Establishment of a Reproducible Rat Model to Evaluate the Roles of Synovium and Infrapatellar Fat Pad in Meniscal Injury and Healing

Ming Wang, Ting Li, Chang H. Lee
Regenerative Engineering Lab, Columbia University Irving Medical Center, New York, 10032, NY
mw3908@cumc.columbia.edu

Disclosures: Disclosures: Ming Wang(N), Ting Li (N), Chang H. Lee (N)

INTRODUCTION: Meniscal injuries are common in clinical practice, particularly among physically active individuals. Although arthroscopic surgery remains the mainstay treatment, its long-term efficacy is often limited by the meniscus's poor vascular supply, especially in the inner avascular zone, where intrinsic healing capacity is minimal. Our *ex vivo* knee joint model demonstrated potential interaction between the meniscus, infrapatellar fat pad (IFP), and synovial tissues, suggesting that these peri-meniscal structures might influence meniscal healing. To further validate these findings *in vivo*, we aimed to establish a rat model to investigate the effects of the IFP and synovial tissues on meniscal healing. This study also sought to assess the feasibility, reliability, and reproducibility of the surgical techniques used to develop this model.

METHODS: Forty male SD rats were randomized into five groups (n=8/group). The groups consisted of a sham group, where only arthrotomy was performed without meniscal injury; a control group, in which a standardized full-thickness meniscal tear was created; a synovium cryoablation group, in which the meniscal tear was followed by targeted cryoablation of the synovial tissue; a fat pad resection group, in which the meniscal tear was followed by surgical removal of the IFP; and a combined intervention group, in which both synovium cryoablation and fat pad resection were performed following meniscal tear creation. The left knee joint was approached through a medial parapatellar incision, and the patella was gently dislocated laterally to expose the anterior compartment of the knee. A full-thickness longitudinal tear was created on the anterior horn using a scalpel, following the same orientation and depth across all animals to ensure consistency. For synovium cryoablation, a stainless-steel forceps frozen with liquid nitrogen was applied to the targeted synovial region until tissue blanching was observed, indicating localized ablation. For fat pad resection, the IFP was carefully excised using fine microsurgical scissors under direct visualization, preserving the surrounding meniscal and ligamentous structures. In the combined group, both procedures were performed sequentially after the creation of the meniscal tear. Upon completion of the assigned intervention, the joint capsule and skin were closed in layers. At 2- and 4-weeks post-surgery, animals were euthanized, and the medial menisci were harvested for gross morphological and histological evaluation. After careful dissection and removal of adhering soft tissues, the specimens were examined under a stereomicroscope to assess tissue integrity, tear morphology, and overall healing. To visualize healing outcomes and surface irregularities, the specimens were stained with India ink. Gross morphological images were captured through the eyepiece of a dissection microscope. All procedures were approved by IACUC.

RESULTS SECTION: Gross evaluation of India ink-stained medial menisci revealed distinct differences in surface integrity and tear morphology among groups and across recovery time points. The India ink staining clearly highlighted surface irregularities, enabling visualization of tear margins and degenerative changes. At two weeks post-surgery, the tear sites were clearly distinguishable across all experimental groups, appearing as clefts within the anterior horn of the medial meniscus. In the control and fat pad resection groups, early reparative tissue was observed partially filling the tear gaps, with thin, translucent bridging across some portions of the lesion. In contrast, no visible reparative tissue was detected in the synovium cryoablation or combined synovium cryoablation and fat pad resection groups, where the tear margins remained widely separated. The combination group exhibited particularly evident fissures with deep, darkly stained grooves, indicating persistent surface disruption and lack of integration. The surrounding meniscal surface in these groups appeared rough, uneven, and irregularly stained compared with the smoother, more homogeneous surface seen in the sham specimens. By four weeks, the sham menisci retained an intact appearance, while the control group still showed a visible tear line but without progression of degenerative changes, suggesting stabilization of the lesion site. In the synovium cryoablation group, however, severe degenerative changes were evident, including deep clefts, expanded defects, and partial fragmentation of the adjacent meniscal tissue. The fat pad resection group displayed areas of surface irregularity and mild deformation at the tear site; although the original cleft was no longer clearly identifiable, dense scar-like tissue was present, accompanied by localized depression and distortion of the meniscal anterior horn. The most pronounced pathology was observed in the combined synovium cryoablation and fat pad resection group, which exhibited extensive structural deterioration of the inner meniscal edge, widespread fissuring, and large surface defects without repair tissue. The meniscal surface in this group appeared grooved and discolored, consistent with advanced degeneration and poor healing response. These macroscopic observations were consistent in each group, suggesting the reproducible surgical interventions. A comprehensive histology, immunohistochemistry, cytokine assays, and scRNA-seq analysis are underway for the recently harvested *in vivo* samples.

DISCUSSION: This study confirmed that the established *in vivo* rat model of meniscal injury with targeted modification of peri-meniscal tissues is both viable and reproducible. All surgical procedures were technically feasible and well tolerated, with no postoperative complications observed, demonstrating the model's safety and stability. Distinct gross morphological differences were observed among groups, supporting the influence of the synovium and infrapatellar fat pad on meniscal healing. We are currently working on quantitative analysis, comprehensive cytokine/gene assays, and histomorphometry, to describe specific roles of synovium and IFP in progression of degenerative changes in meniscus injury and healing process.

SIGNIFICANCE/CLINICAL RELEVANCE: Understanding the contribution of synovium and IFP to meniscal injury and healing may help optimize surgical and regenerative strategies by preserving or restoring these supporting structures to enhance healing outcomes after meniscal injury.

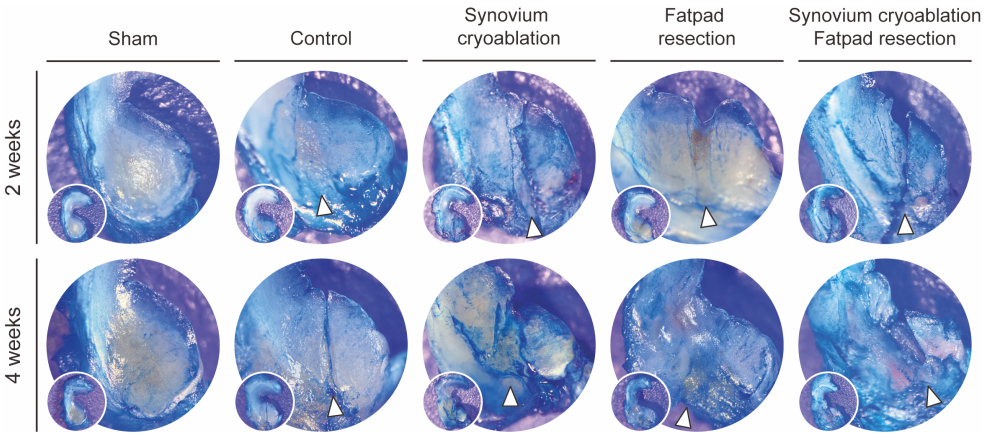

Figure 1. Gross evaluation of the medial meniscus following India ink staining at 2 and 4 weeks after surgery. Representative images from each experimental group are shown: sham, control (meniscal tear only), synovium cryoablation, fat pad resection, and combined synovium cryoablation with fat pad resection. White arrowheads indicate the location and orientation of the meniscal tear.