Post-traumatic joint stiffness (PTJS) is common and can cause significant pain and loss of motion. Functional impairment caused by joint contracture and increased stiffness can occur in several different joints. From a clinical perspective, PTJS of the elbow is an especially challenging condition. The elbow joint is anatomically and biomechanically one of the most complex in the body. Three separate bones form a complex interaction of articulations to provide precise and comprehensive motion of the forearm and hand. Because of a high degree of natural congruency, the elbow joint is especially prone to stiffness after traumatic insult. Minor alterations in ligament tension, bone anatomy, or cartilage congruity can lead to rapid onset of joint contracture. Stiffness is poorly tolerated in the elbow; relatively minor contracture can impart large functional deficiencies in the simplest daily and vocational activities. In spite of the clinical relevance and high incidence of elbow contracture, the elbow is one of the most poorly studied joints in all of orthopaedics.

PTJS is a difficult clinical problem because the nature of the injury (e.g., severity, location) often does not correlate with the degree of functional impairment, making it difficult to predict which patients are at risk. In the elbow, joint contracture is an especially challenging clinical problem because unlike in the knee or shoulder, treatment options for reconstructing a severely pathologic joint are problematic. Total elbow arthroplasty is not a desirable option for most patients and joint fusions are very debilitating. More comprehensive research on this difficult clinical problem is needed, particularly in a model system that considers the complexity and challenges unique to the elbow.

While the etiology of PTJS is likely to be multifactorial, the capsule clearly plays a role. Analyses of capsule biopsies from patients with contracted joints have revealed significant biological changes including capsular thickening, increased turnover of extracellular matrix, presence of disorganized/fibrotic collagen, and hypercellularity. While clinical biopsies are insightful, biological variation of human tissue makes identification of causative factors difficult. These studies are also limited to evaluation of end-stage disease, so are unable to elucidate the etiology and time-dependent progression of capsular contracture and joint stiffness. In contrast, animal models enable the study of disease pathogenesis and permit evaluation of treatment strategies. However, no animal model has been developed to evaluate the elbow, which is necessary to be able to fully examine and understand clinical challenges specific to this complex joint. Previous animal models evaluating joint contracture in other joints (i.e., knee) have focused exclusively on the capsule and have not considered the contribution of other joint structures to PTJS.

The objective of this study was to develop an animal model of post-traumatic elbow contracture, and evaluate its potential for studying the etiology of joint stiffness and treatment of elbow injuries. To develop this model, an appropriate species/breed of animal was selected, and a clinically relevant surgical injury and immobilization protocol was developed and evaluated for its ability to replicate characteristics similar to the human condition.

Grant sponsor: NIH; Grant number: P30 AR057235. Correspondence to: Spencer P. Lake (T: 314-935-3161; F: 314-935-4014; E-mail: lake@seas.wustl.edu) © 2015 Orthopaedic Research Society. Published by Wiley Periodicals, Inc.
MATERIALS AND METHODS

Species Selection

A number of animal species were evaluated for relevance as a model of the human elbow. The characteristics determined to be most essential were as follows: (1) functional range-of-motion (ROM) of the joint; (2) anatomical similarities of bony architecture and soft tissues; and (3) prehensile use of the upper extremities. Important criteria included elbow joints with a separate radius and ulna, three distinct joint articulations (radioulnar, humeroulnar, humeroradial), pronation/supination in addition to flexion/extension, the presence of a capsular tissue, and the ability to use the limb in a non-weight bearing fashion. Several important practical considerations also factored into species selection including ability to acquire animals, ethical implications, ease in handling, and availability of appropriate biological assays and reagents. Further evaluation focused on the following species: mouse, rat, hamster, gerbil, ferret, guinea pig, rabbit, and cat.

Evaluation of these candidate species was performed using a variety of different resources, including extensive Medline searches and review of journal articles, textbooks, and online videos, photos and blogs. Several veterinarians were consulted regarding species selection. The Long-Evans rat was selected for use in this study (Fig. 1); refer to Results for detailed justification.

Animal Care

Rats arrived 2 weeks prior to the scheduled surgery day, allowing for time to acclimate to room conditions and cage mates, and alleviate any stress from delivery. Animals were housed three per cage with ad libitum access to food and water. In addition, nylabones (Nylabone Products, Neptune City, NJ) and fruit flavored cereal were added to each cage throughout the study.

Injury Model

This study was approved by the Institutional Appropriate Care and Animal Use Committee (IACUC). Twenty-four Long-Evans rats (320–370 g) were randomized into three surgical groups (Injury I, Injury II, Sham) and a group of age-matched (Control) animals (n = 6/group). The injury protocol performed replicated varying degrees of soft tissue injury seen in elbow subluxation/dislocation injuries. For the surgical groups, rats were anesthetized using isoflurane at 2.5–4% with oxygen carrier via nasal inhalation using a nose cone. Animals received pre-operative doses of non-steroidal anti-inflammatory drug (NSAID) (5 mg/kg carprofen, Pfizer Animal Health, New York, NY) and antibiotic (7.5 mg/kg enrofloxacin, Bayer Health LLC, Shawnee Mission, KS). The left elbow was prepped and draped under sterile conditions. A 2 cm incision was made over the lateral elbow and necessary skin flaps were developed. The triceps was retracted posteriorly and the lateral column of the humerus was identified. The forearm extensors were elevated from the lateral epicondyle leaving the capsule intact. The Injury I group had an anterior capsulotomy performed by incising and elevating the capsule from the anterior humerus using the tip of an 11-scalpel blade (Fig. 2A). The Injury II group

Figure 1. Several key anatomical similarities of the rat elbow were identified, including the (A) lateral collateral ligament (outlined in black) and the (B) humeroulnar articulation; (C) µ-CT images show the size and shape of the bones in the rat forelimb (insets: posterior view of the humerus and medial view of radius and ulna).
had an anterior capsulotomy combined with transection of the lateral collateral ligament (LCL) and subsequent elbow subluxation to induce a more severe injury (Fig. 2B). The forearm was supinated and the elbow subluxed with postero-lateral rotation followed by reduction. For the Sham group, the left elbow capsule was exposed similar to the injury groups but no injury was induced. Following skin closure with staples, operated limbs were immobilized (see next section). For all surgical groups, contralateral (CL) limbs served as uninjured comparisons.21 A group of age-matched control animals were allowed unrestricted cage activity for the duration of the study.

Immobilization
Immediately after skin closure and a post-operative dose of NSAID, injured limbs from the Injury I, Injury II, and Sham groups were immobilized for 6 weeks. A piece of tubular elastic netting (Nich Marketers Inc., Gulf Breeze, FL) was cut to ~3 in. and placed around the upper torso of each rat (Fig. 3A). An access hole was cut in the right side of the netting to allow the uninjured right arm unrestricted use. A 2 in. × 20 in. piece of self-adhering Vetrap bandaging (3MTM, St. Paul, MN) was wrapped around the same portion of the torso three times, with access holes consistently cut to leave the right limb unconstrained (Fig. 3B). The composite wrap was carefully pinched together and trimmed to ensure consistent immobilization yet minimize discomfort. Animals were closely monitored for 48 h following surgery to check for potential complications. During the course of the 6-week immobilization period, rats were checked daily to ensure successful constraint of the immobilized limb and to identify any indications of pain or distress. In addition, they were examined twice a week by a veterinary technician for activity level and general health. If any signs of distress were apparent, or if the wrap became loose or soiled, animals were anesthetized using isoflurane and bandages replaced. If symptoms persisted, animals were left unwrapped and observed closely for 24–48 h before reapplying immobilization bandages. Each time animals were rewrapped, any sores or cuts were treated with antibiotic powder/cream (nitrofurazone (Neogen Corporation, Lexington, KY), silver sulfadiazine (Dr. Reddy’s Laboratories Louisiana, Shreveport, LA)) and/or chafing cream (PrestigeBrands, Tarrytown, NY). At a minimum, immobilization bandages were replaced biweekly. Animals were also weighed weekly.

After 3-weeks of immobilization (i.e., halfway point of study) radiographs were taken of animals to quantify the position at which their injured limbs were immobilized (Fig. 3C). After applying clean immobilization bandages under anesthesia, rats were laid on their right (uninjured) side and a sagittal radiograph was acquired (MX-20, Faxitron Bioptics, Tuscon, AZ). Radiographs were analyzed to determine the flexion angle of each joint. Each radiograph was placed against a white computer screen (acting as a light box) and the angle of the immobilized elbow was traced three times using the angle tool in ImageJ (NIH, Bethesda, MD). Angle values were averaged to determine the degree of flexion for each elbow.

Mechanical Testing
Rats were sacrificed 6 weeks after injury via CO2 inhalation and immediately stored in a −20°C freezer. Animals were thawed 24 h prior to dissection. Bilateral forelimbs were prepared for testing by removing all skin, disarticulating the glenohumeral joint, and sharply resecting the paw. The humeral head and distal ulna/radius were cleaned of all tissue. The proximal humerus was potted in a 1 in. long polycarbonate tube (3/8 in. outer diameter; 1/4 in. inner diameter), while the distal end (ulna/radius) was potted into a larger 1 in. long polycarbonate tube (1/2 in. OD; 3/8 in. ID). Limbs were prepared by allowing a layer of cyanoacrylate to dry on the exposed bone ends, then placing each side into tubes filled with hardening putty (Bondo, 3M, Maplewood, MN) and drying for 1 h.

A custom mechanical test system for evaluating rat elbow stiffness and joint contracture was designed and built (Fig. 4A), similar to systems used for testing rabbit knees.22,23 The device utilizes one actuator of a planar biaxial mechanical test system (TestResources, Shakopee, MN) to apply linear displacement and measure force with a six-degree-of-freedom sensor (ATI Industrial Automation, Apex,
A rack and pinion gear converts linear displacement to rotational motion and allows for load-controlled cyclic testing of the rat elbow in flexion-extension. After securing the potted ends of each limb in custom fixtures of the mechanical test system, five cycles of loading to ±0.75 N (±11.25 N mm of torque) were applied. A digital camera mounted above the test system was used to capture images at the peak flexion and extension angles (Fig. 4B,C). After testing, peak angles were measured from the digital images using the angle function in ImageJ. Angle values were computed in terms of degrees of flexion, defined relative to a horizontal line representing 0˚ flexion (or full extension) (Fig. 4B,C). Force and displacement data from the first cycle were converted to torque and angular position, respectively. Torque-angle loading curves were analyzed using a custom written Matlab program (Mathworks, Natick, MA) to quantify elbow joint motion (Fig. 5A).

Total ROM, maximum flexion, maximum extension, and neutral zone length are all measures of joint contracture, while flexion, extension and neutral zone stiffness values are indicative of overall joint stiffness. ROM is defined as the difference between the angular positions corresponding to maximum torque values in flexion and extension (e.g., low ROM values indicate joint contracture). The ROM midpoint quantifies the relative shift of the overall torque-displacement curve, which can indicate altered joint motion relative to control, even without a loss in total ROM. The neutral zone (NZ) is the flatter region of the curve that falls between linear fits of flexion and extension stiffness. The neutral zone stiffness is equal to the average slope of the loading and unloading curves in that region and corresponds to the relative stiffness of the joint through its functional range (e.g., high values indicate a stiffened joint). Mechanical testing was performed on both injured and uninjured CL limbs from each animal so paired comparisons could be made, in addition to comparisons with controls.

**Histological Analysis**

Following mechanical testing, a subset of samples (\(n = 3\) group) was prepared for histological assessment using standard protocols. Briefly, elbow joints were fixed with 10% neutral buffered formalin for 72 h followed by decalcification.
for 14 days in 14% EDTA. Following processing and paraffin embedding, 5 μm sagittal sections were cut and stained with hematoxylin and eosin (H&E). On blinded sections from each elbow joint, anterior capsule tissue was evaluated by a musculoskeletal pathologist using semi-quantitative scores for several biological characteristics of interest. Analysis focused on capsule tissue because of the role of the capsule in PTJS and because we sought to compare results from this animal model to previous histological data of capsule tissue from human patients with contracted elbows. Evaluating criteria included adhesion, cellularity, inflammation, synovial proliferation and vascularity, which were scored as summarized in Table 1. Capsular thickness was also measured on each section; since these values can vary for sections cut at different depths, we converted thickness measurements to a semi-quantitative scoring scheme (similar to other evaluated metrics). Thus, capsular thickness was scored as a relative change compared to the average thickness of control capsules (Table 1). Following evaluation, numerical scores were averaged across each group and converted to a symbolic grading scheme (−, +, ++, ++++, +++++) for comparison between different treatment groups.

Statistical Analysis
One-way ANOVA tests were utilized to compare mechanical test parameters between all animal groups. In addition, one-way ANOVAs were used to compare measured body weights at each time point. When significance was found, post-hoc Bonferroni analyses were used to compare each experimental group to control. Within treatment groups, paired t-tests were used to compare uninjured right limbs to injured left limbs. For all statistical tests, significance was defined as p < 0.05.

RESULTS

Species Selection
The Long-Evans rat was identified as the best species for an animal model of elbow pathology. Gross dissections of the forelimb and imaging analysis using μ-CT showed anatomical similarities of bones, articulations and soft tissue (Fig. 1). One critical factor was the ability to pronate and supinate the forearm, which distinguishes Long-Evans rats from other breeds including Sprague-Dawley and Wistar rats (other commonly used rats for research). Long-Evans rats have ~90° of pronation and ~60° of supination, and regularly supinate during feeding, pronate during reaching, and rotate with other cage activities. While all evaluated animals exhibited flexion/extension motion, the ability to pronate/supinate was much less common. Since motion in both flexion-extension and pronation-supination is vitally important for full use of the elbow joint in humans, the selected animal needed to be capable of both.

Anatomical Evaluation
The elbow anatomy of the Long-Evans rat was evaluated grossly and with CT scans in three normal specimens. Average flexion of the elbow is ~150°, and average extension is ~28°. The Long-Evans rat is able to pronate to ~90° from neutral during grasping activities, and can supinate to nearly ~80°, as seen during feeding activity. This pronation-supination motion is a major difference from other rat breeds where grasping is more rudimentary because of lack of pronation, and feeding is through the side of the mouth because of lack of supination. Analogous to the human elbow, the rat elbow has three bones forming a complex articulation, humerus, radius, and ulna, forming the humeroulnar, humero radial, and proximal radioulnar joints. The ulna has a large olecranon process, extending 2-3 mm proximal to the articular surface of the ulna, facilitating a robust triceps insertion.

The lateral collateral ligament is visible as a distinct structure originating from the lateral epicondyle and inserting on the sublime tubercle of the ulna. The medial collateral ligament is likewise visible on the medial aspect of the ulna in anatomic location analogous to the human elbow. The musculature surrounding the elbow includes the triceps, biceps, and brachialis muscles.

Immobilization Protocol
The immobilization protocol was successful in constraining rat elbow joints over the six-week period:

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<td>0</td>
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<td>2</td>
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<td>Inflammation</td>
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<td>Synovial proliferation</td>
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<td>Thickness (change from control)</td>
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<td>Vascularity</td>
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Table 1. Semi-Quantitative Scoring Scheme for Evaluating Capsule Tissue of Rat Elbows. Not All Possible Scores Were Used for Each Evaluated Metric (Unused Scores Marked With a “—“). In General, Higher Scores Correspond to More Severe Differences Compared to Healthy Tissue.
immobilized limbs were constrained an average of 93 ± 7% of the time. The immobilization wraps maintained the limb close to the animals’ torso in flexion. The 3-week radiographs showed that elbow joints were immobilized at an average angle of 155.9 ± 5.0˚ of flexion. Following sacrifice and dissection, injured limbs were grossly observed to be more contracted than uninjured limbs with much less extension at a resting state (Fig. 6).

Following a small drop in weight the week after surgery, the body weights of the animals in all treatment groups increased steadily throughout the remainder of the study (Fig. 7). There were no differences in body weight between treatment groups at any time point (p > 0.05), although they were significantly decreased compared to controls at the time of sacrifice (average weight at sacrifice of 397 ± 26 g for treatment animals and 505 ± 66 g for controls). According to the growth chart on the breeder website (Charles River Laboratories International, Wilmington, MA), the average weight of Long-Evans rats of this age is ~375–590 g. In this study, all groups were within this range at the time of sacrifice. All animals were checked regularly throughout the duration of the study by the researchers (5 ×/week) and veterinary technicians (2 ×/week), and no signs of animal distress were observed. All animals were active in their cages and were observed to have good overall health and welfare. Thus, the difference in the rate of weight gain between treatment groups and controls was not likely to be evidence of poor health or pain. Although there was no evidence of a struggle to access feed, continuing work with this model has changed the location of the feed to allow for easier access in hopes of decreasing the weight differences between the control group and injury groups.

Mechanical Testing
Mechanical test data showed dramatic differences in the shapes of torque-angle curves for injured limbs compared to their uninjured CL limbs (Fig. 5B). Specifically, ROM curves for treated limbs were shifted towards increased flexion (and decreased extension) and were more compacted along the angle axis (i.e., demonstrating less ROM). Quantitatively, limbs from all injury groups exhibited significantly smaller ROM values than controls, with the most severe injury (Injury II) demonstrating smallest ROM values overall (Fig. 8A; Control = 107.3 ± 10.2˚; Sham = 71.2 ± 28.5˚; Injury I = 67.6 ± 16.1˚; Injury II = 50.0 ± 10.6˚). Injury I and Injury II groups also showed significant differences in ROM between injured and contralateral limbs (Injury I CL = 94.5 ± 16.4˚; Injury II CL = 93.4 ± 8.5˚). ROM midpoint values also showed significant differences by group (Fig. 8B): All immobilized joints exhibited increased ROM midpoints relative to controls (Control = 87.2 ± 6.0˚; Sham = 113.6 ± 20.0˚; Injury I = 113.9 ± 8.3˚; Injury II = 125.3 ± 5.8˚), and both Injury I and Injury II groups had significant differences between injury and CL joints (Injury I CL = 88.4 ± 6.0˚; Injury II CL = 91.3 ± 5.4˚). Similar to total ROM, midpoint ROM values were most dramatically altered for the most severe injury.

For neutral zone stiffness, Injury II had significantly larger values in injured limbs compared to uninjured CL limbs, while both Sham and Injury II were significantly different from uninjured controls (Fig. 9A). Neutral zone length also decreased for limbs that were immobilized/injured, with values following trends similar to the ROM values (Fig. 9B). In this case, all surgery groups showed inter-limb differences,
and groups that included a soft tissue injury in addition to immobilization (i.e., Injury I and Injury II) had significantly smaller neutral zone lengths compared to control limbs (Control = 75.0 ± 8.8°; Injury I = 34.6 ± 13.8°; Injury II = 26.0 ± 15.3°).

Regarding the mechanical behavior at the limits of motion (Table 2), there were few statistical differences in flexion: no significant differences between any groups for flexion stiffness, and no significant differences from control for maximum flexion. Only Injury I and Injury II showed significant differences in maximum flexion between injured and uninjured limbs. While stiffness in extension also showed few differences (only inter-limb for Injury II), there were large differences in maximum extension. Injured joints from all groups had significantly smaller maximum extension values than control, and both groups with soft tissue injuries were significantly different between immobilized and uninjured CL limbs. Importantly, none of the output parameters from mechanical testing showed significant differences between uninjured CL limbs and controls (Figs. 8&9, Table 2).

**Histological Analysis**

Immovilized limbs (i.e., Sham, Injury I, Injury II) exhibited altered tissue histology in the anterior capsule compared to control and CL joints (Table 3, Fig. 10). Specifically, immobilized limbs showed increased adhesion to osseous surfaces, hypercellularity, and thicker capsule/scar tissue compared to non-injured joints. Hypercellularity appeared to be mostly attributed to fibroblast and/or myofibroblast proliferation, however, further staining/evaluation would be necessary to distinguish cell types. No groups showed evidence of inflammation or increased vascularity, nor was significant synovial proliferation observed. CL limbs were very similar to control for all assessed metrics. Surprisingly, there were few differences between any of the immobilization groups: Sham joints exhibited similar results to both Injury I and Injury II limbs.

**DISCUSSION**

We have developed an animal model for elbow injury and induced post-traumatic elbow contracture after injury and immobilization. After examining a number of potential animal species, the Long-Evans rat was identified as the most appropriate species and breed for this purpose. Key considerations in this evaluation included similar bony and soft tissue anatomy, prehensile use of the upper extremity, and similarities in functional use of the joint, including both pronation-supination and flexion-extension.

Having identified a candidate animal for model development, micro-surgical techniques were developed for creating two clinically relevant soft tissue injuries representing elbow subluxation/dislocation injuries that occur in humans. Combined with post-injury joint immobilization for 6 weeks, these surgical procedures successfully led to stiff and contracted elbows. The injured/immobilized joints of all treatment groups were stiffer and more contracted than uninjured CL limbs and control animals. Importantly, the severity of the induced injury correlated with loss of motion and joint stiffness. For nearly all metrics of joint function (including total ROM, ROM midpoint, NZ stiffness, length of NZ, extension stiffness, and limits in flexion/extension), Injury II animals exhibited the most dramatic differences compared to age-matched control animals and uninjured CL limbs. Thus, the injury/immobilization protocol developed in this study led to significantly altered joint mechanics (e.g., decreased ROM, increased stiffness) that mimic...
symptoms common to human patients, with the largest quantitative biomechanical changes corresponding to the most severe injury. As a first step in examining the biomechanical consequences of the injury/immobilization protocol, this study evaluated flexion-extension through joint testing. Future work will also investigate issues related to pronation-supination and how PTJS alters the full range of elbow function.

To our knowledge, there are no previous animal models of elbow injury/pathology. Instead, previous work aimed at understanding PTJS in the elbow have evaluated rabbit or rat knees and then extrapolated the results to the elbow joint. Hildebrand and

Table 2. Range of Motion Limits and Stiffness in Extension and Flexion Showed Increasingly Altered Joint Mechanics With Increasing Injury Severity (Average ± standard Deviation; * = Different From Control; Bolded values = Different From Contralateral; $p < 0.05$)

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<tr>
<th></th>
<th>Limits (°)</th>
<th>Stiffness (N/mm)</th>
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<tr>
<td></td>
<td>Max. Extension</td>
<td>Max. Flexion</td>
</tr>
<tr>
<td>Control</td>
<td>33.6 ± 6.9</td>
<td>140.8 ± 8.7</td>
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<tr>
<td>Sham</td>
<td>78 ± 33.9*</td>
<td>149.2 ± 7.5</td>
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<tr>
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<td>47.6 ± 3.6</td>
<td>138.7 ± 5.1</td>
</tr>
<tr>
<td>Injury I</td>
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<td>147.7 ± 2.6</td>
</tr>
<tr>
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<td>41.2 ± 10.5</td>
<td>135.7 ± 9.7</td>
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<tr>
<td>Injury II</td>
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<tr>
<td>Injury II CL</td>
<td>44.6 ± 9.2</td>
<td>138 ± 3.2</td>
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Table 3. Histological Evaluation of the Anterior Capsule Showed Altered Tissue Properties for Immobilized Limbs Compared to Control and CL Joints (Refer to Text for Summary of Grading Scheme)

<table>
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<th></th>
<th>Adhesion</th>
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<tr>
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<td>Injury I</td>
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Figure 10. Representative sagittal histological sections (H&E stain) of control and injured elbow joints. Low magnification images (2.5 ×) demonstrate joint anatomy and general morphological characteristics of sections from (A) control and (B) Injury II joints (C = capsule; M = muscle; H = humerus; R = radius; scale bar = 0.5 mm). Higher magnification images (10 ×, corresponding to dashed boxes in A and B) show (C) normal anterior capsule in a control joint, and (D) visible differences in an Injury II joint including increased cellularity, adhesion, and thickness (scale bar = 100 μm).

JOURNAL OF ORTHOPAEDIC RESEARCH FEBRUARY 2016
colleagues developed a rabbit model wherein the knee was exposed via medial and lateral para-patellar arthrotomies, 5 mm² cortical windows were removed from the non-articulating portions of the medial and lateral femoral condyles, and the joint was immobilized in a flexed position using a Kirschner wire (K-wire) to secure the tibia and femur. Using this procedure, rabbit knees lost ~25–30˚ of ROM when loaded to 0.2 Nm torque in extension following 8-weeks of immobilization. Another group reported similar changes in rabbit knees using this surgical protocol and immobilization, with ~20˚ loss of extension motion at the conclusion of the immobilization period. This study also evaluated a more severe injury protocol, in order to initiate joint contractures that would be more likely to persist long-term following remobilization. Specifically, in addition to removing 3 mm cortical windows from the femoral condyles and immobilizing using K-wire fixation, the anterior and posterior cruciate ligaments were incised and the joint hyperextended to disrupt the posterior capsule during the surgical procedure. As expected, this extremely severe injury resulted in more dramatic joint contracture following 8-weeks of immobilization, with a total reduction of ~75˚ in extension. Similar quantitative changes were also observed using a similar surgical protocol in rat knees, with an average of ~95˚ ROM loss when loaded in extension.

Compared to these previous studies, our current work found similar biomechanical results consistent with contracture. Sham and Injury I animals exhibited a reduction of ~45˚ in extension at the level of force utilized for evaluation, while Injury II animals lost ~65˚ of extension compared to Controls (Table 2). Different from previous studies, we measured the total ROM of analyzed joints; reductions in total ROM were ~36˚, ~40˚, and ~57˚ for Sham, Injury I, and Injury II, respectively, when accounting for slight changes in flexion limits for injury groups compared to Control (Fig. 8). In addition to measuring the loss of motion apparent in flexion-extension cyclic loading, the present study also quantified joint stiffness through the total ROM (flexion, extension, and NZ), thereby providing a more in-depth assessment of functional joint mechanics.

Histologically, the protocol developed in this study induced changes in the capsule tissue similar to what has been reported for biopsies taken from human patients with contracted joints. Specifically, previous studies of human tissue have reported thickened capsular tissue, disorganized dense/fibrous collagen, and increased fibroblast-like cell proliferation, which are consistent with our findings (Table 3; Fig. 10). In addition, limited evidence of neovascularization and synovial proliferation in human data also agrees well our observations. One surprising result was the similar histological characteristics observed for all three immobilization groups (i.e., Sham, Injury I, Injury II), even though the two injury groups included direct disruption of the anterior capsule during surgery. While the capsule/scar tissue in these groups may indeed be similar, more in-depth biological evaluation may also identify important distinctions between these groups. The histological similarities to human specimens establish the clinical relevance of this model. Future research will focus on regulation/presence of key ECM proteins and growth factors, and increased density of specific cell types in contracted capsules.

While previous studies of joint contracture using animal models have yielded significant insight, the anatomical and functional characteristics of the rabbit or rat knee are a poor representation of the human elbow, so these experimental models have limited applicability in investigating questions of clinical relevance specific to the elbow joint. In addition, compared to previous injury models of PTJJS, our approach utilizes much less severe and more clinically relevant injury and immobilization protocols to initiate stiffness and contracture. Specifically, we instigated soft tissue damage similar to what can occur in humans during joint dislocation, and utilized a more relevant wrapping/bandaging procedure to achieve joint immobilization, compared to much more severe and invasive surgical techniques used previously (e.g., bony fixation via implanted K-wires). These changes were possible, in part, because of relative differences in functional use of the elbow compared to the knee in quadruped animals, which are likely to result in long-term joint disease in our model for less severe induced injuries. Thus, the present study developed and utilized a clinically-relevant injury and immobilization protocol to enable the first in vivo analysis of elbow pathology using an animal model.

This study is not without limitations. First, any animal study has associated limitations when comparing results to human conditions. For example, rats are quadruped animals that use their upper extremity in ways that are different from humans (e.g., locomotion), and they also exhibit some anatomical differences from humans (e.g., extended olecranon process of the ulna). However, our careful selection of species/breed has matched key similarities (e.g., range/types of joint motion, similarity of joint articulations) to maximize the clinical relevance of this animal model. In addition, rats have been used for over 20 years as an established and accepted model of shoulder injury and pathology, suggesting that they may also serve as an appropriate model for study of the elbow. Second, this study did not evaluate the persistence of joint dysfunction following remobilization. Ongoing work is investigating whether the detrimental biomechanical changes observed in this study are transient (and recover when the joint motion resumes) or if they remain long-term following remobilization. Finally, there were significant differences in body weight between control animals and treatment groups (Sham, Injury I, Injury II) at the time of sacrifice (six weeks.
following surgery). The weights of control and treatment animals were similar at the beginning of the study. Both control and treatment animals gained weight throughout the study, however they appear to have gained weight at different rates. Unfortunately, control animals were only weighed at week 0 and week 6, so direct comparisons at intermediate time points was not possible. Based on consistent and regular observations of animals throughout the study by researchers and veterinary technicians, we are confident that differences in weight gain were not due to animal distress or pain. As in our previous studies involving immobilized limbs, food and water were accessible and consumption confirmed by veterinary and research staff.

In conclusion, this study developed an animal model of post-traumatic elbow contracture, and evaluated its potential for studying the etiology of joint stiffness and treatment of elbow injuries. To our knowledge, this is the first animal model capable of examining challenges unique to the anatomically and biomechanically complex elbow joint. More in depth basic science investigations are needed to better understand the etiology and pathophysiology of PTJS. Elbow injuries cause significant pain, loss of motion and function, secondary conditions (e.g., osteoarthritis), and lead to very high rates of disability. Further investigations will use this in vivo animal model to evaluate hypotheses related to mechanisms responsible for the development and progression of post-traumatic joint stiffness and contracture. Evaluation of the time course of changes within all anatomical structures of the elbow joint, something only possible using an animal model, will enable characterization of this condition and allow for formal statistical evaluation of mechanistic hypotheses.

AUTHORS’ CONTRIBUTIONS
S.P.L. and L.M.G.: designed the experiments. S.P.L., R. M.C., and S.B.: acquired data. S.P.L., R.M.C., C.L.D., and N.H.: analyzed the data. S.P.L., R.M.C., C.L.D., and L.M.G.: interpreted the data and wrote the manuscript. All authors have read and approved the final submitted manuscript.

ACKNOWLEDGEMENTS
The authors thank Daniel Lieb for assistance with µ-CT and radiograph imaging, Margaret Liu for helping with animal handling/immobilization, and the Washington University Musculoskeletal Research Center (NIH P30 AR057235).

REFERENCES
15. Hildebrand KA. 2013. Posttraumatic elbow joint contrac-
25. Sacrey LA, Alaverdavshili M, Whishaw IQ. 2009. Similar hand shaping in reaching-for-food (skilled reaching) in rats and humans provides evidence of homology in release,


