid and trypsin resulted in greater apparent porosity in the tendon matrix. Distributions of the newly formed mineral on the surface of the tendon samples remain on the surface of the tendon, with a thicker coating formed on the acetic acid treated group (Fig. 1, lower panel).

Calcium Phosphate Formation: crystal structure of the CaP formed was evaluated with XRD (Fig. 2, left). In addition, ESEM images of uncoated and coated samples at 15kV, 500X mag. (top); Von kossa stain on coated samples (5X mag.; bottom) and XRD spectra of CaP coated tendon control, uncoated tendon, and HA standard (left). Comparison of ultimate tensile stress and tensile moduli on all groups (‘coated vs uncoated); *among treatments; p<0.05).

DISCUSSION:
The results of this study demonstrate that immersion in the simulated body fluid resulted in calcium phosphate deposition on the surface of the tendon matrix. Moreover, solution treatment with either trypsin or acetic acid appeared to increase the porosity of the tendon matrix. Both treatment methods resulted in increased penetration of calcium phosphate deposition within the tendon matrix, accompanied by decreased apparent porosity after the coating process. XRD analysis confirms the formation of a semi-crystalline phase of calcium phosphate on the tendon samples. The presence of the two broad peaks in 29 locations 32.0535-34.152, and 46.788-53.5459 (rather than 4 distinct peaks as identified in the ICDD database for HA) suggests that the coating contains both crystalline and amorphous calcium phosphate phases, which is similar to that found in native bone.

While tensile properties of the patellar tendon decreased after immersion in SBF, treatment with either trypsin or acetic acid helped to preserve the graft mechanical properties, with both the ultimate tensile stress and tensile moduli showing no significant difference from the uncoated control tendon. The results of this study collectively demonstrate that SBF immersion coupled with either acetic acid or trypsin treatment may be used to promote tendon mineralization and ultimately promote the integration of tendon grafts utilized for ACL reconstruction. Future work will involve optimization of the tendon pre-treatments to enhance calcium phosphate formation within the tendon matrix, and to evaluate osteointegration of the tendon grafts in vivo.

REFERENCES: