Published in final edited form as:

Muscle Nerve. 2018 December ; 58(6): 843–851. doi:10.1002/mus.26344.

Muscle does not drive persistent post-traumatic elbow contracture in a rat model

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Abstract

Introduction—Post-traumatic elbow contracture is clinically challenging because injury often disrupts multiple periarticular soft tissues. Tissue specific contribution to contracture, particularly muscle, remains poorly understood.

Methods—This study utilized a previously developed animal model of elbow contracture. After surgically inducing a unilateral soft tissue injury, injured limbs were immobilized for 3, 7, 21, and 42 days (IM) or for 42 IM with 42 days of free mobilization (42/42 IM-FM). Biceps brachii active/passive mechanics and morphology were evaluated at 42 IM and 42/42 IM-FM, while biceps brachii and brachialis gene expression was evaluated at all time points.

Results—Injured limb muscle exhibited significantly altered active/passive mechanics and decreased fiber area at 42 IM but returned to control levels by 42/42 IM-FM. Gene expression suggested muscle growth rather than a fibrotic response at 42/42 IM-FM.

Discussion—Muscle is a transient contributor to motion loss in our rat model of post-traumatic elbow contracture.

Keywords

Contracture; Elbow; Fibrosis; Gene Expression; Mechanics; Muscle

Introduction

Post-traumatic elbow contracture is difficult to manage and treat due to the extensive periarticular soft tissue pathology that contributes to contracture. The affected periarticular

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Ethical Publication Statement: We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

Disclosure of Conflicts of Interest: Aaron M. Chamberlain has received support for work outside the submitted manuscript from Zimmer-Biomet, DePuy, Arthrex, and Wright Medical. The remaining authors have no conflicts of interest.

soft tissues may include muscles, tendons, capsule, ligaments, and cartilage¹. Muscles near the elbow stabilize and align joint surfaces during articulation by force transmitted through active contraction and passive contributions from extracellular matrix (ECM)^{2–5}. In response to traumatic injury and immobilization, muscle may contribute to reduced joint mobility through multiple adaptations. Decreased loading can lead to loss of muscle fiber contractile protein (atrophy), thereby reducing muscle active force and joint torque⁶. Additionally, in response to post-traumatic inflammatory signaling, muscle may experience a pathological increase in ECM content (fibrosis) causing its passive resistance to joint motion to increase⁷.

Previously, immobilization-only rat models of knee contracture evaluated how muscles/tendons contributed to overall limb mechanics^{6,8,9}. In these models, joint motion loss immediately following immobilization was believed to be caused by muscles/tendons, but these changes were reversible^{6,8}. Thus, muscles/tendons were suggested to be transient contributors to knee contracture^{6,9,10}. However, these models did not evaluate the effect of local injury with immobilization on muscle. Clinical observations have suggested that the close proximity of the elbow capsule, ligaments, and muscles could cause these tissues to contribute to motion loss following traumatic joint injury¹¹. Specifically, local inflammation combined with immobilization could cause maladaptation and persistent pathology. No work has evaluated the role of local tissue injury in muscle adaptation to joint immobilization. However, tenotomy models of unloading in the upper extremity, which involve tendon injury, demonstrate persistent muscle atrophy and fibrosis following reloading^{12,13}, suggesting that inflammation and unloading may play a synergistic role in the development of persistent pathology.

Our group previously developed a rat model of post-traumatic elbow contracture, which resulted in significant motion loss in flexion-extension and pronation-supination that persisted following free mobilization^{14–16}. While the injury induced in our rat model of elbow contracture did not directly damage the tendons or muscles, the inflammatory environment surrounding them was altered due to the injury induced in the anterior capsule and lateral collateral ligament. Since muscle is sensitive to inflammation, it could influence muscle adaptation to immobilization and subsequent free mobilization, and drive muscle contribution to contracture¹⁷.

The objective of this study was to evaluate the contribution of muscle to post-traumatic elbow contracture. Specifically, the goal was to evaluate temporal changes in active and passive muscle structure-function-composition relationships with immobilization and free mobilization in the context of soft tissue injury. We hypothesized that active and passive muscle properties would be significantly altered and contribute to contracture following injury and immobilization, and that these changes would partially persist following free mobilization.

Methods

Animal and Injury Model

Long-Evans rats (Charles River Laboratories International, Wilmington, MA) were selected based on previously described criteria, including similarities to human anatomy, functional

range-of-motion (ROM), and upper extremity use especially in flexion-extension and pronation-supination^{14–16}. In this IACUC-approved study, 78 male Long-Evans rats (250–300g, 8–10 weeks old) were randomized into a surgical (injured) and control group. The same animal injury and immobilization protocol was used as previously developed by our group^{14,15}. Briefly, animals in the injured group were anesthetized and unilateral surgery consisting of an anterior capsulotomy with lateral collateral ligament transection was performed to replicate the soft tissue injuries that occur during elbow dislocation. The surgical approach included: (1) splitting the muscular layer between the anconeus and extensor carpi ulnaris and, more deeply, splitting the extensor carpi radialis brevis, (2) isolating and dividing the anterior capsule, (3) severing the lateral collateral ligament complex, and (4) closing the muscle fascial layer with suture and skin with staples. The brachialis and biceps brachii muscles were not violated during the procedure. Following surgery, injured limbs were immobilized in a flexed position ($\sim 153^\circ$)^{14,15}. Control animals were neither injured nor immobilized and served as comparisons to injured animals.

Animals were randomized into two groups, including injured and age-matched controls, and biceps brachii muscles were mechanically evaluated after 42 days of immobilization (IM) or after 42 days of immobilization with 42 days of free mobilization (42/42 IM-FM) during which the immobilization bandage was removed and the animals were allowed unrestricted cage activity ($n = 7/\text{group}$ per time point; 28 total animals) (Supplemental Figure 1A). After mechanical testing, these muscles were subsequently used for histology and immunohistochemistry. Additional animals were randomized into two groups for gene expression analysis, including injured and age-matched controls, with the biceps brachii and brachialis muscles evaluated after 3, 7, 21, and 42 IM, and 42/42 IM-FM ($n = 5/\text{group}$ per time point; 50 total animals) (Supplemental Figure 1A). Significant elbow contracture was previously established in our animal model after 42 IM¹⁴ and we also showed persistent motion loss up until 42/42 IM-FM¹⁵. Thus, these time points represent key points of evaluation following contracture development and long-term contracture, respectively. Early time points were evaluated to detect biological changes following injury that might support changes observed in joint mechanics.

The biceps brachii and brachialis muscles from our rat model of elbow contracture were isolated for evaluation because they are major elbow flexor muscles¹⁸. While both muscles were used in gene expression analysis, pilot testing revealed that only one muscle per animal could be reliably evaluated using mechanical testing due to technical limitations. The biceps brachii was selected for mechanical evaluation because its ability to generate force has been reported to be compromised in flexed elbow positions, while the brachialis showed intermediate force generation over all elbow positions¹⁸. Since our animal model included immobilization in a flexed position, resulting in a flexion contracture^{14,15}, the biceps brachii was deemed most likely to exhibit altered mechanical properties. Animals for mechanical testing were sacrificed by thoracotomy and aortic dissection, while animals for gene expression were sacrificed via CO₂ inhalation overdose.

Mechanical Testing

Active and passive mechanical testing were performed on whole biceps brachii muscle of both injured and control limbs using an ex-vivo muscle physiology testing system (Aurora Scientific, Aurora, Ontario) (Supplemental Figure 1B). Animals were anesthetized using isoflurane at 2.5–4% via nasal inhalation. The biceps brachii of the left limb for injured and control animals was isolated and sutured through the proximal and distal tendons. The muscle was then excised and immediately placed in Ringer's solution (pH ~7.5; 37°C). The proximal tendon was secured to a fixed post while the distal tendon was attached to the lever arm. A load cell in-line with the lever arm enabled force measurement throughout mechanical testing. Once secured in the test system, the muscle was flanked by parallel plate electrodes enabling electrical stimulation.

Muscle slack length (L_s) was set as the length at which passive tension first registered above zero. Maximum tetanic tension was determined by increasing muscle length incrementally from L_s and stimulating tetanic contraction (0.3ms pulse width, 330ms duration, 225 Hz) at supramaximal voltage until a plateau in force was reached. Supramaximal voltage was set at slack length by increasing the voltage input until a plateau in force occurred demonstrating full activation of the muscle. Active mechanical testing was completed within 30 minutes of muscle excision and 15 minutes of muscle relaxation/equilibration was allowed between active and passive testing. Passive mechanics were evaluated via stress-relaxation tests, in which muscle was stretched at a rate of $2 L_s/s$ in 10% L_s increments to either failure or to 120% L_s , whichever was reached first, with 2 minutes of relaxation between each increment. Active and passive mechanical properties were normalized to muscle physiological cross-sectional area (PCSA): $PCSA = \frac{\text{Muscle Weight} \cdot \cos(\text{Pennation Angle})}{\text{Optimum Fiber Length} \cdot \text{Muscle Density}}$ ¹⁹. Based on previous literature, pennation angle and muscle density were defined as 0° and 1.05 g/cm³, respectively¹⁸. Muscles were weighed after all mechanical testing was completed.

Histology and Immunohistochemistry

After mechanical testing, all muscles were flash frozen in liquid nitrogen-cooled isopentane, then immediately stored at –80°C. Three muscles per group, which exhibited average active and passive mechanical testing data, were cut in 10µm transverse sections on a cryostat at –24°C. Serial sections ($n = 5/\text{muscle}$) were stained with hematoxylin and eosin (H&E) to view overall fiber morphology. To quantify muscle fiber cross-sectional area and ECM area fraction, serial sections were immunolabeled with primary antibodies for laminin and collagen type I, respectively (Ab11575 and Ab34710, respectively; Abcam, Cambridge, United Kingdom). Muscle sections were treated with blocking buffer (2% BSA), incubated with primary antibodies for 1 hour, followed by incubation with an Alexa Fluor 499 goat anti-mouse secondary antibody (Life Technologies, Carlsbad, CA) for 30 minutes. Quantification of muscle fiber cross-sectional area was performed on laminin stained sections using a custom macro in ImageJ (NIH, Bethesda, MD). Quantification of ECM area fraction was performed on collagen type I stained sections using an ImageJ Pixel Counter Plugin. Image processing settings were standardized across all sections.

Quantitative real-time PCR

The biceps brachii and brachialis muscles were harvested from injured and control animals via sterile dissection within 20 minutes after sacrifice. After dissection, tissue was immediately flash frozen and stored at -80°C until RNA isolation was completed using RNeasy Plus and RNase-free DNase Set (Qiagen, Hilden, Germany). RNA quality was evaluated by a RNA Nano Kit (Agilent, Santa Clara, CA); all samples had at least 300ng RNA with a RNA integrity number (RIN) > 7 . cDNA was synthesized using a SuperScript VILO kit (Invitrogen, Carlsbad, CA) and TaqMan primers were used to evaluate the expression of genes related to fibrosis, ECM regulation, collagen, proteoglycans and myogenesis (Supplemental Table 1). All reactions were performed in triplicate and quantification was performed using the $2^{-\text{CT}}$ method, with expression levels normalized to GAPDH²⁰ and control animals at each time point.

Statistical Analysis

Unpaired *t*-tests compared injured to control at each time point for (1) active stress, (2) passive stress, (3) fiber area, and (4) ECM area fraction. Gene expression was evaluated across immobilization time points via one-way ANOVA; when significance was found, post-hoc *t*-tests with Bonferroni corrections were used to compare data at individual time points. Unpaired *t*-tests were used to compare gene expression at 42/42 IM-FM to 42 IM. GraphPad Prism was used to perform all statistical analyses (GraphPad Software Inc., La Jolla, CA), and significance was defined as $p < 0.05$.

Results

Mechanical Testing

In general, muscle from injured limbs exhibited differences compared to control at 42 IM, but by 42/42 IM-FM there were no differences between injured and control muscles (Figure 1). Active stress was significantly decreased in injured limb muscle at 42 IM compared to control ($p = 0.0086$) (Figure 1A). Peak passive stress at 42 IM was significantly increased in injured limb muscle at 10% strain as well as from 50% to 70% strain compared to control (Figure 1B). Equilibrium passive stress at 42 IM was significantly increased in injured limb muscle at 20% strain and from 40% to 70% strain compared to control (Figure 1C). Qualitatively, similar results were evident for peak and equilibrium passive stress versus muscle length curves (Figure 1D–E; not evaluated statistically). Injured limb muscle length at maximum tetanic tension was significantly decreased compared to control at 42 IM ($p = 0.0011$) (Figure 2A), but muscle weight was not significantly different at either time point (Figure 2B). Raw forces exhibited similar results at 42 IM and 42/42 IM-FM for active and passive mechanical testing (data not shown).

Histology and Immunohistochemistry

H&E stained sections revealed moderate fiber atrophy at 42 IM but no gross morphological pathology (Figure 3A). Fiber area was quantified on laminin-labeled sections (Supplemental Figure 2A), wherein injured limb muscle was significantly decreased compared to control at 42 IM ($p = 0.0005$), but was not different from control at 42/42 IM-FM (Figure 3B). ECM

area fraction, computed using collagen type I labeled sections (Supplemental Figure 2B), was not significantly different in injured limb muscle at either 42 IM or 42/42 IM-FM compared to control (Figure 3C). Additionally, markers of muscle regeneration (i.e., centrally located myonuclei) were not observed at either time point²¹.

Quantitative real-time PCR

A heatmap summarizing all gene expression results (Figure 4A–B) illustrates small changes in injured limb muscle, where values ~1 indicate no difference compared to control. Statistical analyses (Figure 4C) and more detailed results are also provided (Figure 5). Interestingly, gene expression varied by muscle, with different expression patterns evident for biceps brachii and brachialis.

A few gene regulators of fibrosis and ECM exhibited small but significant changes. TGFβ3, which is pro-fibrotic²², was the only gene significantly altered during immobilization in both muscles (Figure 4C). Significant post-hoc comparisons demonstrated early upregulation during immobilization, specifically at 3 IM compared to 7 IM in the biceps brachii (Figure 5A) and at 7 IM compared to 42 IM in the brachialis (Figure 5F). Another profibrotic gene²², CTGF, was also upregulated early during immobilization for both muscles (Figure 4A–B) but was only significantly altered in the biceps brachii (Figure 4A, 4C). Specifically, CTGF was significantly upregulated at 3 IM compared to 7, 21, and 42 IM (Figure 5B). Following free mobilization, the only significant change in expression was the upregulation of MMP2 and TIMP2, which regulate ECM remodeling⁷, in the brachialis (Figure 4B–C). Brachialis α-SMA expression also varied significantly during immobilization and was upregulated at 42 IM compared to 3 IM ($p = 0.0098$).

ECM genes for collagens and proteoglycans exhibited the largest number of significant changes in expression over time. Several collagens varied significantly with time during immobilization, but only Col IV was significantly altered in both muscles (Figure 4C). Col IV was significantly increased at 42 IM compared to 7 IM in biceps brachii (Figure 5D) and at 21 IM compared to 3 IM in the brachialis (Figure 5I). Col III in the biceps brachii and Col I and VI in the brachialis also varied significantly during immobilization (Figure 4C). However, only Col I in the brachialis exhibited significant post-hoc comparisons where expression was upregulated at 21 IM compared to 3, 7, and 42 IM (Figure 5H). Interestingly, all types of collagen were significantly upregulated at 42/42 IM-FM compared to 42 IM in the brachialis only (Figure 4C, 5H–I). DCN, a proteoglycan, was also significantly upregulated in the brachialis at 42/42 IM-FM compared to 42 IM (Figure 4C).

Similar to regulators of fibrosis and ECM, myogenic genes exhibited few significant fold changes. MyoD, a myoblast differentiation regulator²³, and MSTN, a negative regulator of muscle growth and regeneration²³, only varied significantly with time during immobilization in the biceps brachii (Figure 4A, 4C). Post-hoc comparisons revealed that biceps brachii MyoD expression was downregulated at 21 IM compared to 3 and 7 IM ($p = 0.0285$ and 0.0488 , respectively), while MSTN expression was significantly upregulated at 7 IM compared to 42 IM (Figure 5E). In both muscles, MSTN expression was significantly downregulated at 42 IM compared to 42/42 IM-FM (Figure 4C) but returned to control/baseline levels by 42/42 IM-FM (Figure 4A–B, 5E, 5J).

Discussion

In this study, the evaluated muscles in our rat model of post-traumatic elbow contracture exhibited significantly altered tissue-level mechanics immediately following immobilization, that returned to normal following free mobilization. Changes in passive and active mechanics occurred concurrently with tissue-level structural alterations (i.e., decreased muscle length and fiber cross sectional area), but these changes resolved under free mobilization. Gene expression yielded small but significant changes throughout immobilization and following free mobilization, highlighting the complexity of gene regulation in morphologic and functional muscle adaptations to joint contracture.

Muscle mechanical properties have been shown to depend on muscle length in addition to muscle activation and tension²⁴. When muscle was immobilized in a shortened position, altered active and passive forces correlated with decreased muscle length, weight, and fiber diameter^{24–26}; similar to our muscle at 42 IM (Figure 2, 3B). But, these changes were not permanent^{24,25}, and muscle was able to recover morphologically and functionally following a free mobilization period. However, the role of soft tissue injury in functional recovery was not evaluated.

Compared to control, injured limb biceps brachii exhibited significantly decreased fiber area and active stress, and increased passive stress at 42 IM (Figure 1, 3). Atrophic changes (i.e., decreased muscle length and fiber area) were expected following immobilization, as previous studies reported muscle atrophy in the rodent ankle after 4–6 weeks of ankle immobilization^{25,27}. Thus, decreased muscle length and fiber area likely contributed to the decreased active force generation at 42 IM (Figure 1–3), which could reduce joint torque and ultimately contribute to the decreased joint motion at 42 IM¹⁴. Additionally, injured limb muscles began passive mechanical testing in a shortened position compared to control at 42 IM (Figure 1D, 1E); thus, moving the injured limb to the same joint angle as control would require increased passive stress, demonstrating impaired joint function at 42 IM due to the shortened muscle length. Even though muscle forces were normalized to PCSA, there was still a deficit in mechanics following immobilization, demonstrating that other factors might also compromise muscle force generation at 42 IM.

The ECM plays a key role in passive muscle mechanics by transmitting force generated by muscle fibers and providing passive resistance to joint motion⁵. Previous studies reported increased ECM production (i.e., collagen type I and III) in muscle after immobilization^{25,27}. In our rat model of elbow contracture, passive stress was increased at 42 IM in injured limb muscle, but was not supported by a significant increase in ECM area fraction (Figure 3C). However, ECM accumulation may be influenced by passive stretch and muscle activation²⁴. For example, previous work showed no change in ECM content when muscle was periodically activated over a reduced range of motion²⁸. While the injured limb of our animal model was immobilized using a bandage wrap¹⁴, the animals may have still activated their muscles in this fixed position, which may have inhibited excessive ECM accumulation.

Despite no difference in the amount of ECM between injured and control muscles (Figure 3C), the ECM still likely contributed to the altered muscle passive mechanics because the

raw forces and passive stresses were both significantly increased compared to control. Therefore, altered collagen organization and crosslinks, or changes to other ECM components (i.e., proteoglycans) may be responsible for the increased passive stiffness at 42 IM^{6,29,30}. The passive properties could also be influenced by changes to cross-links between actin and myosin filaments and/or non-contractile proteins within the sarcomere cytoskeleton (i.e., titin, desmin)²⁴. Regardless, changes to muscle mechanics, length and fiber area were not permanent after free mobilization (Figure 1–3). Overall, in our rat model of elbow contracture, the altered morphology and mechanics of injured limb muscle at 42 IM indicate detrimental remodeling of the ECM during immobilization, however, subsequent reparative remodeling likely occurred during free mobilization since changes did not persist at 42/42 IM-FM.

Clinically, joint contracture is believed to result from periarticular soft tissue fibrosis, which is defined as an excessive increase in ECM due to a pathologic tissue repair response^{7,31,32}. Specifically, collagen is synthesized at a rate which exceeds its degradation and may be exacerbated by an imbalance of MMPs and TIMPs^{7,33} which degrade ECM proteins and inhibit ECM protein degradation, respectively. Both muscles exhibited significant changes in collagen during immobilization, but at 42/42 IM-FM significance only existed for the brachialis (Figure 4–5). While MMPs and TIMPs did not vary significantly over time during immobilization in either muscle, MMP2 and TIMP2 in the brachialis were significantly upregulated at 42/42 IM-FM (Figure 4). MMP2 upregulation (1) could be indicative of increased matrix remodeling rather than fibrosis and (2) is associated with the formation of new myofibers which supports the potential of muscle growth⁷.

CTGF was upregulated early during immobilization for both muscles, by only varied significantly with time for the biceps brachii (Figure 4–5). Previously, expression of CTGF was reported to be upregulated in fibrotic tissue where it mimics the effects of TGFβ, increasing the production of ECM proteins like fibronectin and collagen⁷. Therefore, the early increase in CTGF and the significant changes in TGFβ3 throughout immobilization may support the subsequent increase in collagen expression (Figure 4–5); but the significant collagen upregulation did not occur until after free mobilization.

The adaptive matrix expression changes observed in muscle from our rat model of elbow contracture were more likely indicative of muscle remodeling during immobilization and remodeling/growth during free mobilization rather than fibrosis³³. While MSTN, a negative regulator of muscle growth and regeneration⁷, was significantly upregulated early during immobilization in the biceps brachii, it returned to control/baseline expression by 42/42 IM-FM (Figure 4–5). Muscle growth during free mobilization is supported by collagen and MMP2 upregulation at 42/42 IM-FM, which is indicative of ECM remodeling and the formation of new myofibers⁷.

While we did see significant changes in muscle gene expression, these changes were very small. Even though some genes changed significantly over time for both muscles, there were limited significant post-hoc comparisons between specific time points. Interestingly, significant changes occurred most often for the brachialis. The distinction in expression between the injured limb muscles and the time points evaluated may indicate a biological or

functional imbalance in muscle which may contribute to overall joint contracture at 42 IM^{2,5,14,23}.

The induced injury did not include any direct damage to the tendons or muscles analyzed; therefore, changes observed in this study could underestimate muscle contribution to contracture in human patients with more severe injuries. However, the focus of our model was to replicate soft tissue damage following elbow dislocation, which consistently affects the anterior capsule and lateral collateral ligament more than muscle. While the muscles analyzed were not directly injured, the inflammatory environment surrounding them was altered (shown via histological evaluation of the anterior capsule³⁴). Since muscle is sensitive to inflammation, the altered inflammatory response of the capsule may have influenced muscle repair, regeneration, and growth through local and circulating inflammatory cytokines¹⁷. While this study did not directly evaluate inflammation in muscle, it did demonstrate that damage to the anterior capsule and lateral collateral ligament did not induce permanent changes in the muscle²⁵. Future work will evaluate how inflammation affects all periarticular soft tissues in our rat elbow contracture model.

This study is not without limitations. First, mechanics and morphology were only evaluated in the biceps brachii muscle, while gene expression was evaluated in the biceps brachii and brachialis muscles. While there were no significant differences in mechanics, ECM area fraction, and collagen gene expression at 42/42 IM-FM in injured limb biceps brachii compared to control, collagen gene expression in the brachialis was significantly upregulated at 42/42 IM-FM and so could potentially exhibit altered mechanics and ECM area fraction at this time point. Unfortunately, as previously discussed, only one muscle could be reliably used for mechanical evaluation. Second, collagen type I was used to quantify ECM area fraction. While collagen type I is the primary fibrous protein in muscle ECM, the interstitial matrix which surrounds muscle fibers is also composed of fibronectin, perlecan, and collagen type III and V^{7,29}. Third, we only used young adult rats³⁵ which are representative of a young patient population. However, the ability of muscle to recover after a period of unloading declines with age¹⁹, and so it is likely that our data would underestimate muscle contribution to contracture in an older patient population. Lastly, our rat model of post-traumatic elbow contracture did not allow us to determine if changes in muscle were due to either injury or immobilization. Previously, we showed that an immobilization-only group did not cause permanent elbow contracture^{14,15} and hence would not be an appropriate model to evaluate contracture. An injury without immobilization group was not used because (1) the joint would be unstable following injury induction and would likely dislocate causing additional uncontrolled joint damage, and (2) it is not representative of how these injuries are handled clinically.

In conclusion, the altered mechanics, morphology, and gene expression in injured limb muscles after immobilization were likely due to structural alterations (i.e., decreased muscle length and fiber cross area) and ECM remodeling. These changes recovered after free mobilization, thereby suggesting that muscle is not a permanent contributor to elbow contracture in our animal model. Future work will use our rat model of post-traumatic elbow contracture to evaluate the contributions of other periarticular soft tissues (i.e., anterior capsule, lateral collateral ligament) to contracture.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements:

The authors thank the American Shoulder and Elbow Surgeons (ASES) for research funding and the National Institute of Health (NIH) for a training fellowship (NIBIB T32 EB018266) and research funding (NIAMS R03 ARR067504).

Abbreviations

ECM	Extracellular Matrix
FM	Free Mobilization
IM	Immobilization
ROM	Range-of-motion
PCSA	Physiological Cross-sectional Area

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Figure 1.

(A) Active stress of injured limbs biceps brachii at 42 IM was significantly decreased compared to control (average $\pm$ std; * $p < 0.05$). (B) Peak and (C) equilibrium passive stress-strain curves of the biceps brachii for control (squares) and injured limbs (circles); only includes strains at which all samples have data. Evaluation was completed at 42 IM and 42/42 IM-FM. Only 42 IM injured limbs exhibited significantly different stress compared to control; 42/42 IM-FM was not different compared to control (average $\pm$ std; * $p < 0.05$ and ** $p < 0.01$ for 42 IM only). (D) Peak and (E) equilibrium passive stress-muscle length curves of the biceps brachii for control and injured limbs qualitatively represent the effect of muscle shortening.

Figure 2.

Biceps brachii (A) muscle length at maximum tetanic contraction was only significantly decreased at 42 IM compared to control, but (B) muscle weight was not significantly different at either time point (average $\pm$ std; * $p < 0.05$, ** $p < 0.01$).

Figure 3.

(A) Representative transverse histology sections of the biceps brachii (hematoxylin and eosin) from control and injured limbs at 42 IM and 42/42 IM-FM (scale bar = 200μm). Quantitative measures computed from fluorescent muscle sections: (B) fiber area (μm²) and (C) extracellular matrix (ECM) area fraction for injured and control limbs at 42 IM and 42/42 IM-FM. While there were no significant changes in ECM area fraction at either time point, fiber area was significantly decreased at 42 IM compared to its respective control (average ± std; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

Figure 4. qPCR: Fold change in the (A) biceps brachii and (B) brachialis muscle of injured limbs evaluating expression of genes for fibrosis, extracellular matrix (ECM) regulators, collagen, myogenesis, and proteoglycans (yellow and blue correspond to increased or decreased expression, respectively, while white represents no change relative to control). (C) Left: One-way ANOVA during immobilization only, including a heatmap representing p values. Right: Results of unpaired t -tests that compared injured limbs at 42 IM and 42/42 IM-FM. White boxes represent when the p value was not significant (BI = biceps brachii, BR = brachialis).

Figure 5. qPCR: Fold change in the (A-E) biceps brachii and (F-J) brachialis muscle of injured limbs showing expression of (A,F) transforming growth factor beta-3 (TGFβ3), (B,G) connective tissue growth factor (CTGF), (C,H) collagen type I (Col I), (D,I) collagen type IV (Col IV), and (E,J) myostatin (MSTN). (average ± std; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$).