Analysis of collagen fibrillogenesis of a caprine patella tendon with magic angle imaging

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Introduction
Controversy remains about the length and continuity of collagen fibers in skeletally mature tendons and ligaments1. To date, it has not been possible to non-invasively assess changes to collagen fibers during aging or repair2. The structure of tendon and ligaments (e.g. orientated fibre tracts) are normally not visible on Magnetic Resonance Imaging (MRI). However, due to the magic angle effect, if appropriately scanned in multiple directions, 3D collagen networks can be constructed and 3D tractography may be performed to visualise collagen fiber tracts. A study to assess inter-subject, intra-group variability with magic angle directional imaging of five caprine knees is presented.

Hypothesis
All caprine knees demonstrate a highly aligned collagen fiber orientation along the length of the patella tendon.

Methods
Five healthy caprine knees were scanned in 9 positions to the main magnetic field (B₀). Experiments were performed on a Siemens 3T Verio (Magnetom, Erlangen) with a 12 channel head coil. An isotropic 3D T1 FLASH sequence (TR13ms, TE4.9ms, FOV256mm, BW230Hz) was performed in each position. The volumes were registered, aligned then compared to identify large variations of signal intensity. Segmentation using a thresholding technique identified voxels containing collagen. For each collagen-rich voxel the orientation vector was computed using Szeveerenyi and Bydder’s method3. Each orientation vector reflects the net effect of all the fibers contained within a voxel. The assembly of all unit vectors represents the fiber orientation map. All steps are shown in figure 1. Signal intensity variations were measured from the central midline slice of the 3D T1FLASH images using Fiji for ImageJ4. Voxel orientation maps were produced using Matlab (The MathWorks Inc., Natick, MA, USA) and visualized using ParaView5.

Results
Of the five caprine knees scanned, three had mean bone marrow signal intensities of 61±6 and two had mean bone marrow signal intensities of 82±6 (Figure 2). Hypointense haemopoietic red bone marrow6 (B) in the immature caprine knees and hyperintense yellow marrow7 (C) in the older more skeletally mature caprine knees can be seen. Unfused epiphyseal plates (A) were noted in one of the caprine knees suggesting it was less than 3 months of age.

Discussion
The technique presented here was able to detect age-related change in caprine patella tendons. The five caprine knees ranged in age from less than 3 months (femoral and tibial growth plates, red bone marrow) to more than 3 years (skeletal maturity, yellow bone marrow). The expected outcome of highly aligned patella tendons was only demonstrated in skeletally mature caprine specimens. The mean collagen fibril length increases from birth to maturity8; fibrils in mature ligaments and tendons are known to be either continuous or functionally continuous5.

Conclusion
The study demonstrates the first visualisation of the collagen fibrillogenesis in caprine patella tendon using magic angle imaging. It is now possible to use MRI to improve our understanding of the development and degeneration of collagen rich structures.

References
5Ahrens et al. 2005. Elsevier, Burlington, Massachusetts, USA.

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Figure 1 MRI post processing schema.

Figure 2 A 3D T1 FLASH central slice from three of the five specimens showing the variation in signal intensity of the immature and mature bone marrow. The femoral and tibial epiphyseal growth plates are unfused in the very immature specimen which also has a similar signal intensity to the immature specimen.

Figure 3 Matlab output for the computed collagen orientations in a sagittal, coronal and axial plane for an immature and a mature patella tendon. The mature tendon is more aligned than the immature tendon as can be appreciated from the coronal and axial views.

Figure 4 The ParaView streamline tracts of patella tendons from an immature (6 month old) and a skeletally mature (3 years old) caprine. Note that the fibers are more organised and aligned in the older specimen.