Evaluation of Activated Endothelial Cells in a Murine Model of Patellar Tendinopathy

Authors: Samuel J. Green, BA[1,2], Claire D. Eliasberg, MD[1], Camila B. Carballo, PhD[1], Leonardo Albertini Sanchez, BA[1,4], Rebecca Bell, PhD[3], Nelly Andarawis-Puri, PhD[3], Scott A. Rodeo, MD[1]
[1] Orthopedic Soft Tissue Research Program, Hospital for Special Surgery, New York, NY, USA
[2]SUNY Downstate, Health Sciences University, Brooklyn, NY, USA
[3]Cornell University, Ithaca, NY, USA
[4]Weill Cornell Medical College, New York, NY, USA
Email of Presenting Author: GreenS@HSS.edu
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Introduction: Patellar tendinosis (PT), or "jumper's knee," is a common sports injury characterized by pain and tenderness at the proximal part of the patellar tendon. This is often a recalcitrant problem and there is currently no consensus on the optimal treatment plan.[1] There exists a population of endothelial cells that can supply tissue-specific "angiocrine factors" which can stimulate organ regeneration and repair.[2] These activated endothelial cells (AECs) supply tissue-specific growth factors which may stimulate intrinsic tissue-resident progenitor cells, support cellular proliferation, differentiation and tissue repair and regeneration.[3] Previous studies using AECs have shown promising results for application in acute injuries such as rotator cuff repair; however their efficacy in a more chronic, degenerative injury such as PT is unknown.[4] We hypothesize that tissue-specific AECs will promote healing of patellar tendinopathy in a murine model.
Methods: 19 adult female C57BL6/J mice underwent fatigue loading using an established murine patellar tendinopathy model[5] with the approval of the IACUC at Cornell University. After anesthetic induction, the left patella and tibia were clamped to the fatigue loading device and preloaded with cyclical loading from 0.5 to 1.5 N, at a rate of 2 Hz for 420 cycles, followed by loading at 1 to 2.8 N at 1 Hz for 7200 cycles. Immediately following the procedure and just prior to the closure of the knee, the mice were randomized and 10 were assigned to the AEC cell treatment group and 9 assigned to the control. The AEC group received injections of 100,000 AECs suspended in 2.5µL of PBS in the proximal patellar tendon (1.5µL) and midsubstance (1.0µL) and the control group was injected with an equivalent volume of PBS. Contralateral legs served as non-loaded, unoperated controls. 8 mice (4 experimental and 4 control) were sacrificed at 7 days and were prepared for histological analysis of picrosirius-red stained sections viewed under polarized light microscopy using OrientationJ software to quantify collagen fibril alignment (Fig. 1). Histological samples were blinded and graded quantitatively by two independent graders. 11 mice (6 experimental and 5 control) were sacrificed at 4 weeks and utilized for uniaxial tensile testing. 2 samples had to be excluded from the biomechanical analysis due to failure occurring at the tibial physis. Statistical analysis of the biomechanics and histology data was performed on GraphPad/Prism using one-way ANOVA with Tukey multiple comparison tests.
Results: Biomechanical analysis at 4 weeks showed a significant difference in stiffness (P = 0.038) between the AEC-treated group (10.79N/mm ± 2.25) vs PBS treated group (5.40N/mm ± 2.02) (Fig. 2). Load to failure was not statistically significant (p = 0.059) but the AEC-treated group (6.71N ± 1.25) trended towards a higher load to failure compared to the PBS-treated group (4.08N ± 1.40) (Fig. 2). Histologic analysis showed no significant differences in coherency (measure of fibril alignment) between non-loaded (0.66 ± 0.13), PBS-treated (0.78 ± 0.08), and AEC-treated (0.71 ± 0.06) groups (p = 0.24) (Fig. 3).
Discussion: This study evaluated the potential for a unique population of AECs to stimulate healing in the patellar tendon following induction of patellar tendinopathy in a murine model. We found that injured tendons treated with AECs had superior stiffness when compared to injured control tendons. This suggests that tissue-specific AECs may promote structural healing to the damaged area. However, we did not find significant improvements in collagen alignment. It was expected that naïve tendon would exhibit greater alignment compared to the fatigue loaded groups, suggesting that further dosing regimens and time points should be evaluated. This preliminary study provides pilot data to support the feasibility of this model for evaluation of this novel cell source. Further studies are required in this murine tendinopathy model to determine structural as well as functional effects of AECs in the treatment of tendinopathy, and eventually human studies to evaluate AEC cells in PT. Limitations of this study include the relatively small sample sizes and limited timepoints, which may have underpowered the study.
Significance/Clinical Relevance: Tissue-specific activated endothelial cells are of interest as a potential treatment for patellar tendinopathy, a condition for which we currently have limited treatment options. Our preliminary study suggests that AECs may encourage structural repair of damaged tendon.

References: 1) Rees JD et al. Am. Journal of Sports Medicine. 2009. 2) Ding BS et al. Cell. 2011. 3) Crivellato et al. Journal of Anatomy. 2007 4) Lebaschi et al. OJSM 2017. 5) Sereysky et al. J Orthop Res. 2012.

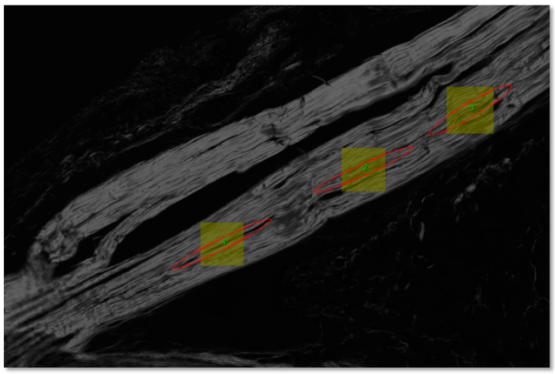

Figure 1: Using OrientationJ, a plugin for ImageJ, as a method of collagen fibril alignment scoring on Picrosirius Red stained patellar tendon.

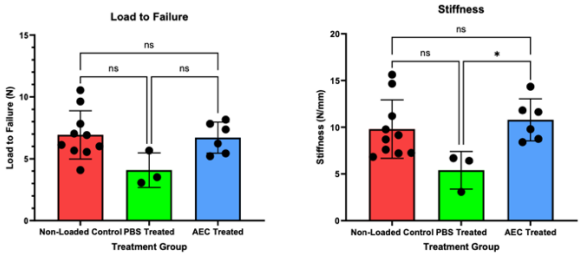

Figure 2: Biomechanical data obtained at 4-weeks timepoint. Tendons were loaded on a uniaxial tensile testing device.

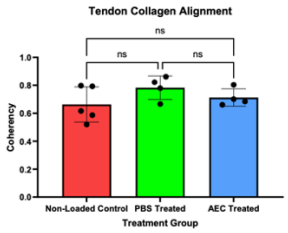

Figure 3: Alignment scores of different treatment groups at 7-days timepoint. Coherency is a measure of the extent of collagen fibril alignment in the major axis of alignment.