Improvement in the Contractility and Muscle Stem Cell Density of the Rotator Cuff Following Surgical Repair
A Case Report

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Large or massive tears of the rotator cuff can cause severe pain and substantially limit mobility, and thus negatively impact quality of life. Following surgical repair, an estimated 50% of patients still have symptoms at six months and 40% have symptoms at one year, demonstrating that surgical repair is often unable to fully restore the normal function and strength of the involved muscles. A common pathophysiological change that occurs in torn rotator cuff muscles is atrophy of muscle fibers and an accumulation of fat in the muscle extracellular matrix, collectively referred to as “fatty atrophy.” Despite improvements in surgical repair techniques to achieve biomechanically strong repairs to bone in a minimally invasive arthroscopic approach, studies of rotator cuff muscles with use of magnetic resonance imaging (MRI) or computed tomography (CT) scans often fail to demonstrate a reduction in fatty atrophy following surgical repair. While these imaging modalities are commonly used as a benchmark of rotator cuff healing, there is little information regarding the cellular and molecular changes associated with fatty atrophy in patients who sustain rotator cuff tears.

Several different animal models have been used to study the natural history of rotator cuff injuries and the impact of repair on tissue regeneration. While the nature of these models allows for invasive analysis of the muscles before and after the repair, the ability to analyze the cellular and molecular changes at multiple time points is difficult in human subjects. We have been studying the physiology of torn rotator cuff muscles with use of biopsy specimens obtained at the time of surgical repair, but ethical and practical issues have limited our ability to study changes in rotator cuff contractility and morphology that occur subsequent to the repair. Our patient, who underwent surgical repair for a massive, traumatic rotator cuff tear, sustained a traumatic reinjury in the acute postoperative period. This event required a timely revision of the rotator cuff repair and provided the opportunity to perform a second biopsy of the supraspinatus muscle. Since previous studies have demonstrated that there was no improvement in the appearance of fatty atrophy in patients who underwent rotator cuff repair, we hypothesized that the muscle obtained from the second biopsy would show minimal improvements at the cellular level following repair. To test this hypothesis, we measured the contractility of single muscle fibers and also analyzed the density of muscle stem cells and the lipid content of supraspinatus muscle biopsies from the patient’s first and second rotator cuff repair surgeries. The study was approved by the University of Michigan Medical School Institutional Review Board. The patient was informed that data concerning the case would be submitted for publication, and he provided consent.

Case Report
A forty-four-year-old physically active man sustained a traumatic full-thickness supraspinatus tendon tear and a partial-thickness infraspinatus tendon tear. The medical history was negative, and he reported no current use of medications or any drug allergies. Physical examination demonstrated full passive motion of the left shoulder symmetric to the right shoulder. However, active forward flexion in the scapular plane was restricted to approximately 80°, and manual supraspinatus muscle strength testing revealed only antigravity strength (3/5) on the left side, compared with full strength (5/5) on the right side. Right shoulder active external rotation with the arm...
adducted was 80° on the right and 45° on the left. Lift-off and belly press examinations were normal. Radiographs demonstrated mild acromioclavicular joint degenerative changes, no glenohumeral degenerative changes, and no superior migration of the humeral head. An MR arthrogram (Fig. 1) revealed a massive full-thickness tear of the supraspinatus tendon and a partial-thickness tear of the infraspinatus tendon.

Given the traumatic rotator cuff injury as well as the impaired active motion and strength, the patient consented to an arthroscopic, double-row rotator cuff repair. Only the most anterior margin of the rotator cable just posterior to the biceps tendon was intact. There was a very large U-shaped tear extending through the entire supraspinatus tendon and the cephalad two-thirds of the infraspinatus tendon. The majority of the greater tuberosity footprint was clearly exposed. Adhesions on both the bursal side and the articular side of the rotator cuff were fully divided to restore normal mobility to this retracted tear. With use of a grasper, the mobilized rotator cuff tear was completely reducible with a posterior-to-anterior vector of pull. The medial and lateral margins of the footprint were defined and carefully prepared to expose a bleeding osseous surface. A double-row rotator cuff repair technique was subsequently performed with use of multiple 5.5-mm double-loaded suture anchors medially; horizontal mattress sutures were passed and secured into the supraspinatus and infraspinatus tendons. These sutures were subsequently secured in a crossed fashion on the bursal side of the tendon and secured to transosseous-equivalent anchors placed along the lateral margin of the footprint to achieve an anatomic, watertight repair. Following surgery, the patient underwent a supervised rehabilitation program and progressed through the program on schedule with minimal symptoms of pain or discomfort. Nine weeks following the repair, he sustained a traumatic retear as a result of shoulder trauma when he was struck by a vehicle while walking. The patient consented to a revision double-row repair that was performed by the same surgeon. A time line of events is presented in Figure 2.

Biopsy specimens of the muscle fibers and surrounding connective tissue of the supraspinatus muscle were carefully obtained with use of an arthroscopic biopsy punch at the time of the second surgery. These specimens were taken from the distal third of the supraspinatus muscle, and care was taken to ensure that these specimens came from the same region as those obtained during the first surgery. A portion of the specimens was prepared freshly for single muscle fiber contractility analysis, and the remaining portion was snap frozen in Tissue-Tek (Sakura Finetek USA) for immunohistochemistry analysis.

Permeabilized Fiber Contractility
The maximum isometric force production ($F_o$) and specific maximum isometric force production ($[sF_o]$, $F_o$ normalized by muscle fiber cross-sectional area) of individual muscle fibers was measured with use of previously described techniques that were adjusted to an optimal sarcomere length of 2.7 μm for human muscle.

Immunohistochemistry
Tissue was sectioned at 10 μm and labeled with primary antibodies or small fluorophore-tagged compounds that identify various biomolecules. These included c-Met antibodies (Santa Cruz Biotechnology, Santa Cruz, California) to identify muscle stem cells (satellite cells); wheat germ agglutinin lectin conjugated to Alexa Fluor 488 ([WGA lectin AF488]; Invitrogen, Carlsbad, California) and collagen I antibodies (AbCam, Cambridge, Massachusetts) to identify the extracellular matrix; BODIPY 493/503 (Invitrogen) to identify lipids; and DAPI (Sigma-Aldrich, Saint Louis, Missouri) to identify nuclei. Alexa Fluor-tagged secondary antibodies were used to detect primary antibodies.

Discussion
To our knowledge, this case report is the first to describe changes in the contractile function of muscle fibers and the response of muscle stem cells following the repair of a massive rotator cuff tear in a human. The use of the permeabilized fiber technique allowed the precise measurement of supraspinatus function, which can be difficult to specifically isolate with use...
whole-body biomechanical measurements. There was a 25% increase in muscle fiber size, a 74% increase in $F_o$, and a 46% increase in $sF_o$ at the time of the second biopsy (Figs. 3-A, 3-B, and 3-C). While an increase in $F_o$ would be expected to occur along with an increase in the cross-sectional area, the increase in $sF_o$ is an important finding in terms of muscle regeneration since this indicates that there is an increase in the density of functional myofibrils within the fibers. Muscle stem cells, or satellite cells, play a central role in muscle repair following injury. We detected an increase in muscle stem cell density and centrally located nuclei with the second biopsy, indicating that the muscle was undergoing active regeneration of previously damaged fibers (Figs. 4-A and 4-B). With use of a lipid probe, we were able to observe a reduction in the accumulation of fat in the extracellular matrix in the second biopsy (Figs. 5-A and 5-B).

Contrary to our initial hypothesis and previous studies that have been conducted on a macroscale, the results from this report suggest that surgical intervention improved recovery at the cellular level following the initial massive rotator cuff tear in our patient. We also observed histological changes in fat accumulation and muscle atrophy before they were apparent on MRI. We do acknowledge that there are several limitations to this study. The patient initially had an acute rotator cuff tear and did not have signs of fatty degeneration on the initial MRI; while there were signs of regeneration, it is not clear whether patients with chronic rotator cuff tears who have moderate-to-severe fatty degeneration would have the same capacity to regenerate the diseased muscles. Additionally, it is possible that the observed changes in muscle fiber contractility and histology were highly localized and not reflective of the muscle as a whole, although great care was taken to ensure that the second biopsy specimen came from the same region as the first, and we would not have anticipated changes in muscle fiber contractility, fat accumulation, and satellite cell density similar to the magnitude that was observed due to local regional variability. While there are several limitations to the study, this case provided us with a unique opportunity to perform a repeat biopsy on a previously repaired muscle, and the results raise important issues about rotator cuff

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**Fig. 3**

Compared with the first biopsy, there was an increase in muscle fiber cross-sectional area (Fig. 3-A), $F_o$ (Fig. 3-B), and $sF_o$ (Fig. 3-C) in the second biopsy. Nine fibers had been obtained from the first biopsy, and seven fibers had been obtained from the second biopsy. CSA = cross-sectional area.

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**Fig. 4**

Compared with the first biopsy (Fig. 4-A), there was an increase in muscle stem cell density and centrally located nuclei in the second biopsy (Fig. 4-B). Muscle stem cells are indicated with arrowheads. Red = c-Met (muscle stem cells), green = WGA lectin AF488 (extracellular matrix), and blue = DAPI (nuclei). Scale bar = 25 μm.
muscle regeneration and fat accumulation in humans that warrant additional study.

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