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A clinically realistic large animal model of intra-articular fracture that progresses to post-traumatic osteoarthritis

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SUMMARY

Objective: Translation of promising treatments for post-traumatic osteoarthritis (PTOA) to patients with intra-articular fracture (IAF) has been limited by the lack of a realistic large animal model. To address this issue we developed a large animal model of IAF in the distal tibia of Yucatan minipigs and documented the natural progression of this injury.
Design: Twenty-two fractures were treated using open reduction and internal fixation with either an anatomic reduction or an intentional 2-mm step-off. Pre-operatively, and 3 days, 1, 2, 4, 8, and 12 weeks post-operatively, animals were sedated for synovial fluid draws and radiographs. Limb loading was monitored at the same time points using a Tekscan Walkway. Animals were sacrificed at 12 weeks and the limbs were harvested for histological evaluation.
Results: All animals achieved bony union by 12 weeks, facilitating nearly complete recovery of the initial 60% decrease in limb loading. TNFα, IL1β, IL6, and IL8 concentrations in the fractured limbs were elevated ($P < 0.05$) at specific times during the 2 weeks after fracture. Histological cartilage degeneration was more severe in the step-off group ($0.0001 < P < 0.27$ compared to normal) than in the anatomic reconstruction group ($0.27 < P < 0.99$ compared to normal).
Conclusions: This model replicated key features of a human IAF, including surgical stabilization, inflammatory responses, and progression to osteoarthritic cartilage degeneration, thereby providing a potentially useful model for translating promising treatment options to clinical practice.

Introduction

Post-traumatic osteoarthritis (PTOA) is a common and potentially devastating complication following joint injury. It has been estimated that capsular or ligamentous injury can increase the risk of subsequent osteoarthritis (OA) by 10-fold, and an intra-articular fracture (IAF) can increase the risk of subsequent OA more than 20-fold[1]. In the United States, PTOA accounts for approximately 12% of all symptomatic OA, affecting approximately 5.6 million patients at a cost of more than $3 billion annually[2]. The PTOA burden is dramatically increased in the military, with a prevalence more than double that of the general population, and 94.4% of the OA cases that result in an unfit for duty designation being attributable to traumatic joint injury[3]. Patients from the general population presenting with PTOA are typically 9–14 years younger than patients presenting for primary OA[2], and patients presenting with PTOA in a military population are frequently in their late 20's and early 30's[4,5]. This young and highly disabled patient population is at a much higher risk for implant failure requiring revision in joints treated with arthroplasty, and at a higher risk for development of OA in adjacent joints if treated with a joint fusion.

The belief that anatomic joint reconstruction will prevent PTOA after IAF has been supported by numerous clinical studies in which more accurate articular surface reconstruction improved clinical and functional outcomes in patients with tibial plafond[6,7] and acetabular fractures[8]. Furthermore, finite element models of repaired tibial plafond fractures have demonstrated that abnormal

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cartilage stress distributions[9] and greater degrees of joint incongruity correlate with the development of PTOA 2 years after IAF[10]. However, with the exception of arthroscopically assisted reduction[11], there has been very little advancement in the ability to more accurately reconstruct IAFs. Perhaps more importantly, even with the emphasis on improved articular reduction, there has been very little change in the overall incidence of PTOA after IAF over the past 40 years[12].

These data indicate reduction quality is not the only factor contributing to outcome, and the cartilage impact injury plus the whole-joint inflammatory response are significant factors contributing to the development of PTOA after an IAF[13]. It has been shown that chondrocyte viability decreases dramatically along fracture edges after IAF[14], and over time chondrocyte death propagates from fracture lines into previously viable cartilage[15]. Chondrocyte death induces further inflammatory responses in the joint which can actively degrade the cartilage[13,16,17,18]. Studies of potential mechanisms for blocking cell death and inflammatory response to IAF have been limited by their investigation in explant systems which omit a full inflammatory response, or in non-physiologic *in vivo* models in which the joint is opened and the cartilage is impacted with a metallic surface. Translation of promising treatment methods for PTOA from the benchtop into clinical practice necessitates animal models that replicate both the full spectrum of joint reaction to IAF and eventual degradation to late-stage PTOA.

A mouse model of closed IAF has been used to investigate natural history and potential treatment targets for preventing PTOA. In that model, a materials testing machine drives an indenter into the proximal tibia from outside the joint, causing fracture without subjecting the system to the additional and non-physiologic complications associated with disrupting the joint capsule[19]. That model has been successfully used to demonstrate that chondrocyte death was associated with IAF in a closed setting, synovitis scores increase in joints with higher energy fractures[20], PTOA severity increases in the context of obesity[21], and decreasing particular inflammatory cytokines limits PTOA development[22–24]. The main drawback is that the mouse knee is extremely small, which precludes the ability to replicate human-like treatment of IAF, which typically includes open reduction and internal fixation. Additionally, mice are an extremely resilient species and often heal injuries much faster than other species.

The objective of this work was to develop a large animal model of closed IAF and to document the progression from initial injury to development of PTOA. It was hypothesized that the Yucatan minipig would reliably model the natural history of a distal tibia IAF while allowing human-like fracture treatment. The Yucatan minipig is a species purpose-bred for research, and it was selected for this work because it is unable to totally offload an injured joint via 3-legged gait, it weighs approximately the same as an adult human when fully grown, and has joints large enough to be treated with human-like fracture fixation hardware. This large animal model would fill the gap between small animal studies and clinical implementation of candidate therapies for PTOA treatment.

Methods

IAF creation

With IACUC (1007141 and 1307140) and Department of Defense ACURO approval, unilateral IAFs were created in the left distal tibia of twenty-two male Yucatan miniature swine (minipigs) ($n = 4$ from Sinclair Bioresources, Columbia, MO; $n = 22$ from Exemplar Genetics, Sioux Center, IA). Animals were skeletally mature with an average age of 30 months (range 25–35 months) and an average weight of 77 kg (range 58–110 kg) at the time of surgery. Under general anesthesia (inhaled 2–3.5% isoflurane in oxygen) and local lidocane block, an antero-medial approach was used to expose the anterior tibial surface, and a secondary posterior-medial approach was used to expose the distal talus. Special attention was paid to preservation of the peri-articular soft tissues, including the ligaments and the joint capsule. With fluoroscopic visualization, a custom tripod device was attached to the talus using three 4-mm to 5-mm tapered conical external fixator pins (Electro Biology Inc., Parsippany NJ)[25]. A stress-rising saw cut was made across the full medial/lateral width of the distal tibia stopping 1–2 mm proximal to the subchondral bone, and a small metallic plate with a metallic sphere was press fit into two holes drilled through the anterior tibial cortex immediately proximal to the saw cut (Fig. 1C)[25]. Using a custom-designed pendulum device[26], a 40 J impact was delivered to the talus via the custom tripod device (Fig. 1A). This impact energy had proven sufficient for inducing tibial fracture during extensive cadaveric pilot testing. The impact drove the talus into the anterior distal tibia, fracturing the tibia near the stress-rising saw cut. Energy not associated with causing the fracture passed through the limb and was measured using the compression of a spring construct mounted on the pendulum[26]. The fracture pattern consisted of a single fragment comprising the anterior one-third to one-half of the tibial plafond (Fig. 2).

After IAF, animals were randomly allocated to an anatomic reduction group or a step-off group. In twelve animals, the fractures were anatomically reduced and plated using a veterinary-grade 2.7-mm TPLO plate (VP4400.R3-2.7, Synthes, West Chester PA). Bicortical screws were inserted into the three screw holes proximal to the fracture line, and one bicortical screw was inserted into the distal center hole. A supplementary bicortical screw was inserted medial to the plate to prevent rotation of the fracture fragment. In the other ten animals, an intentional step-off was created by plating the anterior fragment 2 mm proximal to the anatomic position. Joints were held in the step-off position using the 2.7-mm TPLO plate on the anterior aspect of the tibia plus a 2.7-mm L-plate (VP1380.R3-2.7, Synthes, West Chester PA) on the medial aspect of the tibia. All animals were casted for a period of 1 week after fracture and allowed to bear weight as tolerated. Casts were changed on Day 3 when the animals were sedated for radiographs and blood/fluid draws.

Unless animal aggression required single housing, animals were housed 2 per pen in concrete-floored enclosures with rubber mats/wood chips as bedding. Animals were allowed unrestricted pen activity and a hard rubber ball for enrichment. Water was provided *ad libitum*, and animals fed a standard swine feed (3% bodyweight/day split into a morning and evening feeding). All animals were monitored twice daily by veterinary staff blinded to the reduction group. Twelve weeks after IAF and fixation, animals were sedated (telazol/ketamine/xylazine combination − 500 mg/mL, 250 mg/mL, 250 mg/mL, respectively) and euthanized with single injection of Euthasol (120 mg/kg). Tissue harvest and individual specimen analysis were performed in the order of animal sacrifice and conducted in a blinded fashion until final statistical analysis.

Gait analysis

Prior to IAF surgery, and 1, 2, 4, 8, and 12 weeks post-operatively, limb loading was evaluated using a Tekscan Walkway system (Tekscan, Boston, MA). Each animal traversed the Walkway at a self-selected speed while foot pressure data were captured at 60 frames per second. A synchronized standard video and the Walkway