Modulation of Vascular Response after Injury in the Rat Achilles Tendon Alters Healing Capacity

Introduction
Although vascular ingrowth is necessary for tendon healing, hypervascularization following tendon injury is not always considered beneficial as, for example, degenerated tendons are also highly vascularized. We demonstrated that delivery of VEGF and anti-VEGF antibody locally to tendons can increase and decrease the vascular response after injury, respectively. However, the effect of altering the vascular response after healing in the tendon is unknown. Therefore, the objective of this study was to define the alterations to tendon healing following injection of angiogenic factors. We hypothesize that reducing the vascular response will result in reduced scar tissue formation and reduced failure properties while increasing the vascular response will result in the opposite. Further, we hypothesize that in vivo gait and joint functional measures will not be significantly impacted by vascularity changes.

Methods
Study Design: 90 Fischer 344 rats (4 months old, IACUC approved) underwent a bilateral Achilles incisional injury, followed by local injections of vascular endothelial growth factor (VEGF) (Peprotech), anti-VEGF antibody (B20.4.1-1, Genentech), or saline (SAL). In vivo functional measures and ultrasound imaging were performed, and animals were sacrificed at 7, 14, and 28 days after injury. Injury: A 1.5mm incisional injury in the Achilles tendon mid-substance was created without repair. Injections: On days 4-6 after surgery, each animal received either 5μg VEGF or 250μg anti-VEGF antibody (B20) in 20μl saline, or 20μl saline only, injected bilaterally intratendinously. Imaging: Imaging was performed on days 7, 14, 21, and 28 (n=12/group) after injury using a Vevo LAZR ultrasound system (MS550D and MS250 transducers, VisualSonics). Animals were anesthetized and positioned with the transducer parallel to the tendon long axis. For contrast-enhanced ultrasound imaging, a 200 sec ultrasound clip was initiated at the start of a bolus injection of 100μl Definity (Lantheus Medical Imaging) microbubble contrast agent. The echo-power vs. time data was fit to a perfusion model. Color Doppler images were taken to quantify percent area of signal and blood flow velocity measures.

Results
Ultrasound: The B20 group demonstrated a decrease in contrast peak enhancement, wash-in rate, and wash-in perfusion index at day 14. When evaluating only the injury area, this group also had a decreased wash-in area under the curve at day 14 and decreased rise time at day 28 (Fig1A,B). The VEGF group showed no changes when evaluating the whole tendon, but an increase in rise time (Fig1A) at days 7 and 14 and a decrease in wash-in rate at day 7 in the injury region. There was a decrease in the Doppler mean color level at day 14 (Fig1C), corresponding to blood flow velocity, but an increase at day 21 in this group. Similarly, there was a decrease in Doppler fractional area (Fig1D) and color weighted fractional area in the B20 group in both the whole tendon and at the injury site at 7 and 14 days, but an increase in these properties at day 21 in the whole tendon. Finally, mean color level and fractional area increased in the VEGF group in the whole tendon at day 21 (Fig1C,D). Photoacoustics imaging (data not shown) revealed an increase in blood oxygenation in the B20 group at day.
increase in vessel size, but no change in vessel density from our histology. While we previously demonstrated that VEGF increased the vascular response after injury, these smaller changes could be due to the use of younger animals, potentially with an already robust vascular response to injury. It is also possible that our ultrasound measures are more sensitive to changes in vessel density than vessel size. Ongoing work includes implementing these vascular modifications on aged animals, which could yield larger changes in the case of VEGF delivery.

Significance
A decrease in vascular response after injury reduced mechanical outcome in early healing, which recovered over time. Future studies will evaluate the effect of vascular modulation after injury with aging to potentially determine therapeutics for improved tendon healing in this population.

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References
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