Monitoring Changes in Tissue Perfusion by Micro-bubble Contrast-enhanced Ultrasound in a Rat Model of Tendon Healing

INTRODUCTION:
Tendon is relatively hypovascular as compared to other connective tissues except cartilage. It is suggested that this may contribute to its lower healing capacity. However, anatomical studies revealed significant vascularization in patellar tendons and increased vascular supplies to tendons were detected after acute tendon injuries. Neovascularization is vital for the healing process by resuming supply of oxygen and nutrients to the tissue, it is thus important to measure tissue perfusion in addition to measurement of neovascularization.

Gas-filled microbubbles have been widely used by ultrasonographer to measure blood perfusion in certain organs. Microbubbles have a high degree of echogenicity as comparing to soft tissues; thus the ultrasound backscatter will be enhanced when microbubbles from circulation enter the region of interest. In the present study, we developed the method to measure tissue perfusion by microbubble contrast-enhanced ultrasound imaging in a rat model of tendon healing, and the results will be compared to the extent of neovascularization detected by 3-Dimensional Doppler ultrasound imaging.

METHODS:
Animal surgery
This study was approved by the Animal Research Ethics Committee of the authors’ institution. SD male adult rats of 8 weeks old (n=9) were operated on the right limbs. The central one-third of the patellar tendon (1 x 4mm) was removed from the distal apex of the patellar to the insertion of the tibia tuberosity. The fascia and skin were then closed. Ultrasonographic imaging was performed 1 day before injury was induced, and at 1, 2, 4 and 6 weeks post injury.

Ultrasound imaging
All ultrasonographic imaging were performed with a High resolution ultrasound imaging system (Visualsonics, Toronto, Canada). Before ultrasonographic image acquisition, the rat was anaesthetized with abdominal injection of ketamine/xylazine and the hair over the knee was removed. The rat was positioned with a standardized protocol 7 with a set of acquisition parameters (scanhead RMV712, 75 MHz, focal length; 6mm, field of view: 9mm; 3-D scanning range of 10 mm with a step size of 0.032 mm). A 3-D ultrasound image was acquired first to confirm the positioning to image the rat’s knee including patella, patellar tendon and tibia insertion (Figure 1). A 3-D Doppler ultrasound image was then performed with Wall filter and scan speed set at 2.5 and 2 mm/s respectively in order to detect flow volume with medium flow velocity. Neovascularization was measured as increase in percentage volumetric vascular flow (%) vascularity) within the patellar tendons, with 2 independent ultrasonographers to define the tendon volume of interest (VOI) in the ultrasound images with the Doppler signals turned off.

RESULTS:
Our results showed that significantly decreased tissue perfusion was detected in the injured patellar tendon at day 1 post injury (p =0.007). The tissue perfusion restored to preinjury level at day 14 post injury, but a further decrease of tissue perfusion was noticed at day 28 post injury, which was coincided with a secondary increase in vascularity. At day 42 post injury, both the tissue perfusion and vascularity tended to restore the pre-injury level.

DISCUSSIONS:
A previous study on rabbit model reported that blood flow and vascularity was increased in injured patellar tendon at as early as day 3 post injury.2 With a more advanced kinetic measurement of tissue perfusion by contrast-enhanced ultrasound imaging, our findings showed that despite neovascularization was increased at early tendon healing, tissue perfusion was restored only at a later time point. It is possible that invasion of major blood vessels into the injured tendon did not immediately improve tissue perfusion. We also observed a secondary increase of neovascularization in the healing tendon at day 28 post injury, which was accompanied with a drop in tissue perfusion. Vascular remodeling was implicated at the remodeling phase with a temporary disruption in the local tissue perfusion.

REFERENCES:
1. Fu SC et al. (2010) Monitoring neovascularization in tendon healing by three-dimensional doppler ultrasound imaging. ISL&T, Hong Kong