Murine Cruciate Ligament Pathology During Osteoarthritis Development

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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/ort mice**, C57Bl/6 mice following **DMM surgery**, and CBA mice following **non-invasive knee trauma** [3]. Samples were stained with Tol. Blue.
- Immunohistochemistry (IHC) was performed at different progression stages; markers included cartilage matrix (collagen type II, and sox9).
- 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
- Mechanical testing clamp was designed using ProEngineer to test femur-ACL-tibia complex with an Instron (10N load cell).

Results

Histology

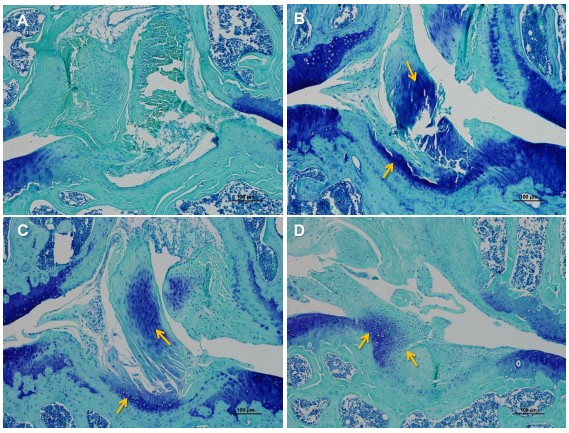


Fig 1. Histology staining of murine cruciate ligaments during OA development.

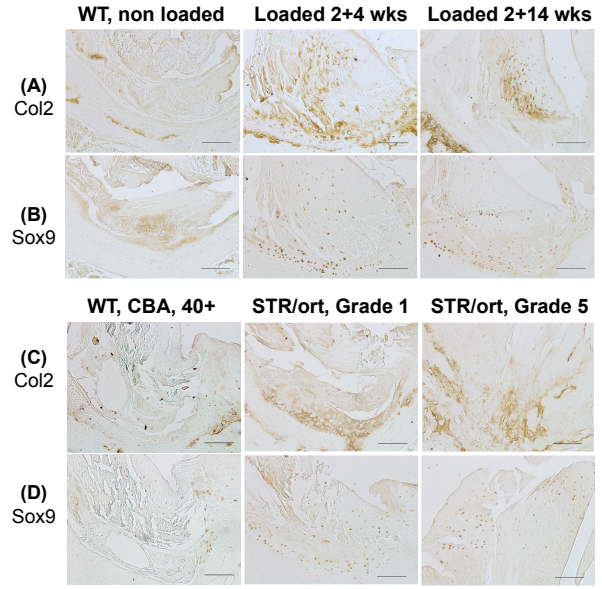
(A) WT anterior cruciate ligament shows low matrix staining and alignment. In STR/ort **(B-C)** and after DMM surgery **(D)** there is an increase in Toluidine Blue or Safranin O, disorganization of the matrix **(C)** and hypertrophy of cells **(C-D)**.

Immunohistochemistry

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

(A) Col2 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/ort mice Col2 deposition was also seen in the attachment site of the ACL, which was not expressed in the aged-matched CBA control. The Col2 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/ort mice at all OA grades, similar to the results from the non-invasive loaded OA models.



Mechanical Tests

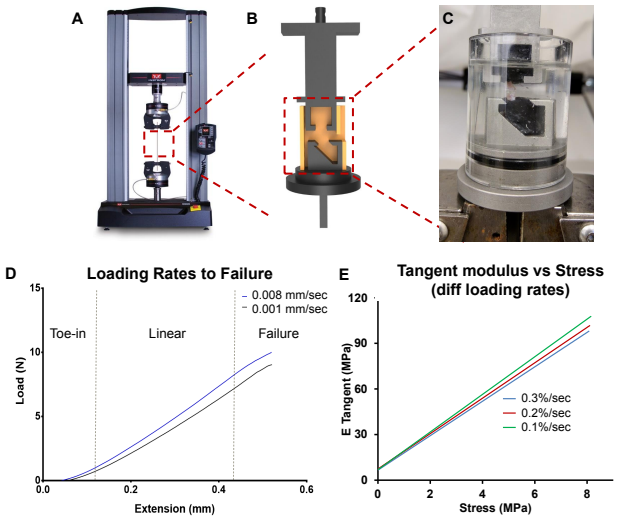
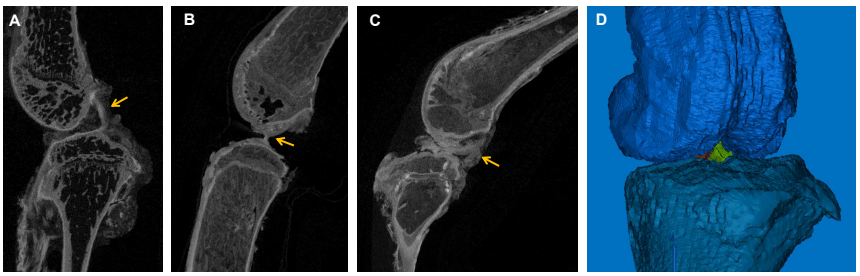


Fig 4. Testing set up **(A-C)**, and preliminary results **(D-E)**.

(A) Instron machine, **(B)** clamp design and **(C)** final set-up with femur-ACL-tibia at 45º angle under 37ºC PBS. **(D)** Load and extension showed viscoelastic behavior at different loading rates. **(E)** Tangent modulus increased over stress and varied with different loading rates.

µCT and 3D Models



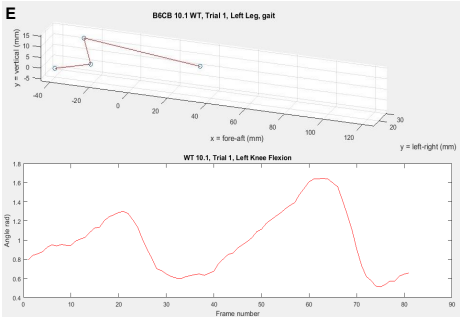


Fig 3. µCT images **(A-C)**, and 3D model created with Mimics **(D)**. Gait analysis **(E)** was done with MATLAB to measure knee flexion.

(A-C) µCT images at different angles (0º, 45º, and 60º), the ACL is indicated by the yellow arrow. From these scans a **(D)** 3D model was created using Mimics, which can be used for mechanical testing. **(E)** Another 3D model was also created of the knee flexion during gait. Preliminary results of knee flexion can be seen in radians over time. This data was used to optimize and justify our mechanical testing setup seen in Figure 4. The Mimics 3D model can be used for further mechanical analysis.

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/ort 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

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