

## Assessment of healing of grade III posterolateral corner injuries: an in vivo model

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### Abstract

The primary purpose of this study was to test the hypothesis that an in vivo model of posterolateral knee instability could be created in the rabbit and to develop a natural history model in animals.

The biomechanical and gross features of the rabbit knee 12 weeks after rupture of the fibular collateral ligament (FCL) and popliteus tendon were investigated in 14 skeletally mature New Zealand white rabbits. In the operated leg both the FCL and popliteus tendon were traumatically ruptured near their respective femoral insertions and the contralateral leg served as the control. At 12 weeks, the legs were removed for analysis of healing by both gross analysis and by biomechanical testing of knee joint stability. Biomechanical testing of varus–valgus knee rotation as well as concurrent coupled external rotation was performed to measure the amount of force necessary to produce a uniform amount of displacement.

Grossly, only one of the FCLs and none of the popliteus tendons healed. Biomechanical testing revealed a statistically significant difference in the amount of force necessary to achieve 10 mm displacement for the operative versus the contralateral control knee for varus at 30° ( $p < 0.001$ ), 60° ( $p < 0.006$ ), and 90° ( $p < 0.01$ ).

Our data supports the clinical observations of human grade III posterolateral corner (PLC) injuries, which appear to undergo minimal healing and generally result in poor outcomes with conservative treatment, that the FCL or popliteus tendon rarely heal when torn. This initial study of healing and knee stability after PLC injuries in rabbits supports the further study of in vivo animal models for evaluation of posterolateral knee injuries.

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**Keywords:** Posterolateral knee; Fibular collateral ligament; Popliteus tendon; Knee instability

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### Introduction

The complexity of the posterolateral corner (PLC) of the knee has become better understood over the past several years. While isolated PLC knee injuries are uncommon, PLC instability with concomitant injury to the anterior or posterior cruciate ligament is more commonly encountered [18,24,26,27]. These PLC injuries can be difficult to identify in the face of other ligamentous injuries. O'Brien et al. noted that undiagnosed PLC injuries are not only a cause of frank instability, but are also a major cause of failure of ACL reconstructions [27]. Research on cadaveric knees has shown increased forces on ACL and PCL grafts after a PLC

injury [12,23,22]. The inherent bony instability of the lateral aspect of the knee created by a convex lateral femoral condyle articulating with a convex lateral tibial plateau, combined with a very mobile lateral meniscus (compared to the medial meniscus) [34] may explain why complete injuries to the PLC do not appear to heal when completely torn [16,17].

There has been a relatively limited number of studies looking at the healing of posterolateral structures in humans. Kannus noted that grade III injuries to the fibular collateral ligament (FCL) treated nonoperatively resulted in severe persistent laxity concurrent with muscle weakness and development of osteoarthritis [17]. Animal studies looking directly at the healing of posterolateral structures after injury are even more rare. In one study performed four decades ago [28], a canine model, in which the FCL was transected without subsequent repair, demonstrated that only diffuse scar tissue formed in its place. The same pattern was recognized

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whether or not the operated legs were casted. To our knowledge, this is the only study in the literature [28] utilizing an animal model to assess healing of the posterolateral structures.

Despite increased research on the anatomy, biomechanics, and diagnosis of PLC injuries, the management of these injuries is still debated [8,10,11,13,14,24,19–22,29,31,30,32,33,35]. This is likely compounded by the fact that PLC injuries commonly occur with concomitant injuries to other structures, allowing a great number of different presentations [16–18,24,26]. The wealth of data on injuries to the medial collateral ligament (MCL) in animal models has provided useful information to our understanding of the healing of this ligament, and ligament healing in general, and prudent clinical management of MCL injuries [1,3,7,6,9,15,36–38]. However, there has not been an animal model for PLC injuries. A recent study has demonstrated that the rabbit knee has similar attachment sites and structure courses as the human posterolateral knee [4]. With this in mind, our goal was to develop an *in vivo* animal model of posterolateral knee instability to help determine the natural history of posterolateral knee injuries and to potentially allow for future testing of posterolateral knee treatment methods.

## Materials and methods

### Posterolateral knee surgery

Approval for this study was first obtained from the University of Minnesota Animal Institutional Review Board (#0109M07901). Fourteen young adult New Zealand white rabbits weighing an average of 4.3 kg (3.9–4.8 kg) were used in our study. This number was chosen as we originally anticipated some rabbit deaths and we anticipated needing a minimum of 10 rabbits to determine if a significant increase in instability could be seen in rabbits with PLC injuries. The animals were anesthetized with 3.5 cc of an intramuscular injection of 80% ketamine, 8% xylazine, and 12% acepromazine.

Each rabbit had one knee operated on, and the contralateral knee served as a control. We alternated between right and left knees so seven had right knee and seven had left knee operations. With the knee in approximately 60–70° of flexion, a 4 cm longitudinal incision was made approximately 1 cm lateral and parallel to the patellar tendon to expose the posterolateral structures (Fig. 1). The biceps fascia, which is the broad attachment of the biceps muscle to the anterolateral aspect of the knee, was incised and retracted posterolaterally to expose the FCL.

To create a traumatic tear similar to a knee injury one would see clinically, we slightly modified the method used by Woo [36] to simulate a MCL injury in the rabbit knee. To tear the ligament, an incision was made in the fascia anterior and posterior to the FCL. A curved hemostat was then placed under the FCL [4]. The hemostat was then used to pull a K-wire into place medial to the FCL (Fig. 2). Several small longitudinal incisions were made in the ligament to weaken it sufficiently to allow it to be ruptured. A lateral and upward traction force was used to rupture the ligament near the femoral attachment which created a mop-end tear of the ligament. The FCL ends were left *in situ*.

The popliteus tendon, which is intraarticular and attaches anterior and distal to the FCL attachment on the femur [4], was exposed by a small vertical incision through the posterolateral joint capsule after the FCL was ruptured. It was ruptured in a similar manner to the FCL. The rod was placed under the tendon near its femoral attachment,

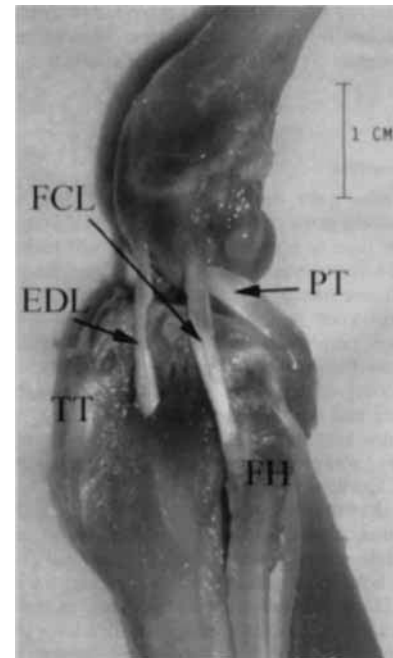


Fig. 1. Photograph of rabbit posterolateral knee (FDL—flexor digitorum longus tendon; FCL—fibular collateral ligament; PT—popliteus tendon; TT—tibial tubercle; FH—fibular head) (lateral view, left knee) [27, Fig. 3].

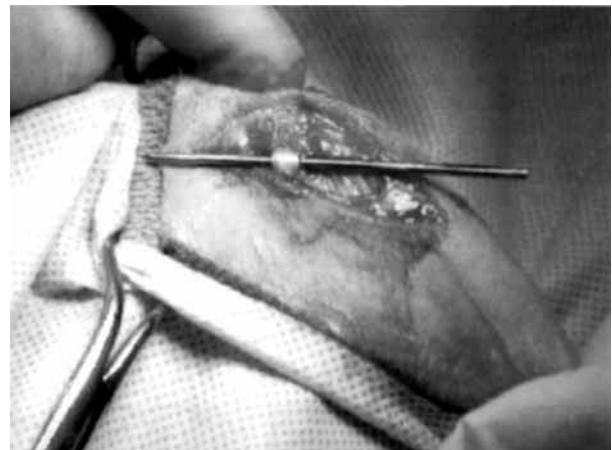


Fig. 2. Method of tearing FCL in rabbit knee. A K-wire is medial (under) to the FCL, while lateral traction ruptures the ligament (lateral view, right knee).

several small incisions made in the tendon, and the rod pulled upward to rupture the ligament.

For the sham operated knees, the ligaments were exposed in the same manner. The K-wire was also placed under the FCL and the popliteus tendon and then removed.

Hemostasis was obtained with direct manual pressure as there was very little bleeding associated with this procedure. The biceps fascia was sutured with a 5-0 absorbable suture with care not to suture any portion of the torn FCL or popliteus tendon. The skin was closed with a 5-0 subcuticular absorbable suture. An injection of buprenex (0.2 ml IM) was given for pain relief. Cefezolin (0.4 ml IM) was given immediately after surgery and continued for 3 days postoperatively.

Acetomenophin (1–2 mg/ml) was also provided in their drinking water for 3 days postoperatively. All rabbits were allowed unrestricted cage activity. The animals were checked daily for infection and use of the operated leg.

#### Specimen preparation

The hindlimbs were disarticulated at the hip joint following euthanasia 12 weeks postoperatively. We chose this time period as this was the average time to a return to no significant laxity differences in sectioned MCLs versus sham knees in the rabbit model [3,7,36], and that this time frame would be sufficient to determine if healing occurs in posterolateral knee injuries in the same species. Gross manual examination was performed on both knees to assess for any joint laxity. To assess for healing, the skin was removed revealing the superficial anatomy of the knee. Each knee was examined and the extent of healing and scar tissue recorded. The knees were then photographed. Gross assessment of deeper layers, the menisci, and the articular cartilage was performed after biomechanical testing so as not to disrupt the capsule of the knees.

To prepare the knees for biomechanical testing, the biceps femoris, semimembranosus, medial and lateral gastrocnemius, and flexor digitorum superficialis muscles were carefully removed from their bony attachments without interrupting the deeper structures. The knees were wrapped with saline soaked gauze at all times, except during actual testing, and kept moist. To create an appropriate lever arm at the distal tibia, a small intramedullary hole was drilled between the lateral and medial malleoli of the distal tibia. The marrow cavity was aspirated with a syringe. The shaft of the tibia was injected with polymethylmethacrylate, and a threaded 1 mm diameter K-wire was screwed into the shaft of the tibia. Polymethylmethacrylate was also used to pot the proximal femur, in which 2 short K-wires were placed to prevent any rotational slippage.

#### Varus–valgus testing

A specially designed testing machine was used to test the varus–valgus stability of the knees at 30°, 60°, and 90° of flexion (Fig. 3). The potted femurs were secured in a metal jig attached to the testing table. The legs were mounted with the MCL complex facing up. In order to find a reproducible zero point, the weight of the leg and rod in the tibia was used to tension the MCL. The rod in the distal tibia was then attached to a MTS Materials Test Machine (MTS, Eden Prairie, MN) (Fig. 3) for testing.

#### Rotation testing

Coupled external rotation which occurred with varus and valgus testing of the operative and control knees was concurrently measured

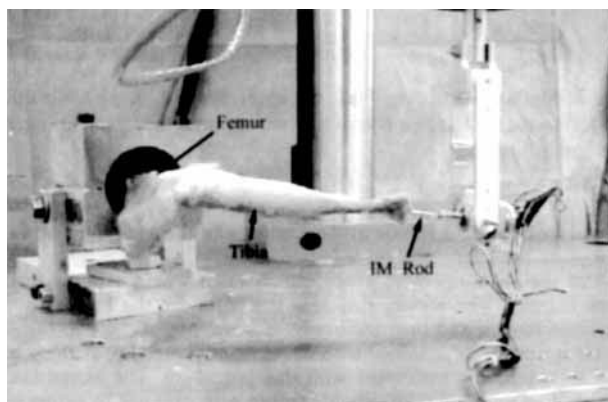


Fig. 3. Biomechanical testing assembly for assessment of varus/valgus rotation and coupled external rotation with varus rotation in a rabbit knee (anterior view, right knee).

with a potentiometer. Measurements of coupled external rotation were obtained at 30°, 60°, and 90° of knee flexion.

#### Statistical analysis

Statistical analysis using Student's *t*-test was performed to assess for differences in varus and coupled external rotation laxity between the operative and control knees. Statistical significance was assumed for *p* values <0.05.

## Results

One rabbit needed to be killed 3 weeks postoperatively due to health reasons. All other rabbits did well postoperatively with little evidence of pain in the early postoperative period. There was no observable decrease in normal cage activity, or favoring of the operative extremity, noted by the veterinary technicians after 2 weeks postoperatively.

On gross examination, there was no evidence of healing of the popliteus tendon. In all but one knee, there was no evidence of healing of the FCL. A mild amount of scar tissue formation was present deep to the biceps femoris muscle in all knees. In no case was there a large hypertrophic scar tissue healing response covering the sham or torn structures knees. In all rabbits, increased varus and external laxity could be appreciated on the operative knees on manual testing. There were no meniscal tears. While histology was not performed in this study, superficial chondromalacia of the articular cartilage of the medial compartment of the operative knees was present in the operative and not the control knees.

Biomechanical testing revealed a significant difference in the amount of force to achieve a 10 mm varus displacement at 30° ( $p < 0.001$ ), 60° ( $p < 0.006$ ), and 90° ( $p < 0.01$ ) (Fig. 4) for the control versus the operative knees. At 30° of knee flexion, an average of  $5.7 \pm 2.0$  N was necessary to result in 10 mm of varus displacement for control knees compared to  $2.4 \pm 2.3$  N for operative knees. No significant difference was found for the coupled external rotation which occurred with varus between groups at any flexion angle (Fig. 5).

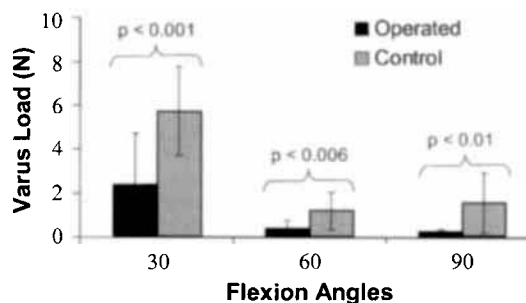


Fig. 4. Graph of force needed to create 10 mm varus displacement in rabbit knee in controls versus posterolateral structures sectioned (FCL and popliteus tendon) knees at 3 months.

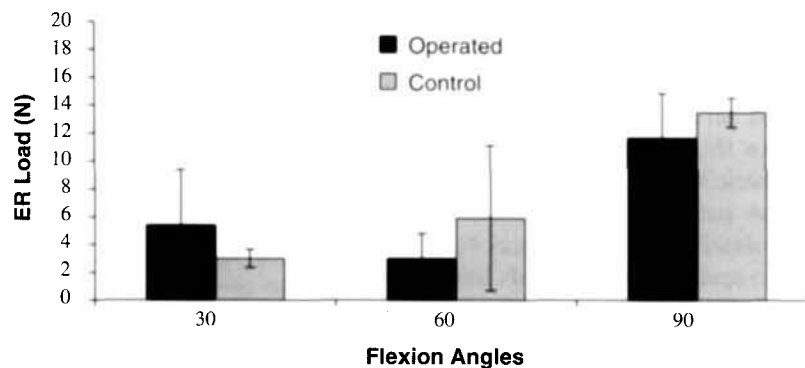


Fig. 5. Graph of coupled external rotation seen with 10 mm varus displacement in control versus posterolateral structures sectioned (FCL and popliteus tendon) rabbit knees at 3 months.

## Discussion

Posterolateral rotatory instability of the knee, first described by Hughston in 1976 [13], is a difficult problem to diagnose and treat. Initially thought to be rare [25], it is now recognized that the overall incidence of PLC injuries is underestimated and underreported [5,24,26,27].

While the implications of an untreated PLC injury appear to be significant [17,24,26,30], the true natural history of posterolateral knee injuries is not well understood. Kannus [17] has reported that untreated grade III injuries do not heal. Along with the functional limitations due to knee instability and a varus thrust gait [24,26,30], chronic posterolateral knee instability has been demonstrated to be associated with meniscal tears [24] and degenerative arthritis [17,30]. In addition, the role of acute or chronic combined cruciate and PLC injuries in causing significant instability of the knee has been increasingly recognized. Fanelli [5] has observed that the majority of patients with PCL tears who describe functional limitations with activities have a concurrent PLC injury which accentuates their functional instability compared to an isolated PCL tear. Several biomechanical and clinical studies have also reported the deleterious effects of untreated or unrecognized PLC injuries on cruciate ligament reconstruction grafts due to the increased force seen on the grafts, due to increases in varus and/or external rotation, which could contribute to their failure [12,23,22,26,27].

To date, scores of articles have been published on the use of in vivo animal models for studying the healing pattern of the MCL of the knee [1,3,7,6,9,15,36–38]. The method has been refined over the years and is felt to have been very useful in aiding the understanding of ligament repair processes and helping to rationally select the appropriate method of treatment for MCL injuries. Specific methods of sectioning and tearing of the MCL, measuring postoperative laxity, and quantitative measurements of the structural and mechanical properties of

the healing MCL complex have been reported in rabbit and canine knees for up to one year after injury [36,37]. These articles on MCL injuries have been translated to human clinical practice in that we now treat isolated, and some combined, MCL injuries in humans nonoperatively and with early knee motion compared to previous techniques of operative repair and/or cast immobilization.

In order to both determine the outcome of untreated PLC injuries on knee function, histology, outcome, and stability, to assess different treatment methods of surgical repair or reconstruction, and to test previous biomechanical studies on the effects of posterolateral knee injuries on cruciate ligament grafts in the multiple ligament injured knee [12,23,22], we felt an in vivo model of posterolateral rotatory instability of the knee was necessary, similar to in vivo MCL animal models [1,3,7,6,9,15,36–38]. After an anatomical study of the rabbit posterolateral knee [4] was completed (Fig. 1), we felt that there were enough similarities in the bony and ligamentous architecture of the rabbit knee to proceed with in vivo testing. The rabbit knee has been found to have similar bony anatomy and attachment morphology for the FCL and popliteus tendon of the posterolateral knee [4]. Based upon the fact that we were able to create a grade 3 posterolateral knee injury pattern at 3 months, we believe that the rabbit model, and larger animal models, will prove useful to further longer term biomechanical and histologic studies on the effect of posterolateral knee injuries on knee function, the potential development of osteoarthritis, assessment of different treatment methods of these injuries, and the effect on concurrent cruciate ligament knee injury treatment. While the rabbit model appears to be useful for the assessment of healing and natural history studies, we also believe that a large animal model is necessary to assess the in vivo effects of PLC injuries in knees with concurrent cruciate ligament reconstructions.

We chose the method of applying a given displacement to the rabbit knee and measuring the change in

force present to create this displacement both before and after cutting a specific structure as it was the best method in our biomechanics lab to determine the functional role of the tested structures in this small animal model. In addition to this same technique for both small animals and cadaveric knees utilized by other investigators [2,10,36,37], the technique of sequential sectioning of specific posterolateral knee structures to determine the displacement to specific applied loads has also been utilized in cadaveric knees [8,10,12,23,22,35]. Based upon the successful creation of this posterolateral knee instability model, we have now devised a small animal model of our human knee testing machine [23, 21,22] to test the amount of displacement (5 degrees of freedom) which occurs with a specific applied force.

In conclusion, we found that the FCL and popliteus tendon of the rabbit knee rarely heal when sectioned and there is a significant amount of increased varus motion in the rabbit knee at 3 months after injury. We believe that the rabbit model will prove valuable to the in vivo study of the natural history of posterolateral knee injuries. In addition, extrapolation of this model to larger animal species will potentially allow for verification of in vitro cadaveric studies of posterolateral knee injuries and the development of models of combined posterolateral and cruciate ligament injuries of the knee.

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