Biomechanics And Micro-ct Scanning Of Normal Mouse Knee Ligaments

Camila Carballo, MSc, Xiangyu Gu, MSc, Zoe Album, Scott Rodeo Jr, Michael Mosca, Arielle Hall, Hongsheng Wang, Lilly Ying, DVM, Xiang-Hua Deng, MD, Scott Rodeo, MD.
Hospital for Special Surgery, New York, NY, USA.


Introduction: Ligaments and tendons are fibrous composite biomaterials that are critical to normal joint stability and movement, respectively. The native tendon or ligament insertion site to bone is a highly specialized and organized tissue that functions to transmit complex mechanical loads from soft tissue to bone. The structure and composition of the native enthesis is not recapitulated during healing of tendon to bone, resulting in a mechanically and structurally inferior interface that may predispose tendon-to-bone repairs to an increased rate of failure (Brophy RH, 2011). The basic cellular and molecular mechanisms of ligament and tendon healing remain relatively poorly understood. Our long-term goal is to establish the feasibility of using a mouse model of ligament reconstruction, to enable future studies using transgenic animals.

Methods: After approval by our Institutional Animal Care and Use Committee, 30 male inbred wild-type (C57B16) mice, 12 weeks of age, weighing between 25-30g, were euthanized using carbon dioxide and each mouse hind leg was carefully dissected from surrounding tissues to keep the ligament of interest intact. The tibia and femur (n=28) were embedded in cement and were tested into a custom-designed materials testing system (MTS) and the load-to-failure data were recorded. The two remaining animals were evaluated via micro-computed tomography (micro-CT).

Results: The anatomical and structural analyses of the mouse knee ligaments (ACL, PCL, LCL and MCL) as well as knee joint and bone (tibia, femur and patella) structure are similar to that of human knee; however, there are some minor differences. We were able to observe by the micro-CT in a mouse knee the anatomical similarities in the shape of the tibia and femur. We found that the curvature of the femoral condyles increases markedly posteriorly and there is a distinct intercondylar fossa. There are normal appearing medial and lateral menisci. Unlike the human knee, the intercondylar area of the mouse tibia forms a distinct depression and the persistence of the growth plate (seen on the tibial side) in the long bones forming the knee joint seems to represent a general feature of the mouse and rat knee (Hildebrand C.,1991). The similar pattern to that in the rat of the lateral-anterior face of the tibia and to the extensor digitorum longus muscle has also been found in mouse. This muscle bridges the knee joint, originating from the lateral femoral epicondyle and likely plays a role in knee stability. Micro-CT measurements of the dimensions (length and width) of the mouse femoral condyle found the following: 4.9mm - sagittal and 2.5mm - coronal plane, and tibial plateau (6.1mm - sagittal and 6.8mm -coronal). The biomechanical testing showed that the anterior cruciate ligament (ACL) had the highest failure force among the four ligaments, the posterior crucial ligament (PCL) and the medical collateral ligament (MCL) had approximately half the strength of the ACL, and the lateral collateral ligament (LCL) had the lowest failure load. The ACL, PCL, and MCL seemed to have similar stiffness, while the LCL had lower stiffness than the other ligaments. All ACL and PCL samples failed at the mid-substance. Most MCL specimens failed at the tibial insertion, while all LCL specimens failed by fibular avulsion. Micro-CT image of the
normal mouse knee showed clear structure of epiphyseal bone on both femoral and tibial sides and a
growth plate on the tibial side.

Discussion: The four ligaments of the mouse knee joint are analogous to the human knee and they are
responsible for its stabilization and as a consequence are essential to preserve its function. The ACL and
PCL are intra-articular ligaments, their primary function is to prevent antero-posterior translation and to
resist internal/external rotation of the tibia, respectively. The ACL is the strongest compared to the
other 3 and the PCL seems to have the same strength that the MCL. If one or both of the cruciates are
disrupted, the biomechanics of gait may be impaired (Chhabra A., 2001). The medial and lateral stability
is achieve mainly through the collateral ligaments, which are integrated in the joint capsule. The MCL
courses straightly distally from the medial femoral epicondyle to the tibia and the LCL extends between
the lateral femoral epicondyle and fibular head, which may explain why they failed in our test by fibular
avulsion. We also found that the LCL was the weakest and least stiff of the ligaments with approximately
half the strength of the PCL and the MCL; however, MCL injuries are more frequent than the LCL in
humans and it was significant lower than the literature report (Warden S.J., 2006). Several recent
reports discuss the role of joint ligaments in bone-tendon healing. Many of these are based on studies in
dog (Rodeo S.A., 1993), rabbit (Panni A.S., 1997) and rat (Hettrich CM, 2014) models, which have been
used to examine the bone-tendon healing after ACL reconstruction. To our knowledge, no one has used
a mouse models to investigate insertion site healing. Our study demonstrated the feasibility of using a
mouse model to study ligament healing. This initial report may provide a new bridge to further studies
considering the mouse for surgical models.

Significance: Based on our knowledge this is the first study to present data on the anatomy of the
normal mouse knee joint and to demonstrate the feasibility of using murine models to study ligament
healing.

<table>
<thead>
<tr>
<th>Type</th>
<th>Failure force (N)</th>
<th>Stiffness (N/mm)</th>
<th>Failure site (number of samples)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACL(n=7)</td>
<td>5.60 ± 0.75</td>
<td>3.44 ± 1.47</td>
<td>substance (7)</td>
</tr>
<tr>
<td>PCL(n=7)</td>
<td>3.33 ± 1.45</td>
<td>3.99 ± 0.98</td>
<td>substance (7)</td>
</tr>
<tr>
<td>MCL(n=7)</td>
<td>3.45 ± 0.84</td>
<td>3.02 ± 1.08</td>
<td>tibia insertion (5); substance (2)</td>
</tr>
<tr>
<td>LCL(n=7)</td>
<td>1.44 ± 0.37</td>
<td>1.35 ± 0.87</td>
<td>fibular avulsion (7)</td>
</tr>
</tbody>
</table>
Failure Force (N)

ACL  PCL  MCL  LCL