INTRODUCTION:
Cartilage transplantation is used extensively in reconstructive surgery. Many cartilage grafts, however, experience post-surgical resorption. Improved understanding of extracellular anisotropy of septal cartilage may prove to be important for understanding the host-graft relationship and the potential for graft resorption.

Collagen anisotropy in articular cartilage has been associated with the depth-dependent physical properties of the tissue [1]. Although septal cartilage is generally assumed to be isotropic, the existence of collagen anisotropies in septal cartilage remains largely undefined and may play an important role in post-surgical resorption of septal grafts.

The current investigation studied septal cartilage in three planes; vertical, medial, and caudocephalic (Fig 1) using microscopic magnetic resonance imaging (µMRI), polarized light microscopy (PLM), and mechanical compression to identify the potential anisotropies of collagen structure within the tissue.

METHODS:
Bovine Nasal Cartilage: Cartilage were obtained from the central region of the bovine nasal septa and analyzed in three orientations and planes (vertical, medial, and caudocephalic, as shown in Fig 1a and 1b).

Microscopic MRI (µMRI): The µMRI experiments were performed on a Bruker AVANCE II 300 µMRI. A solenoid coil, with a rotation device, was used in the experiments. The rotation axis, in the direction of the long axis of the cartilage block, remained unchanged and perpendicular to the static magnetic field (B0) during experiment (Fig 1c). Each sample was rotated 180°, at an interval of 15°. At each angle, a T2 imaging experiment was performed using a CPMG magnetization-prepared T2 imaging sequence. The anisotropy of T2 relaxation was simulated using a fan model in literature.

Polarized Light Microscopy (PLM): Samples for PLM were cut along three orthogonal planes (Fig 1d) and embedded in the OCT before being cryo-sectioned to 6 µm thick. A Leica polarizing light microscope fitted with a CRI Abrio imaging system was used, where the computer controlled compensator allowed the construction of two quantitative images, Angle and Retardation. A 5x objective was used in the imaging, which yielded a pixel size of 2.0 µm.

Biomechanical Protocols: The compressive mechanical properties of septal cartilage were assessed using indentation to measure the load-strain behavior of the tissue. A semi-spherical indenter (0.3 mm diameter) attached to the actuator of an Enduratec ELF 3200 system compressed the sample to a maximum load of 0.1 N. This level of compression was held for 5 min and the relaxation behavior of the tissue was recorded. Each sample was loaded in this manner in each of the three orthogonal planes mentioned previously.

RESULTS:
Fig 2 shows a set of proton intensity images in both sagittal and axial planes and the calculated T2 image in the axial plane, which show that the intensity and T2 images in septal cartilage is rather homogeneous. T2 anisotropy analysis indicates that the collagen distribution in septal cartilage is relatively homogeneous and macroscopically anisotropic, aligned in the medial plane (direction y).

DISCUSSION:
The current study shows marked collagen anisotropy in cartilage from the bovine nasal septum using three multidisciplinary techniques (µMRI, PLM, and mechanical compression). The existence of such anisotropy bears critical relevance to clinical procedures where collagen misalignment in cartilage grafts may hinder the long-term success of the transplant.

REFERENCES:

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