Morphometric and Functional Analysis of Healthy and ACL Deficient Rat Knee Joints

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Summary

Rodent models of the knee joint are often used to explore the effects of injury on musculoskeletal health due to their similar anatomy and genetic tunability. Yet, analysis of these models is hindered by prior experimental limitations in measuring 3D morphometry and multiaxial joint function. This work leverages innovations in high-field magnetic resonance imaging (MRI) and multiaxial robotic testing to better assess the implications of anterior cruciate ligament (ACL) injury in the rat knee. Increases in multi-directional laxity were observed in the ACL deficient knees, highlighting the importance of assessing multiaxial functional analyses.

Introduction

Rat models are critical to understanding risk factors, injury mechanisms, and healing responses of musculoskeletal injuries [1]. For example, the ACL rupture model highlights how joint instability leads to an increased risk of developing post-traumatic osteoarthritis [1]. Current techniques in large animal models employ MRI and multiaxial robotic testing systems to comprehensively characterize knee structure and function [2]. However, these techniques have yet to be appropriately scaled for use in rodent models. Therefore, the objective of this study was to 1) leverage a 9.4T MRI to measure rat knee joint morphometry and 2) employ a 6-degree of freedom (DOF) force sensing system to quantify changes in knee function following ACL transection.

Methods

Knee joints were dissected from 5-month-old female Long-Evans rats and stored in saline-soaked gauze at -20°C prior to imaging (n = 10) and mechanical testing (n = 5). Knees were thawed and imaged in a Bruker Biospec 94/20AV 9.4T MRI machine (0.05mm^3 voxels). Images were segmented and 3D models of soft tissues were generated for morphometric analysis (i.e. cross-sectional area (CSA), meniscus height and width) as previously described [3]. Next, soft tissues were dissected away from the joint, set in custom printed clamps, wrapped with saline-soaked gauze, and stored at -20°C.

Robotic testing was performed using a tabletop robotic system composed of a Meca 500 robotic arm (Mecademic), a multiaxial load cell (ATI Nano 25 IP65), and commercial software (simVITRO). Briefly, the knee was mounted on the robot, anatomical coordinate systems were established, and the joint was preconditioned. The intact joint was set to 40° of flexion and manipulated through anterior-posterior (AP; peak load: 2.5N) and varus-valgus (VV; peak torque: 0.025Nm) paths under hybrid kinetic-kinematic control. Following this protocol, the "intact path" was repeated in kinematic control. The ACL was transected, and the protocol was repeated to establish the "injured path." Lastly, the "intact" and "injured" paths were repeated in kinematic control on the injured knee to assess ACL contributions. Statistical analysis included ANOVAs to compare tissue morphological parameters and tissue function between injury states ($\alpha = 0.05$).

Results and Discussion

There was a tissue-dependent relationship in ligament CSA (Fig 1A; $p < 0.05$) and an anatomical location-dependent relationship in meniscus width (Fig 1B; $p<0.05$). Application of an AP force resulted in 2.02mm±1.30mm, or a 141% increase in anterior tibial translation (ATT) in the ACL deficient knee, from a baseline ATT of 1.43±0.79mm (Fig 1C). Similarly, the VV torque resulted in 13.9°±8.1°, or a 119% increase in rotation in the ACL deficient knee. Increases in AP translation and VV rotations highlight the ACL as a pivotal stabilizer of the knee in the sagittal and coronal planes. Ongoing work is focused on expanding the current cohort of animals to compare how common injury types (e.g. ACL and meniscus tear) affect knee joint structure-function in rodents.

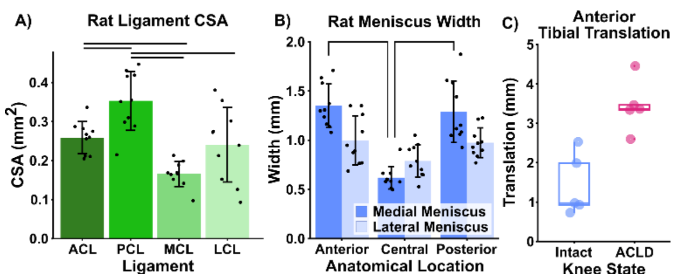

Figure 1: Normative rat A) ligament CSA B) meniscus width, and C) biomechanical function before and after injury. Points represent individual specimens, error bars represent standard deviations, bars represent significant differences ($p < 0.05$).

Conclusions

High-field MRI revealed tissue- and region- specific morphometry and 6-DOF robotic testing highlighted decreases in whole joint stability after injury. These findings establish a comprehensive understanding of rat knee structure and the acute changes in mechanical stability after injury.

Acknowledgments

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References

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