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Chelsey L. Dunham

Ryan M. Castile

Aaron M. Chamberlain

Spencer P. Lake

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The Role of Periarticular Soft Tissues in Persistent Motion Loss in a Rat Model of Posttraumatic Elbow Contracture

Chelsey L. Dunham, BS, Ryan M. Castile, BS, Aaron M. Chamberlain, MD, and Spencer P. Lake, PhD

Investigation performed at Washington University in St. Louis, St. Louis, Missouri

Background: Elbow injuries disrupt the surrounding periarticular soft tissues, which include the muscles, tendons, capsule, ligaments, and cartilage. Damage to these tissues as a result of elbow trauma causes clinically significant contracture in 50% of patients. However, it is unclear which of these tissues is primarily responsible for the decreased range of motion. We hypothesized that all tissues would substantially contribute to elbow contracture after immobilization, but only the capsule, ligaments, and cartilage would contribute after free mobilization, with the capsule as the primary contributor at all time points.

Methods: Utilizing a rat model of posttraumatic elbow contracture, a unilateral soft-tissue injury was surgically induced to replicate the damage that commonly occurs during elbow joint dislocation. After surgery, the injured limb was immobilized for 42 days. Animals were evaluated after either 42 days of immobilization (42 IM) or 42 days of immobilization with an additional 21 or 42 days of free mobilization (42/21 or 42/42 IM-FM). For each group of animals, elbow mechanical testing in flexion-extension was completed post-mortem with (1) all soft tissues intact, (2) muscles/tendons removed, and (3) muscle/tendons and anterior capsule removed. Total extension was assessed to determine the relative contributions of muscles/tendons, capsule, and the remaining intact tissues (i.e., ligaments and cartilage).

Results: After immobilization, the muscles/tendons and anterior capsule contributed 10% and 90% to elbow contracture, respectively. After each free mobilization period, the muscles/tendons did not significantly contribute to contracture. The capsule and ligaments/cartilage were responsible for 47% and 52% of the motion lost at 42/21 IM-FM, respectively, and 26% and 74% at 42/42 IM-FM, respectively.

Conclusions: Overall, data demonstrated a time-dependent response of periarticular tissue contribution to elbow contracture, with the capsule, ligaments, and cartilage as the primary long-term contributors.

Clinical Relevance: The capsule, ligaments, and cartilage were primarily responsible for persistent motion loss and should be considered during development of tissue-targeted treatment strategies to inhibit elbow contracture following injury.

Posttraumatic contracture develops in 50% of patients who experience elbow injury (i.e., dislocation or fracture)[1], in part because the congruent joint architecture and soft-tissue constraints of the elbow are often disrupted as a result of the injury[2]. Restoring elbow range of motion (ROM) following injury and contracture is a difficult, time-consuming, and costly challenge because it is a multi-tissue pathology. Current treatment strategies such as physical therapy or surgical intervention do not target all fibrotic tissues in the elbow, which include muscles, tendons, capsule, ligaments, and cartilage[3]. However, the extent to which these tissues contribute to contracture over time in the elbow is unknown.

Previous studies of immobilization-induced knee contracture animal models (i.e., no joint injury) showed that the contribution of capsule/ligaments/cartilage and muscles/tendons to contracture exhibited a time-dependent increase and decrease, respectively, throughout the course of immobilization and subsequent free mobilization (i.e., joint no longer immobilized)[4-7]. However, these findings in the knee cannot be directly translated to the elbow because of the anatomical and functional differences

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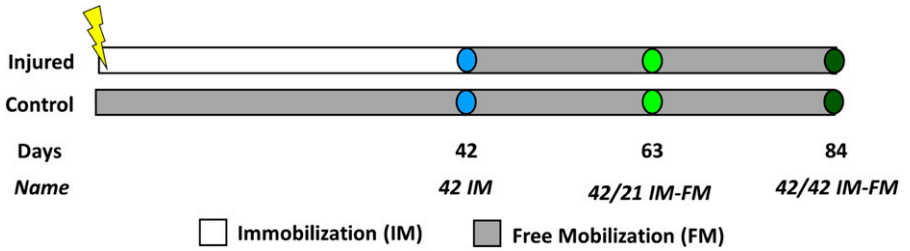


Fig. 1

Timeline of experimental evaluation for injured and control animals. The lightning bolt indicates the time of surgical injury and an oval indicates an analysis time point.

in these 2 joints. To our knowledge, no study has evaluated these specific tissue contributions to contracture in the elbow. Despite clinical evidence indicating the capsule is a primary contributor to persistent elbow contracture on the basis of biological changes observed in the tissue, no study has specifically isolated the mechanical contribution of the capsule to loss of elbow ROM[2,8-10].

We previously developed an animal model of posttraumatic elbow contracture that exhibited significant loss of flexion-extension ROM[11,12]. In addition to the altered joint mechanics, biological changes were histologically observed in the anterior capsule (i.e., increased thickness, adhesions, and myofibroblasts) and non-opposing joint surfaces (i.e., cartilage-capsule interactions indicative of arthrosis), consistent with clinical observations[11-13]. This animal model allows evaluation of the role of periarticular soft tissues in elbow contracture and elucidation of which soft tissues primarily cause motion loss will aid development of tissue-targeted treatment strategies to prevent elbow contracture. The objective of this study was to evaluate the passive contributions of muscles/tendons, capsule, and ligaments/cartilage to loss of elbow extension caused by contracture. We hypothesized that all tissues would substantially contribute to elbow contracture after immobilization, but that only the capsule, ligaments, and cartilage would contribute after free mobilization because we previously reported that altered muscle mechanics recovered after free mobilization[14]. At all time points, we hypothesized that the capsule would be the primary contributor to contracture on the basis of persistent biological changes observed in human patients[2,8-10] and in our rat model[13].

Materials and Methods

Animal and Injury Model

On the basis of previously described criteria including similarities to human anatomy and functional upper-extremity ROM, male Long-Evans rats (250 to 350 g; 8 to 10 weeks old; Charles River Laboratories International) were selected and randomized into injury and control groups[11,12]. A power analysis (power = 0.8; $\alpha = 0.05$) determined that 7 rats per group were required for joint mechanical tests to detect differences in

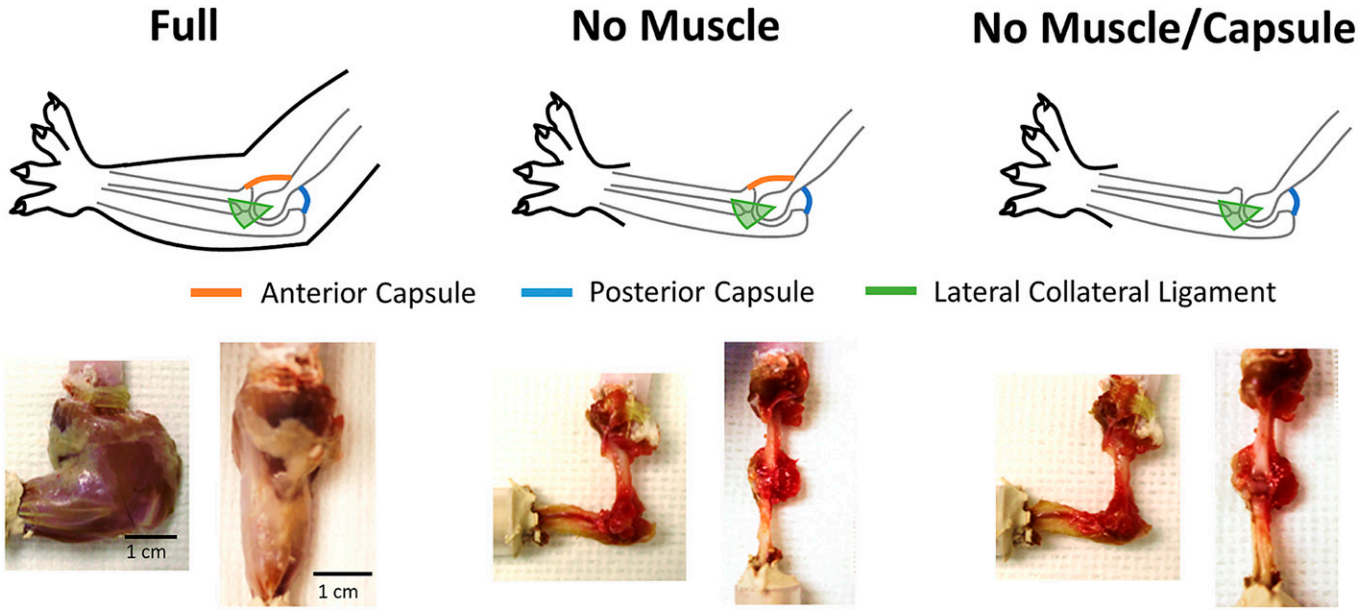


Fig. 2

Schematic and images of a rat forelimb at each testing condition: full (all soft tissue intact), no muscle (muscle and tendons removed), and no muscle/capsule (muscle/tendons and anterior capsule removed). For each condition, the left and right images are a sagittal and coronal view of the rat forelimb.

ROM of 15° with a standard deviation of 10°; a slightly more conservative group size was chosen (8 rats per group per time point). The animal injury and immobilization protocol used in the present study was previously developed by our group and was approved by the Institutional Animal Care and Use Committee[11,12]. Briefly, animals in the injury group were anesthetized and a unilateral surgical procedure, including an anterior capsulotomy with lateral collateral ligament transection, was performed to replicate the soft-tissue damage that occurs during elbow dislocation. The injured limbs were then immobilized in a flexed position immediately after the surgical procedure. The contralateral limbs and the animals in the control group were neither injured nor immobilized.

Animals were evaluated after 42 days of immobilization (42 IM) to understand the contribution of soft tissue to contracture development or after 42 days of immobilization with either 21 or 42 days of free mobilization (42/21 or 42/42 IM-FM, respectively) to understand how the soft-tissue contribution changes after joint reloading (Fig. 1). During free mobilization, the immobilization bandage was removed and animals were allowed unrestricted cage activity. At each time point, animals were killed via CO_2 inhalation overdose and stored immediately in a −20°C freezer.

Mechanical Testing

Post-mortem, the forelimbs were prepared and subjected to flexion-extension mechanical testing with use of previously described protocols[11,12]. Each limb was tested a total of 3 times to evaluate the contribution of periarticular soft tissues to elbow contracture. Flexion-extension mechanical testing was completed with (1) all soft tissues intact (full), (2) muscles and tendons removed (no muscle), and (3) muscles/tendons and anterior capsule removed (no muscle/capsule) (Fig. 2). Because the synovial membrane is a few cell layers thick, it was difficult to remove it from the capsule during dissection, so both were released for the third testing condition[15]. One individual performed all dissections to ensure consistency within the study.

To start each test, limbs were placed at 90° of flexion and then cyclically loaded to ±0.75 N (±11.25 N-mm of torque) for 5 cycles at 0.3 mm/sec. Force-displacement data from the fifth cycle

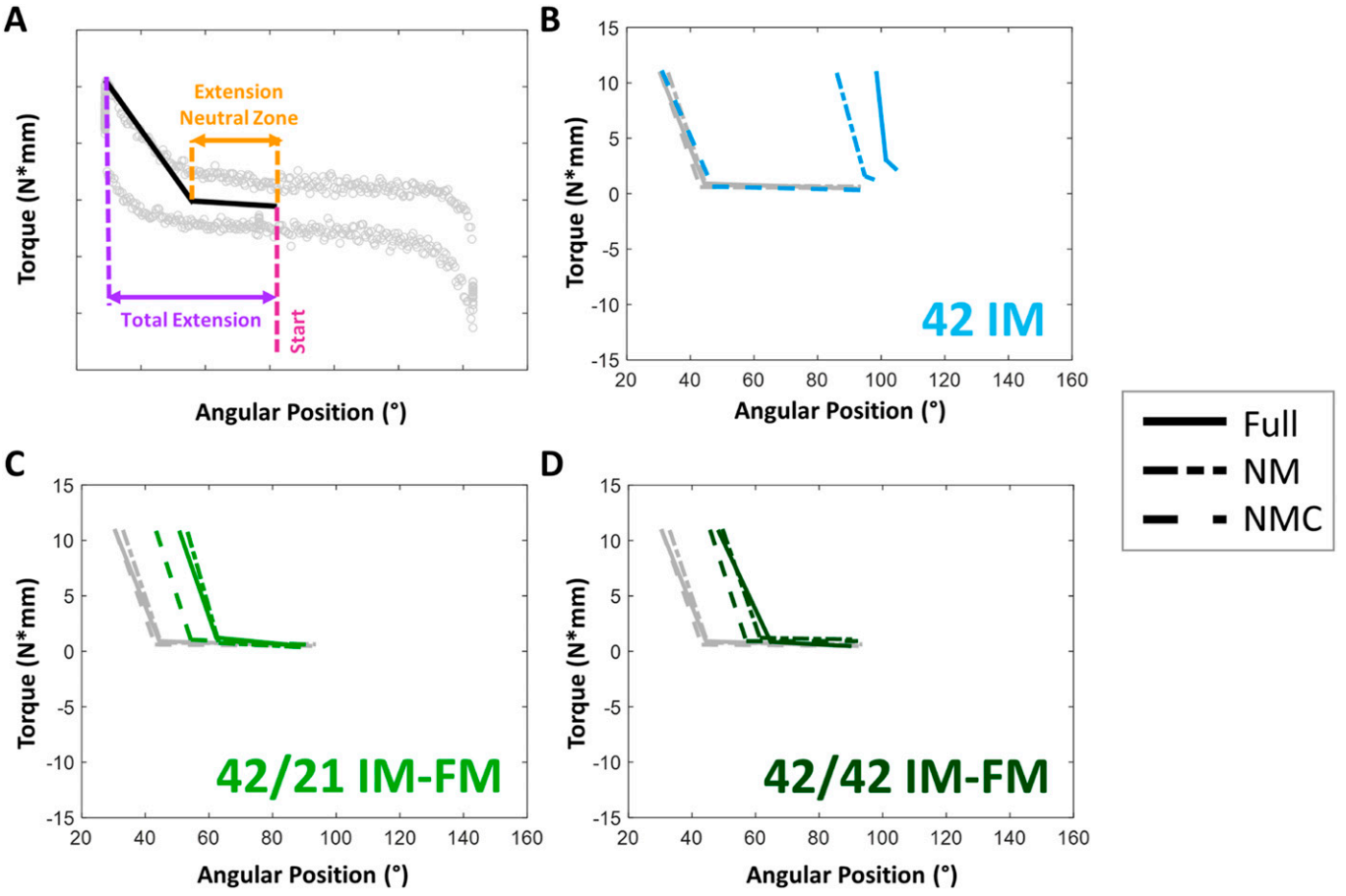


Fig. 3

Fig. 3-A Graph showing torque-angle loading curve in flexion-extension with parameters identified for a representative data set (light gray circles) and corresponding average curve (black line). **Figs. 3-B, 3-C, and 3-D** Average curves are shown in blue for 42 IM (**Fig. 3-B**), light green for 42/21 IM-FM (**Fig. 3-C**), and dark green for 42/42 IM-FM (**Fig. 3-D**) to qualitatively illustrate extension contracture for each testing condition. NM = no muscle and NMC = no muscle/capsule. For each time point, the control group is represented by gray lines.

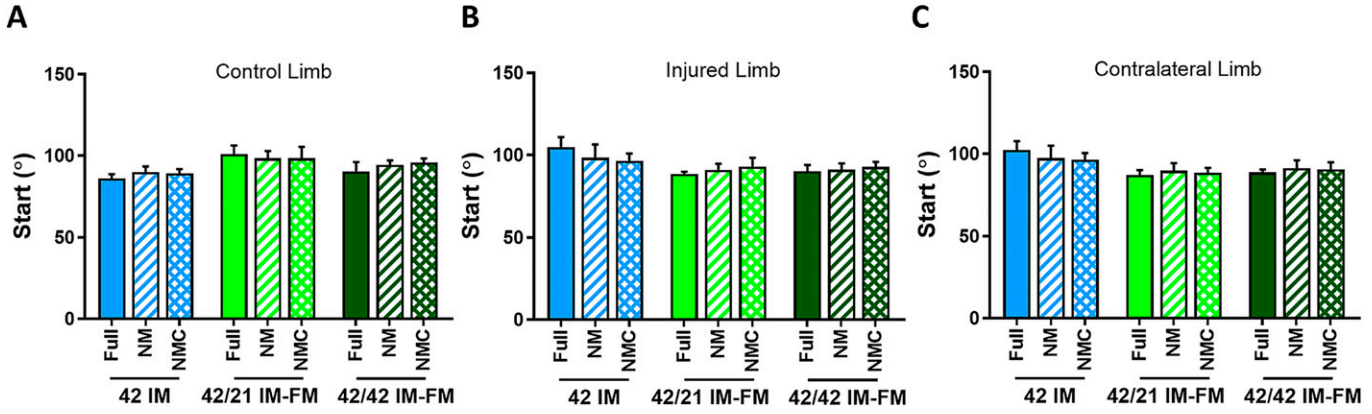


Fig. 4

Quantitative results for the starting position of the control (**Fig. 4-A**), injured (**Fig. 4-B**), and contralateral (**Fig. 4-C**) limbs for each condition at each time point, demonstrating consistent orientation across dissection states in the setup of each test. Data are presented as the average ± standard deviation. There were no significant differences between the full, no-muscle (NM), and no-muscle/capsule (NMC) conditions at each time point for the control, injured, or contralateral limbs.

were converted to torque-angular position and analyzed with use of a custom MATLAB program (MathWorks). Measurements included start position, total extension, and extension neutral zone length (Fig. 3-A). The extension neutral zone length is the linear region between the start position and the loading/unloading curves of maximum extension. Clinically, extension neutral zone length represents the amount of motion possible in elbow extension before a larger external force is applied to move the joint further. Analysis was focused on extension because data from our previous studies showed no significant change in flexion with contracture[11,12]. Total extension and extension neutral zone length data are presented as a percentage of the control, where a value of 100% represents no difference between the injured or contralateral limb and the control limb. The average point of maximum extension and both end points of the extension neutral zone were calculated to present a qualitative representation of joint motion. Group-average values were utilized to plot average curves for each testing condition at each time point. Extension lost is the difference in total extension between injured and control limbs in the full condition.

Statistical Analysis

At each time point, repeated-measures 1-way analysis of variance (ANOVA) was used to compare mechanical test parameters for each group for the 3 test conditions: (1) full, (2) no muscle, and (3) no muscle/capsule. When ANOVA analysis showed significant results, post-hoc Bonferroni corrections were used to compare each test condition. No statistical analyses were completed across different time points. Significance was set at $p < 0.05$ and trending at $0.05 < p < 0.1$. All statistical analysis was performed in GraphPad Prism (GraphPad Software).

Results

The starting position was evaluated to determine if joints were consistently placed in the same orientation in the mechanical testing system across the 3 testing conditions. There were no significant differences in the starting positions for the full, no-muscle, and no-muscle/capsule conditions among the control, injured, or contralateral limbs at any time point (Figs. 4-A, 4-B, and 4-C), demonstrating consistent and repeatable testing before and after sequential dissections within a given group.

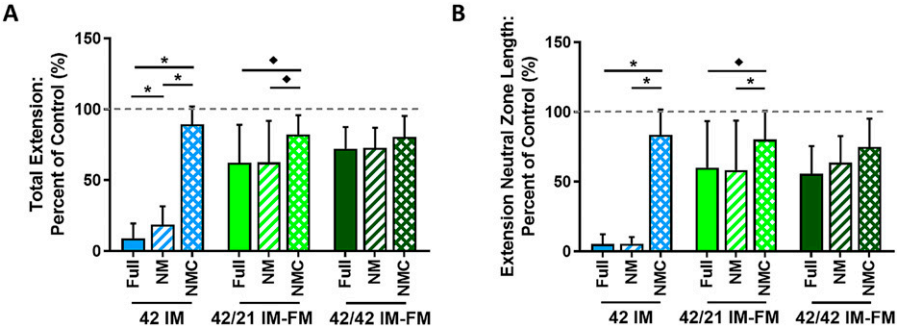


Fig. 5

Quantitative results for the injured limb total extension (**Fig. 5-A**) and extension neutral zone length (**Fig. 5-B**), presented as a percentage of the control at each time point for each testing condition. Data are presented as the average ± standard deviation. The dashed line indicates the control (100%), an asterisk indicates significance ($p < 0.05$), and a diamond indicates a trend toward significance ($0.05 < p < 0.1$). NM = no muscle and NMC = no muscle/capsule.

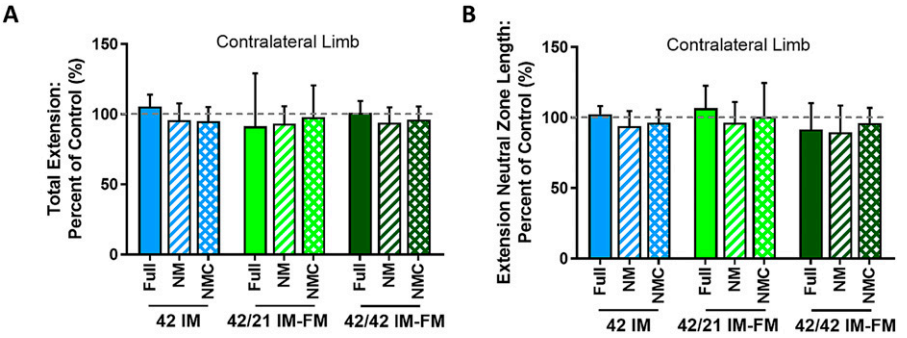


Fig. 6

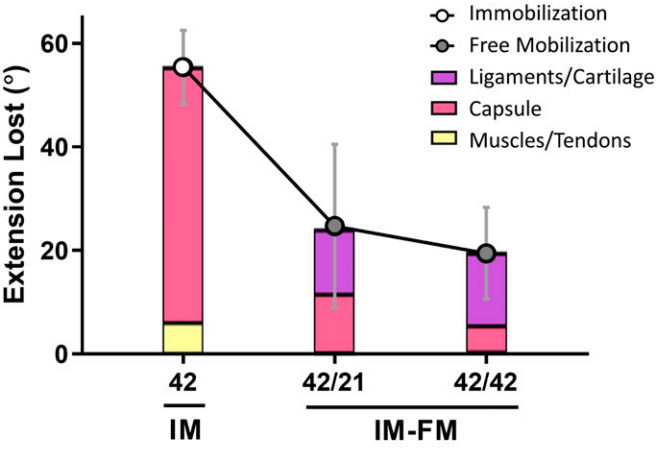
Quantitative results for contralateral limb total extension (**Fig. 6-A**) and extension neutral zone length **(Fig. 6-B**), presented as a percentage of the control at each time point for each testing condition. Data are presented as the average ± standard deviation. The dashed line indicates the control (100%). There were no significant differences for either parameter among the full and no-muscle conditions, the full and no-muscle/capsule conditions, or the no-muscle and no-muscle/capsule conditions at each time point.

At 42 IM, the full condition for the injured limb had a very short extension neutral zone length, the curve of which shifted away from the control (Fig. 3-B). Although the no-muscle condition qualitatively resulted in a small shift toward the control (Fig. 3-B), it represented a 9% increase in total extension compared with the full condition ($p = 0.017$) (Fig. 5-A). With subsequent removal of the anterior capsule, the average curve of the no-muscle/capsule condition nearly overlapped with the control (Fig. 3-B), representing a 71% and 80% increase in total extension compared with the no-muscle and full conditions, respectively ($p = 0.006$ and 0.003, respectively) (Fig. 5-A). For neutral zone length, the no-muscle condition showed no change compared with the full condition; however, the value for the no-muscle/capsule condition exhibited a 78% increase compared with those of both the no-muscle and full conditions at 42 IM ($p = 0.005$) (Fig. 5-B).

At 42/21 and 42/42 IM-FM, there was a qualitative increase in extension for the full condition compared with that of the same group at 42 IM (Figs. 3-C and 3-D). Interestingly, after muscles/tendons were released at 42/21 IM-FM, there was neither a change in the average curve nor a significant difference in either parameter evaluated (Figs. 3-C and 5). A small shift in the average curve occurred only after release of the capsule (Fig. 3-C), representing a 22% significant increase in extension neutral zone length compared with the no-muscle condition ($p = 0.042$) (Fig. 5-B). At 42/21 IM-FM, total extension for the no-muscle/capsule condition trended toward significantly larger values compared with the no-muscle and full conditions (Fig. 5-A), and extension neutral zone length values for the no-muscle/capsule condition showed a trend toward significant increases compared with the full condition (Fig. 5-B). The average curves at 42/42 IM-FM exhibited similar changes: the no-muscle condition did not alter the curve and there was only a slight shift toward the control after removal of the capsule (Fig. 3-D). However, at 42/42 IM-FM neither total extension nor extension neutral zone length exhibited any significant differences among the full, no-muscle, and no-muscle/capsule conditions (Fig. 5). Because removal of the muscles/tendons and capsule at both time points in free mobilization did not cause a return to the control level, it appears that other remaining, full tissues (i.e., ligaments and cartilage) must contribute to contracture at these time points.

In the contralateral limbs, there were no significant differences in total extension or extension neutral zone length across any test conditions at any time points (Figs. 6-A and 6-B). Contralateral data were also not different compared with the control, with average total extension and extension neutral zone length values at 97% of the control.

The amount of extension lost in the injured limbs was 55° at 42 IM, with the muscles/tendons and capsule contributing



	42 IM		42/21 IM-FM		42/42 IM-FM	
Extension Lost	55°		25°		19°	
Ligaments/Cartilage	0%	(0°)	52%	(13°)	74%	(14°)
Capsule	90%	(50°)	47%	(11°)	26%	(5°)
Muscles/Tendons	10%	(5°)	1%	(1°)	0%	(0°)

Fig. 7

Plot of extension lost during immobilization (white circle) and free mobilization (gray circles) (shown as the average ± standard deviation) along with a table showing the percentage contribution of the periarticular soft tissues to elbow contracture at each time point.

10% and 90% to elbow contracture, respectively (Fig. 7). Twenty-one days of free mobilization reduced extension loss to 25°, with the contributions of the muscles/tendons and capsule decreased to 1% and 47%, respectively; the ligaments/cartilage contributed 52% to contracture at this time point. Surprisingly, at 42/42 IM-FM, the contributions of the muscles/tendons and capsule to contracture decreased to 0% and 26%, respectively, whereas the contribution of the ligaments/cartilage increased to 74%.

Discussion

The contribution of periarticular soft tissues to posttraumatic elbow contracture is dependent on time and the relative amount of joint mobility. Immediately following immobilization, the anterior capsule was the primary contributor to elbow contracture. However, reloading the joint during free mobilization shifted the soft-tissue response to be increasingly dominated by the remaining, intact tissues (i.e., ligaments and cartilage). Surprisingly, as the duration of free mobilization increased, so did the contribution of the ligaments/cartilage to elbow contracture.

Muscles/tendons were responsible for 10% of elbow contracture at 42 IM (Fig. 7). This limited yet significant contribution after immobilization was consistent with the findings of our previous study, which showed that active and passive muscle mechanics were significantly altered at 42 IM (Figs. 3-B and 5)[14]. Thus, muscle contributes to early elbow contracture. However, during free mobilization, the contribution of muscles/tendons decreased, demonstrating that prior alterations to these tissues recovered with joint reloading (Figs. 5 and 7). These results were also consistent with previous data that showed active and passive muscle mechanics were not significantly different compared with control at 42/42 IM-FM[14]. Similarly, studies of immobilization-induced knee contracture animal models have demonstrated that the contribution of muscles/tendons after immobilization decreased with time[4-6].

In our previous study utilizing the same rat model of elbow contracture, total ROM in flexion-extension only increased after 21 days of free mobilization[13]. This initial gain in motion was likely the result of increased muscular forces across the elbow[16]. A longer period of free mobilization did not increase elbow ROM in these previous studies because the active/dynamic muscles/tendons had already recovered (Fig. 7)[13]. Thus, in this animal model, muscles/tendons are not permanent contributors to posttraumatic elbow contracture and should not be the focus of tissue-targeted treatment strategies.

After immobilization, the capsule was responsible for 90% of the motion lost as a result of contracture (Fig. 7). In an immobilization-induced knee contracture model, Chimoto et al. similarly reported that the capsule substantially contributed to reduced extension following immobilization[15]. During free mobilization, the percentage of contribution and the amount of extension lost because of the capsule decreased with time, demonstrating that the capsule was not the only periarticular soft tissue responsible for motion loss during this time period (Figs. 5 and 7). Clinically, the contribution of non-capsular tissues to elbow contracture has been shown by the persistence or recurrence of joint-motion loss following either open or arthroscopic anterior capsule release[3,17,18]. In such patients, full ROM is rarely restored and secondary operations are even indicated in 12% to 15% of cases to further extract fibrotic joint tissues[19].

Although the ligaments/cartilage did not contribute to contracture after immobilization, these tissues were responsible for 52% and 74% of the motion lost at 42/21 and 42/42 IM-FM, respectively (Fig. 7). Interestingly, although the percentage of contribution of these structures to elbow contracture increased over time during free mobilization, the amount of extension lost remained the same (Fig. 7). Ligament thickening was visually observed during dissections of limbs at both free mobilization time points, suggesting that ligament scarring/hypertrophy led to the increasing contribution to contracture. The increasing contribution of ligaments/cartilage could also be a result of mechanical and biochemical interactions of the pathological capsule with the surrounding ligaments and cartilage in the joint following trauma. Previous histological evaluation in this animal model found more damage and degeneration in the non-opposing joint surface (i.e., cartilage-capsule) compared with the opposing joint surface (i.e., cartilage-cartilage)[13]. Thus, the cartilage in the non-opposing joint surface could be interacting with the pathological capsule and responding to the altered mechanical and biological environment, resulting in secondary degeneration after contracture[17,20]. An immobilization-induced knee contracture model also exhibited evidence of cartilage-capsule interaction, showing proliferation and adhesion of connective tissue during free mobilization[21]. Reloading the joint during free mobilization could also cause micro-damage to both the capsule and ligaments/cartilage, which could alter tissue mechanical load and biological signaling to ultimately affect the tissue contribution to elbow contracture[7].

Lindenhovius and Jupiter stated that the timing of treatment is associated with the outcome of motion improvement in the elbow, and that the longer intervention is delayed, the larger the contribution of muscles, tendons, and cartilage[3]. Although the present study demonstrates that muscles/tendons were not permanent contributors to elbow contracture, the results did support a time-dependent response of the capsule and ligaments/cartilage. In conclusion, it appears that the capsule, ligaments, and cartilage were all persistent contributors to permanent contracture in our rat model of elbow contracture and should be considered during development of tissue-targeted treatment strategies. Ongoing evaluation of various treatment strategies in this rat model will elucidate concepts that may be clinically translatable.

There are limitations and additional aspects of this study to consider. First, rats are quadruped animals, hence their forearms experience different loads than humans. However, in our animal model, the injured forelimb is immobilized to prevent weight-bearing by using an external bandage which more closely mimics the human condition following trauma. Second, the evaluation of the periarticular soft-tissue contribution to elbow contracture only isolated the muscles/tendons and anterior capsule. However, the results showed that the remaining, intact tissues (i.e.,

ligaments and cartilage) dominated contracture during free mobilization. Future work will evaluate ligament mechanics to understand the way they contribute to contracture. Third, although our previous work evaluated morphological changes in muscle, capsule, and cartilage[11-13], future work will also study biological changes in the capsule, including evaluation of protein content and matrix organization. ■

Chelsey L. Dunham, BS[1]
Ryan M. Castile, BS[1]
Aaron M. Chamberlain, MD[1]
Spencer P. Lake, PhD[1]

[1]Departments of Biomedical Engineering (C.L.D. and S.P.L.), Mechanical Engineering & Materials Science (R.M.C. and S.P.L.), and Orthopaedic Surgery (A.M.C. and S.P.L.), Washington University in St. Louis, St. Louis, Missouri

E-mail address for S.P. Lake: lake.s@wustl.edu

ORCID iD for C.L. Dunham: 0000-0002-8827-5257
ORCID iD for R.M. Castile: 0000-0002-1974-2106
ORCID iD for A.M. Chamberlain: 0000-0002-4622-9797
ORCID iD for S.P. Lake: 0000-0002-7424-947X

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