

## Comparison of the Biomechanical and Biologic Properties Between Fresh and Frozen Bone-Patellar Tendon-Bone Allografts

Tyler Perleberg<sup>1</sup>, Maxwell Vidal<sup>1</sup>, Justin Hollenbeck<sup>1</sup>, Taylor Calibo<sup>1</sup>, Anna Laura Nelson<sup>1</sup>, Noah Knezic<sup>1</sup>, Dane Lind<sup>1</sup>, Rio Castro<sup>1</sup>, Johnny Huard<sup>1</sup>, Tyler Uppstrom<sup>2</sup>, Sam Huntley<sup>2</sup>, Matthew T. Provencher<sup>2</sup>

<sup>1</sup>Steadman Philippon Research Institute, Vail, CO, <sup>2</sup>The Steadman Clinic, Vail, CO

[tperleberg@sprivail.org](mailto:tperleberg@sprivail.org)

Disclosures: Tyler Perleberg (N), Maxwell Vidal (N), Tyler Uppstrom (N), Justin Hollenbeck (N), Taylor Calibo (N), Anna-Laura Nelson (N), Noah Knezic (N), Dane RG Lind (N), Rio Castro (N), Johnny Huard (N), Tyler Uppstrom (N), Samuel Huntley (N), Matthew Provencher (5-Department of Defense (DoD) and National Institute of Health (NIH); 1-Arthrex, Inc and Arthrosurface, Inc; 3B-Arthrex, Inc, Joint Research Foundation (JRF), and Arthrosurface, Inc; 8-SLACK, Inc, AANA, AAOS, AOSSM, ASES, ISAKOS, The San Diego Shoulder Institute, and The Society of Military Orthopaedic Surgeons)

**INTRODUCTION:** Anterior cruciate ligament (ACL) injuries account for over half of all knee injuries among active individuals and athletes, particularly female athletes and those in high-risk sports. ACL reconstruction (ACL-R) is the primary method used to repair ACL injuries, but its success is highly dependent on graft selection. Allografts avoid donor site morbidity and reduce operative time, but have higher failure rates, slower incorporation, and delayed return-to-sport compared to autografts. Most patellar allografts utilized in ACL-R are deep frozen, which may disrupt the extracellular matrix and compromising both the biomechanics and cell viability of the soft tissue within the allograft. However, it is unclear if freezing processes affect the biological or mechanical properties of the graft. The objective of this study is to compare the biomechanics, gene expression, and histological properties between fresh and frozen patellar allografts. It is hypothesized that fresh allografts will demonstrate superior failure load, stiffness, cell viability, and gene expression.

**METHODS:** Three fresh and three frozen bone-patellar tendon-bone (BTB) allografts were acquired. The lateral and medial 0.5 cm of the tendon were dissected from patellar to tibial blocks and then sectioned at 1 cm intervals for full tendon analysis. Gene expression was analyzed by isolating RNA from each sectioned sample using TRIzol reagent and performing qRT-PCR for tendon-specific (SMAD3), fibrosis (TGF-β1), and adipose (FABP4) gene targets. Tissue viability and integrity were assessed histologically with H&E and Herovici's stains. For biomechanical testing, each bone block was potted in polymethylmethacrylate and fixed in custom clamps to ensure axial alignment and prevent slippage. The grafts were loaded vertically in a tensile testing machine under uniaxial tension. After preconditioning to 90 N for 5 minutes, load-to-failure was performed at a constant displacement rate of 20 mm/min. Stiffness (slope of the linear region) and failure load (peak force prior to failure) were calculated from load-displacement curves. Testing was performed at room temperature under uniform lab conditions. Results are presented for preliminary data only. Paired t tests will be performed on biomechanical data after the full sample size (n = 12) has been tested.

**RESULTS:** Graphical display of the results can be found in Figure 1. Between fresh (n=3) and frozen (n=3) allografts, fresh allografts had increased SMAD3 (p=0.13) and FABP4 expression (p=0.02), and decreased TGF-β1 expression (p=0.18) as compared to the frozen allografts. Histologically, Herovici's stain located viable cells within the fresh allografts and demonstrated a native collagen fibril organization, while the frozen allografts lacked viable cells and showed a disorganized collagen fibril organization. Additionally, fresh allografts presented with a greater percentage of collagen I and smaller percentage of collagen III than frozen allografts, although this was not significant. Biomechanically, fresh allografts demonstrated 101% higher failure load (Fresh: 1410.8 ± 383.35 N, Frozen: 701.10 ± 288.37 N) and greater stiffness (Fresh: 237.21 ± 36.89 N/mm, Frozen: 183.40 ± 85.11 N/mm).

**DISCUSSION:** The most important findings of this study are that fresh allografts resulted in higher failure load, greater stiffness, and improved cell viability compared to frozen allografts. Transcriptional analysis of fresh allografts showed increased SMAD3 expression, which promotes type I collagen synthesis and extracellular matrix development. Histological analysis aligned with these findings, as Herovici's stain revealed a greater percentage of collagen I and a more organized collagen fibril structure resembling native tissue when compared to frozen allografts. Together, these findings suggest that fresh allografts may retain a more favorable structural architecture, contributing to their superior biomechanical performance. Interestingly, fresh allografts also showed increased FABP4 and decreased TGF-β1 expression, which are typically associated with fatty infiltration, diminished collagen production, impaired cross-linking, and reduced tendon strength. These results are contradictory to the observed biomechanical advantages. A plausible explanation is that the increased SMAD3 expression may compensate for reduced TGF-β1 signaling, as SMAD3 is a downstream effector capable of maintaining or even enhancing the transcription of key tendon matrix genes. Additionally, the upregulation of FABP4 may represent a nonfunctional change that is not significant enough to affect tendon integrity. Further research with larger sample sizes and clinical correlation is suggested to clarify whether fresh or frozen allografts provide superior outcomes in ACL-R.

**SIGNIFICANCE/CLINICAL RELEVANCE:** Clinicians may consider utilizing fresh bone-patellar tendon -bone allografts over frozen allografts in ACL reconstruction, as they may provide higher failure load and improved cell viability, which may lower risk of ACL graft re-tear.

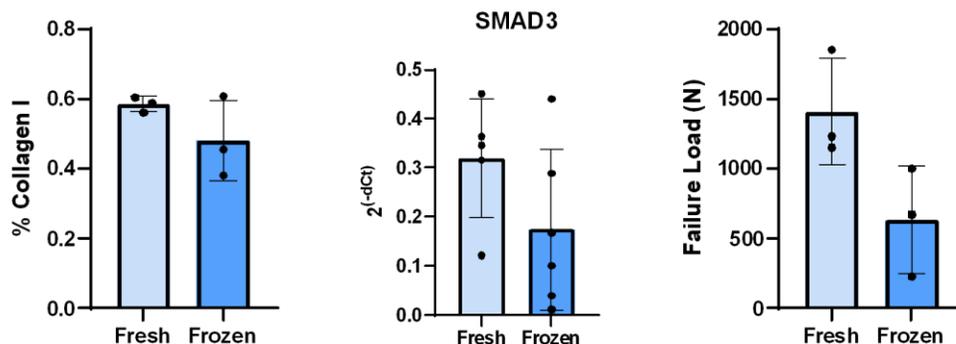


Figure 1. (Left) Herovici's stain of fresh and frozen allografts showed higher collagen I expression in fresh allografts. (Center) qRT-PCR results display a higher trend in SMAD3 expression in fresh allografts. (Right) Biomechanical results demonstrate higher failure load in fresh allografts.