Introduction

Osteoarthritis (OA) is the most common form of arthritis and the leading cause of disability among elderly. It is a multicomponent disease characterized by articular cartilage degeneration, but also affecting surrounding joint tissue including ligaments [1]. Little is known about the role of ligaments in OA. However, trauma to the ligament has been closely linked to OA in humans, and is also seen in OA animal models [2].

Aim: To study the markers and mechanical properties of the anterior cruciate ligament (ACL) during disease progression in spontaneous and posttraumatic OA.

Methods

• Histological sections of 3 mouse knee OA models: STR/orient mice, C57Bl/6 mice following DMM surgery, and CBA mice following non-invasive knee trauma [3]. Samples were stained with Tol. Blue.
• μCT and 3D models. Knee samples were imaged with μCT (1% PTA) to determine area and angle. 3D models were created with MATLAB and Mimics.

Results

Histology staining showed changes in the ligaments which could be consistent with endochondral ossification. IHC showed collagen type II deposition in different locations in both loaded and STR/orient model. Sox9 expression was also noted in the tibial attachment region of both OA mouse models. μCT images showed ACL orientation and cross-sectional area, and allowed us to create 3D models to be used for further mechanical analysis. Mechanical testing optimization showed viscoelastic behavior at different loading rates. (E) Tangent modulus increased over stress and varied with different loading rates.

Histology staining of murine cruciate ligaments during OA development. (A) WT anterior cruciate ligament shows low matrix staining and alignment. In STR/orient (B-D) and after DMM surgery (D) there is an increase in Toluidine Blue or Safranin O, disorganization of the matrix (C) and hypertrophy of cells (C-D).

Immunohistochemistry

WT, non loaded

Loaded 2+4 wks

Loaded 2+14 wks

WT, CBA, 40+

STR/orient, Grade 1

STR/orient, Grade 5

Fig. 3. μCT images (A-C), and 3D model created with Mimics (D). Gait analysis (E) was done with MATLAB to measure knee flexion.

Fig. 2. IHC staining of murine cruciate ligaments from non-invasive loading (A-B) and STR/orient mice (C-D). Scale bar is 50μm.

(A) Co2 deposition was found in the non-invasive loaded mice, near the attachment site and in the mid-region of the anterior cruciate ligament. Expression continued at 14 weeks.
(B) Sox9 expression was found in the trauma loaded mice in the tibial attachment site at both time points.
(C) In the STR/orient mice Co2 deposition was also seen in the attachment site of the ACL, which was not expressed in the aged-matched CBA control. The Co2 expression remained throughout OA progression (OA Grade 1 to OA Grade 5).
(D) Sox9 expression was also seen in the attachment site of the STR/orient mice at all OA grades, similar to the results from the non-invasive loaded OA models.

Conclusions

Histology staining showed changes in the ligaments which could be consistent with endochondral ossification. IHC showed collagen type II deposition in different locations in both loaded and STR/orient model. Sox9 expression was also noted in the tibial attachment region of both OA mouse models. μCT images showed ACL orientation and cross-sectional area, and allowed us to create 3D models to be used for further mechanical analysis. Mechanical testing optimization showed viscoelastic behavior of WT murine ACLs. The full extent of these changes along with the consequences to ligament function and OA remains to be seen.

Acknowledgments

Many thanks to my advisers and to Dr. Keenan and Dr. Ashraf Khuraz and Ashkan Mohammadavali for help in the lab.

This project was funded by the Institute of Ageing and Chronic Disease

References