# Imperial College London 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 ligaments<sup>1</sup>. To date, it has not been possible to non-invasively asses changes to collagen fibers during aging or repair<sup>2</sup>. 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 (B<sub>0</sub>). Experiments

The Matlab output for the net voxel vector collagen orientations were computed for an immature and mature patella tendon (Figure 3). Each vector is coloured depending on its direction to assist in visualising the collagen orientations. The mature specimen is much more aligned and organised when compared to the immature specimen.



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 Szeverenyi and Bydder's method<sup>3</sup>. 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 ImageJ<sup>4</sup>. Voxel orientation maps were produced using Matlab (The MathWorks Inc., Natick, MA, USA) and visualized using ParaView<sup>5</sup>.



Figure 1 MRI post processing schema.

### **Results**

Of the five caprine knees scanned, three had mean bone marrow signal intensities of  $61\pm3$  and two had mean bone marrow signal intensities of  $282\pm6$  (Figure 2). Hypointense haemopoetic red bone marrow<sup>6</sup> (**B**) in the immature caprine knees and hyperintense yellow marrow<sup>6</sup> (**C**) in the older more skeletally mature caprine knees can be seen. Unfused epiphyseal plates (**A**) were noted in one of the

A ParaView<sup>5</sup> streamline visualisation in 3-dimensions of the patella tendon collagen tracts of a mature and immature caprine stifle are shown in figure 4. The immature specimen has less aligned collagen fiber tracts whereas the skeletally mature specimen is highly aligned.



# Discussion

#### caprine knees suggesting it was less than 3 months of age.



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 specimens. 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 maturity<sup>7</sup>; fibrils in mature ligaments and tendons are known to be either continuous or functionally continuous<sup>1</sup>.

This technique could be applied to human clinical practice as it has potential to be used to noninvasively monitor healing post-injury and post-surgical repair.

## **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.

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# References

<sup>1</sup>Provenzano & Vanderby. 2005. Matrix Biology. 25:71-84.
<sup>2</sup>Barros et al. 2002. The Spine Journal. 2(1):57-62
<sup>3</sup>Szeverenyi & Bydder. 2011. MRM.65:463–470.
<sup>4</sup>Schindelin et al. 2012. Nature methods. 9(7): 676-682
<sup>5</sup>Ahrens et al. 2005. Elsevier, Burlington, Massachusetts, USA.
<sup>6</sup>Malkiewicz & Dziedzic. 2012. Polish Journal of Radiology. 77(4):45-50.
<sup>7</sup>Craig, et al. 1989. Connect. Tissue Res. 19, 51-62.