

# ACL Allografts Exhibit a Similar Remodeling Response but Heightened Immune Response to Cyclic Load Compared to Autografts

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**INTRODUCTION:** Anterior cruciate ligaments (ACLs) are one of the most commonly torn ligaments with more than 200,000 people in the United States experiencing an ACL rupture each year<sup>1</sup>. Given the lack of spontaneous tissue repair, reconstruction of the ACL is the standard treatment. Graft options for reconstructing the ACL come in the form of autogenous or allogenic tendon. While outcomes between graft types are similar in the general patient population, the rerupture rate of non-irradiated allografts are 3-4 times higher than autografts in young active individuals<sup>1</sup>. Data suggests that the difference in clinical performance between graft types is due to impaired remodeling in allografts. Graft remodeling is sensitive to mechanical loading, and studies have suggested that allografts and autografts respond differently to mechanical stimulation<sup>2</sup>. Furthermore, fibroblast mechanobiology is influenced by immune cell composition<sup>3</sup>. Since allografts may elicit a heightened immune response as a foreign object<sup>4</sup>, an increased pro-inflammatory immune cell population may be responsible for impaired allograft remodeling and mechanobiology. Despite these data, no study has investigated whether mechanobiological differences exist between cells in allografts versus autografts. Therefore, the **objective of this work** is to compare the response of cells within allograft and autograft reconstructions to mechanical stimuli. We **hypothesized** that allografts will exhibit an impaired remodeling response to load demonstrated by increased catabolic gene expression and a pro-inflammatory immune cell environment compared to autografts.

**METHODS:** Following IACUC approval, a total of 22 male New Zealand rabbits underwent unilateral autograft and allograft ACL reconstruction using semitendinosus tendons following an established surgical model<sup>5</sup>. At 8 weeks post-reconstruction, rabbits were euthanized, and grafts harvested. Reconstructions were placed in a tensile bioreactor with culture media kept at 37°C and 5% CO<sub>2</sub>. After 18 h of acclimating the ACLs to explant culture, the bioreactor cyclically loaded the samples to 2 MPa at 0.5 Hz for 8 h<sup>6</sup>. Control samples were placed in the bioreactor for the same duration but under a minimal (0.1 MPa) static load. **Cell Viability:** After loading, the autograft reconstructions (n = 2-3) were stained with 2 µl/ml fluorescein diacetate (FDA) and 5 µl/ml propidium iodide (PI) in PBS for 10 min to visualize live and dead cells, respectively. Volumetric image stacks were acquired at multiple locations using an inverted confocal microscope. The images were thresholded to determine regions of positive labeling, and cell viability was determined by dividing the area of FDA signal by the total (FDA + PI) signal. **Gene Expression:** After loading, reconstructions (n = 3-5) were rinsed with ice cold RNase free-water and flash frozen in liquid nitrogen. Total RNA was extracted with RNeasy minicolumns, cDNA was synthesized from 10 ng of the total RNA, and real-time PCR was performed using Taqman probes to measure expression of anabolic (*COL1A1*, *COL1A2*, *LOX*, *COL3A1*, *TGFβ1*, *ACTA2*), catabolic (*MMP1*, *MMP2*, *MMP10*, *MMP13*, *TIMP1*, *TIMP3*), inflammatory (*IL-1β*, *PTSG2*), and immune cell (*NOS2*, *MRC1*, *CD4*, *CXCR2*) markers with *GAPDH* as a reference. **Statistics:** Quantification of gene expression was performed via the delta-delta Ct method (after correcting for primer efficiencies) relative to the respective control condition. Mann-Whitney tests were used to determine differential expression of each gene compared to the respective control. Additional Mann-Whitney tests were conducted to compare the gene expression between graft types. Significance for all tests was set at p < 0.05 with statistical trends set at p < 0.15.

**RESULTS SECTION:** Cell viability of the cyclically and statically loaded autografts was 78% and 82%, respectively (data not shown). For the statically loaded samples, allografts trended toward increased expression of *COL1A2* and *TGFβ1* compared to autografts (p = 0.0653) but no differences in immune cell markers (data not shown). With cyclic loading, autografts trended toward increased expression of *TGFβ1* and decreased expression of the T cell marker *CD4* (p = 0.1143) compared to the graft-matched static control (**Fig. 1-2**). Cyclically loaded allografts trended toward increased expression of *CD4* and neutrophil marker *CXCR2* (p < 0.10) compared to the graft-matched static control (**Fig. 1-2**). When comparing the response to load between allografts and autografts, allografts had a significant increase in expression of *CD4* (p < 0.05) and trended toward decreased expression of *TGFβ* (p = 0.1143) (**Fig. 1-2**).

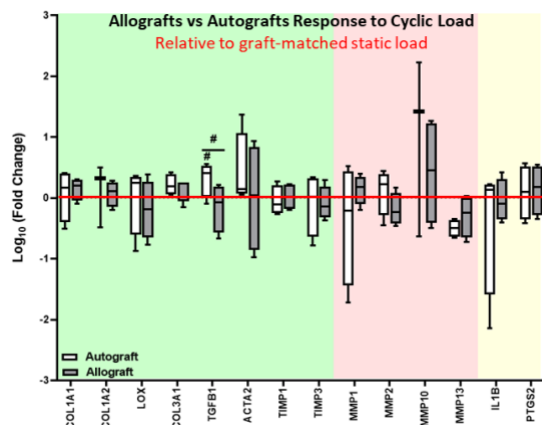
**DISCUSSION:** This study investigated the biological response of reconstructions to cyclic loading in a tissue explant model. Contrary to our hypothesis, preliminary data suggests that there is not a differential anabolic or catabolic gene expression response between graft types. However, in response to load, allografts exhibited an increased response of helper T cell marker *CD4* and trended toward increased expression of neutrophil marker *CXCR2* compared to autografts. Together, these preliminary data suggests that the increased allograft rerupture rate may not be due to an impaired remodeling response to load, but it may be due to a differential immune cell response. A limitation in this study is that 2 MPa may not be sufficient to induce a differential mechanobiological response between graft types. Furthermore, it is not known if the increased expression of *CD4* in allografts is due to increased T cell proliferation or gene expression. Therefore, future studies will compare the immune cell composition between graft types.

**SIGNIFICANCE/CLINICAL RELEVANCE:** Preliminary data provides insight into the mechanobiological response of immune cells in response to load between autografts and allografts. This information could be utilized to help improve allograft reconstruction outcomes through manipulation of signaling pathways mediating the mechanobiological response of cells within allografts (i.e., inhibiting a pro-inflammatory immune response).

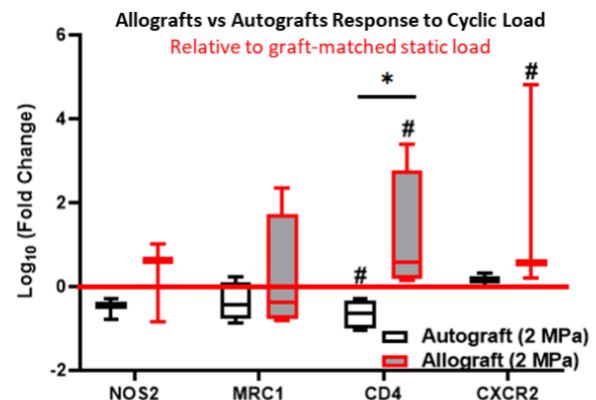
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**IMAGES AND TABLES:**



**Fig. 1:** Expression of remodeling genes in response to cyclic load. Fold change is relative to the graft-matched static control. (# p < 0.15). Data represented as box and whiskers plot with the whiskers representing the min and max data.



**Fig. 2:** Expression of markers of immune cells (NOS2: M1 macrophage, MRC1: M2 macrophage, CD4: helper T-cell, CXCR2: neutrophil) in response to cyclic load. Fold change is relative to the graft-matched static control. (\* p < 0.05, # p < 0.15). Data represented as box and whiskers plot with the whiskers representing the min and max data.