Microcirculation of the Rat Achilles Tendon is Dependent on Particle Size

Jarod M. Forer[1], Yan Carlos Pacheco[1], Kait Link[1], Michael E. Hahn[1], Nick J. Willett[1]
[1]University of Oregon, Eugene, OR
jforer@uoregon.edu

Disclosures: J.M. Forer (N), Y.C. Pacheco (N), K. Link (N), M.E. Hahn (N), N.J. Willett (N)

INTRODUCTION: Microcirculation dynamics in peripheral tissues are known to be critical for maintaining tissue homeostasis. However, the tendon is a poorly vascularized tissue with few lymphatic or blood vessels[1]. Fluid accumulation and unresolved inflammation occur in tendon disease and injury, implicating dysfunction of the tendon's innate microcirculation and clearance mechanisms[2]. There is a gap in knowledge concerning the properties of vasculature-based clearance in these tissues. Moreover, the presence and function of lymphatic vessels in healthy tendons is not well understood, to the point where there is no consensus on their existence in tendons[3,4]. New high resolution near-infrared (NIR) imaging has been used to track particle size dependent transport routes in peripheral tissues using small (blood vessel targeting) and large (lymphatic vessel targeting) fluorescent tracers[5]. We employed these technologies to evaluate clearance rates and routes in healthy rat Achilles tendons. The rats were split into two groups (exercise and sedentary) to elucidate the effect of mechanical stimulation as well as tracer size on the transport dynamics. We hypothesized that the small tracers would clear faster than the large tracers, the small tracers would clear through the vasculature while the large tracers would clear through the lymphatics, and that mechanical stimulation through exercise would noticeably increase clearance rates.

METHODS: All procedures were conducted in accordance with IACUC protocol. While under general isoflurane inhalant anesthesia, female Sprague-Dawley rats (Charles River Labs, 3 months old) received bilateral 15 µL injections of tracer particles into their Achilles tendons. Left legs received IRDye 800CW (LI-COR) and right legs received the same dye conjugated to 20 kDa PEG particles. Animals were imaged using an IVIS Spectrum (PerkinElmer/Revvity) to measure the fluorescence from the Achilles tendon region of interest (ROI). Post-injection, half of the animals underwent a 30-minute exercise protocol on customized NordicTrack treadmills at 10 meters/minute. All animals were subsequently imaged with the IVIS Spectrum at progressive timepoints after the injections (1, 3, 6, 24, 48 hours, …). The radiant efficiency was quantified using a manually drawn ROI in the Living Image Software program (PerkinElmer/Revvity), and normalized to the value at 1 hour after injection. These data were used to analyze tracer particle clearance from the ROI over time by fitting with two-phase exponential decay curves. Longitudinal data were analyzed with two-way ANOVA and parameter data were analyzed with one-way ANOVA in Prism (Graphpad), with an alpha level of 0.05. Data are presented as mean ± standard error of the mean.

RESULTS: The experimental protocol had four groups: Small Tracers + Sedentary (n = 7), Small Tracers + Exercise (n = 7), Large Tracers + Sedentary (n = 8), Large Tracers + Exercise (n = 6). Representative images demonstrate the longitudinal imaging and show a decrease in fluorescent intensity with time for both tracer sizes (Fig. 1). Legs injected with the small tracer showed a decrease in fluorescent intensity faster than legs injected with the large tracer. From 1 to 300 hours post injection both small tracer groups were significantly different than both large tracer groups ($p<0.0291$). At no time were the exercise and sedentary groups within a tracer size significantly different ($p>0.4$) (Fig. 2a). At one hour post-injection, the large tracer showed increased fluorescence while the small tracer decreased ($p<0.001$), and there was no immediate effect of exercise ($p>0.48$) (Fig. 2b-c). The parameters of individual two-phase exponential decay curves show significant differences between tracer groups for area under the curve ($p<0.001$) (Fig. 3a), slow time constant ($p<0.001$) (Fig. 3b), and rate constant ($p<0.001$). The fast time constant of the decay curves showed no differences across any groups (Fig. 3c).

DISCUSSION: Our findings indicate that tracer size does impact clearance rates as hypothesized, with the small tracer fluorescent signals decreasing at a significantly faster rate than the large tracer. These data are consistent with previous experiments conducted in other peripheral tissues showing differences in size-based clearance rates and routes in the knee joint[5]. However, contrary to our hypothesis, and data from the knee joint, a single bout of mechanical stimulation delivered by running exercise did not increase clearance rates. Another interesting result described here is that all groups had a similar decay rate in the early time period after injection, meaning that for a period of time after the first hour, both vasculature and lymphatic clearance pathways operated at similar rates. These findings demonstrate that clearance rates in tendon are different than other peripheral tissues, and insinuate that different approaches need to be taken to understand and potentially regulate fluid transport within tendon as it pertains to healing and rehabilitation. A limitation of this study is the mode of exercise used as a hypothesized driver of fluid transport. Eccentric exercise modalities, commonly used for physical therapy, should be considered for future studies. Further work will investigate clearance mechanisms in disease and injury.

SIGNIFICANCE/CLINICAL RELEVANCE: Treatments of tendon injury and diseases depend on how much is known about the healing mechanisms of the tissue. This study investigates an area of tendon mechanics, fluid transport, that is undescribed as of yet, and therefore provides a new avenue for researchers and practitioners alike to investigate potential new strategies for the clinic. This information will aid clinicians in treating tendon injuries and disease.

REFERENCES: [1] Chen, T. M. et al. *Clin. Anat.* 22: 377-385, 2009. [2] Malmgaard-Clausen, N. M. et al. *J Magnetic Reson Img* 54: 832-839, 2021. [3] Tempfer, H & Traweger, A. *Frontiers in Physiology* 6, 2015. [4] Tempfer, H. et al. *Histochem Cell Biol* 143: 411-419, 2015. [5] Bernard, F. et al. *J Biomed Optics* 26: 126001, 2021.

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IMAGES AND TABLES:

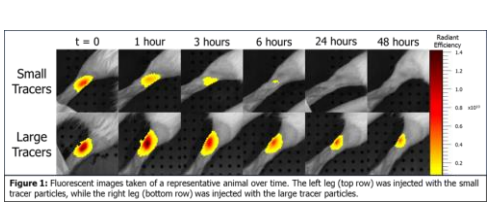

Figure 1: Fluorescent images taken of a representative animal over time. The left leg (top row) was injected with the small tracer particles, while the right leg (bottom row) was injected with the large tracer particles.

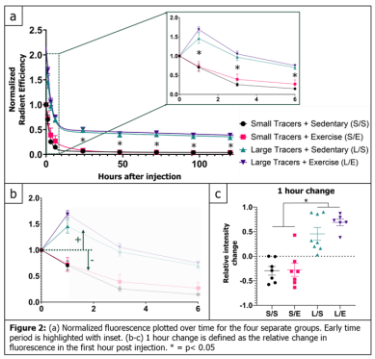

Figure 2: (a) Normalized fluorescence plotted over time for the four separate groups. Early time period is highlighted with inset. (b-c) 1 hour change is defined as the relative change in fluorescence in the first hour post injection. * = p< 0.05

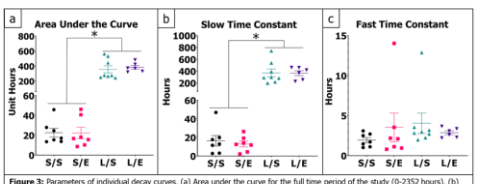

Figure 3: Parameters of individual decay curves. (a) Area under the curve for the full time period of the study (0-2352 hours). (b) Slow time constant of the two-phase exponential decay curve representing the late stage of clearance. (c) Fast time constant of the two-phase exponential decay curve representing the early stage of clearance. * = p<0.05