Developmental Biology and Regenerative Medicine: Addressing the Vexing Problem of Persistent Muscle Atrophy in the Chronically Torn Human Rotator Cuff

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Persistent muscle atrophy in the chronically torn rotator cuff is a significant obstacle for treatment and recovery. Large atrophic changes are predictive of poor surgical and nonsurgical outcomes and frequently fail to resolve even following functional restoration of loading and rehabilitation. New insights into the processes of muscle atrophy and recovery gained through studies in developmental biology combined with the novel tools and strategies emerging in regenerative medicine provide new avenues to combat the vexing problem of muscle atrophy in the rotator cuff. Moving these treatment strategies forward likely will involve the combination of surgery, biologic/cellular agents, and physical interventions, as increasing experimental evidence points to the beneficial interaction between biologic therapies and physiologic stresses. Thus, the physical therapy profession is poised to play a significant role in defining the success of these combinatorial therapies. This perspective article will provide an overview of the developmental biology and regenerative medicine strategies currently under investigation to combat muscle atrophy and how they may integrate into the current and future practice of physical therapy.

Rotator cuff (RC) tendinopathy is one of the most common causes of shoulder dysfunction and one of the most challenging orthopedic disorders to treat. It is estimated to affect nearly 30% of the adult population, and more than 100,000 patients annually seek treatment through either operative or nonoperative interventions.[1,2] Clinical symptoms can vary broadly from minor weakness to debilitating pain and loss of function.[3] Interventional outcomes are equally broad, regardless of whether patients opt for conservative treatments (eg, physical therapy, medication) or surgical tendon reattachment. Although physical therapy is effective in many cases,[4,5] 20% to 50% of patients treated conservatively report a less than satisfactory result.[4,6] Importantly, the best longitudinal data suggest that tears progress over time.[7] Similarly, surgery is effective in many patients, but approximately 30% of surgical repairs will fail.[8,9] This failure rate jumps to as high as 90% in patients with more complex conditions, such as those with large chronic tears.[10]

The variability in clinical outcomes can be explained, at least in part, by progressive and persistent degeneration of RC muscles following a tear. Although tendon deterioration also is correlated with poor functional outcomes, the degree of muscle atrophy and the presence of ectopic fat within the muscle (fatty infiltration) are the strongest predictors of surgical failure.[4,6,9,11–13] This relationship highlights the intimate connection (both literal and figurative) between muscle and tendon: as muscle mass, health, and function decline, so does the ability to form a strong tendon-to-bone attachment. However, even in cases where the surgical reattachment holds, the majority of the atrophic changes to the muscle fail to resolve, and may even progress.[11,12,14,15] Therefore, atrophic muscle changes associated with RC tears are often classified as "irreversible."[12,14] Although patients do report functional improvements despite progressive muscle atrophy (likely the result of changes in pain), long-term outcomes and functional scores are improved if muscle degeneration is at least halted.[16] This finding suggests that adequate functional recovery is dependent, at least in part, on adequate muscle recovery, which currently is a substantial roadblock for surgical and nonsurgical interventions alike.

The features of this degenerative process (ie, muscle atrophy, fibrosis, and fatty infiltration) are not unique to RC pathology; they also are seen, to various degrees, in dystrophies, nerve injuries, unloading, and diabetes. However, there is some indication that RC muscles are unique. Although uncommon, repairs of chronic tears to the Achilles or distal biceps tendon have comparatively high success rates, with good recovery of strength and low re-tear rates.[17,18] Additionally, studies in animal models suggest that the gastrocnemius muscle of the leg experiences less fatty infiltration than the muscles of the RC following tendon detachment and nerve injury.[19] Why the RC muscles are so uniquely susceptible to degeneration and resistant to rehabilitation is a question of ongoing research.

As restoring tendon-bone connectivity and muscle loading appears insufficient to reverse chronic muscle atrophy following large RC tears,[12,14] there is an effort to design new or adjuvant therapies that will promote muscle regeneration. The majority of these strategies fall under the headings of "developmental biology" and "regenerative medicine." Broadly, developmental biology approaches seek to understand the cellular and signaling processes that regulate muscle atrophy and growth, and regenerative medicine approaches are designed to mitigate or reverse these atrophic changes. The aim of these strategies is to provide muscle with the tools it needs to respond to regenerative signals. However, priming muscle to respond to the signals for growth and repair is useful only in the setting where it is receiving the appropriate mechanical signals. Thus, developmental biology and regenerative medicine strategies go hand in hand with physical interventions. In this perspective article, we will highlight current strategies in developmental biology and regenerative medicine for the mitigation and reversal of RC muscle atrophy and discuss the relevance of these strategies to current physical therapy approaches.

Natural History of Tendinopathy and RC Tears

Rotator cuff tendinopathy is typically a progressive disorder evolving from tendon inflammation to degeneration and ultimately rupture.[7,20] However, a subset of RC tendon ruptures occur acutely as the result of a traumatic event. These injuries are more likely to occur in younger individuals and be quickly surgically repaired, a combination of features that leads to improved outcomes and little overt tendon or muscle pathology relative to the chronic tear (reviewed by Mall et al[21]). Thus, the majority of this discussion will focus on challenges specific to the chronic tear and treatment of its progressive pathology. The development of this injury is likely multifactorial, deriving from both extrinsic and intrinsic sources (reviewed by Seitz et al[22]). Extrinsic factors such as anatomical abnormalities (eg, variation in acromion shape) and biomechanical changes (eg, abnormal scapular or humeral kinematics) are thought to drive RC tendinopathy through compression and mechanical wear of the tendon.[23–25] These extrinsic changes also may drive intrinsic pathology in the tendon. Studies in animal models with experimentally controlled extrinsic factors demonstrate extracellular matrix (ECM) degradation and reorganization, alterations in cell behavior, vascular remodeling, inflammation, and edema in response to tendon mechanical wear (reviewed by Lake et al[26]). Likewise, intrinsic factors such as reduced cellularity,[27] reduced vascularity,[28] and ECM reorganization,[29] which are hypothesized to be primary drivers of a subset of RC tendinopathies, ultimately may lead to alterations in RC biomechanics and increased tendon wear as tendon material properties and regenerative capacity are altered.

Progressive degeneration of the tendon, particularly tendon tearing, is associated with progressive muscle pathology. The proposed progression of this pathology is depicted in Figure 1. Atrophy, typically measured as a reduction in muscle cross-sectional area (CSA) relative to bony landmarks,[9] is measurable early in the development of tendon tearing. During the progression of tendinopathy, this atrophy can range from minimal, in the

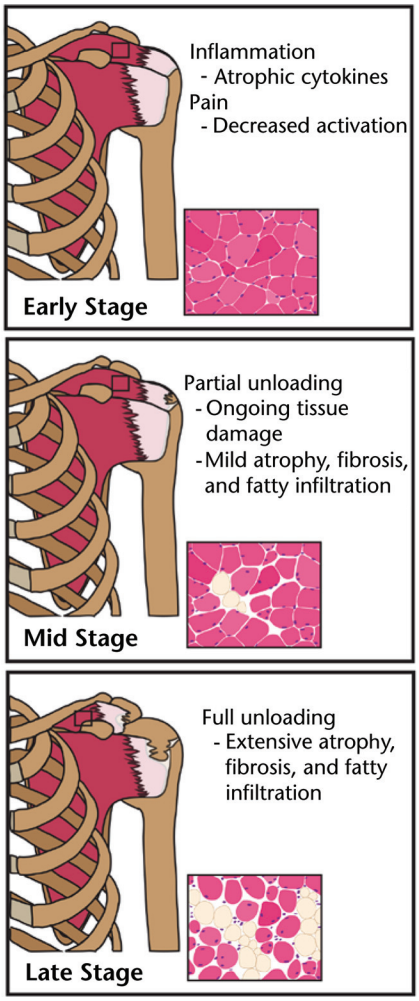

Figure 1.
Schematic depiction of the proposed stages of muscular changes in the chronically torn rotator cuff.

case of small tears, to greater than 75% reduction in CSA in large and chronic tears involving multiple tendons.[11,30] The degree of muscle atrophy is correlated with the size of the tendon tear,[31] suggesting that progressive reduction in the mechanical load through the tendon is a likely driver of this effect. Similarly, as the tendon tears, the muscle retracts onto the scapula, reducing muscle length.[32,33] This retraction of muscle onto the scapula is important because muscle force production is length sensitive.[34]

In addition to an overall reduction in muscle CSA and length, many adaptations occur within the muscle microstructure in response to tendon tear. These adaptations may include changes to fiber type, fiber size, and sarcomere structure. This is important to note, as gross muscle size is not always a good predictor of strength in the context of aging or pathology.[35] Although we are unaware of any study that has measured it directly in the RC, chronic unloading in muscle is typically associated with a shift toward faster fiber types, which have unique contractile and metabolic properties (reviewed by Pette and Staron[36]). More research is needed to investigate the potential for fiber type to influence muscle function in the torn RC. Other changes to the muscle microstructure, however, have been demonstrated to result from RC tears. Fiber CSA and sarcomere length decrease significantly as a function of tear size,[32,37] and large areas of connective tissue and fat develop between the fascicles and fibers.[11,15,38,39] These features, generally termed "fibrosis" and "fatty infiltration," are frequently seen in conjunction with muscle atrophy and are difficult to address because little is known about the signals that regulate their formation or resolution.

Fibrosis, in all organ systems, is primarily driven by fibroblast cells that reside in the ECM, and the process is likely mediated by inflammation. In muscle, fibroblasts surround and mechanically couple fibers. Inflammatory signals and tissue damage are thought to activate fibroblasts to expand their numbers and increase the quantity and stiffness of their surrounding matrix (principally collagen) (reviewed by Serrano et al[40]). This extra ECM alters the transmission of internal and external mechanical forces to fibers and creates a physical barrier to growth of existing fibers (hypertrophy) and the formation of new fibers (hyperplasia). Additionally, increased volume and stiffness of the ECM alters the passive properties of the muscle, compromising its ability to properly stabilize the glenohumeral joint. Studies in animal models suggest that fibrosis resulting from RC tears significantly stiffens the muscle, more than compensating for the reduction in muscle size.[41]

Much less is known about the process of fatty infiltration. There is ongoing debate over which cells generate the fat, with the prevailing theories being transdifferentiation of satellite cells (SC), non-SC myogenic populations, or a specialized population of muscle-resident "fibroadipogenic" progenitors.[42–44] Little also is known about what signals drive fatty infiltration, but its tight association with atrophy has led to theories that either the unique mechanical or metabolic environment of atrophied muscle is driving adipogenesis.[45,46] However, in the torn RC, fat expansion is not limited to the muscle itself, but occurs outside the muscle boundary (epimuscular fat) as well, suggesting a non–muscle-specific mechanism also may be at play. Therefore, it is unknown how closely related epimuscular and intramuscular fat are, but addressing this question could shed light on both the origin and regulation of fatty infiltration.

The strong correlation between tendon degeneration and muscle pathology has mostly led to the conclusion that tendon damage causes pathological muscle adaptations. This conclusion is likely true, as unloading is one of the most potent drivers of muscle atrophy. However, some of the muscle-tendon interplay in this pathology is almost certainly reciprocal. Muscular imbalance or deficits in RC muscle performance can cause shifts in glenohumeral kinematic patterns linked with tendon impingement.[47,48] Furthermore, healthy muscle activity is a source of dynamic loading and growth factors involved in regulating tendon health.[49] Tendon quality and integrity of the tendon-bone interface are both compromised if muscle activity is blocked either during embryonic development or during healing following surgical repair.[50,51] This muscle-tendon interdependence suggests that repairing a tendon may not be sufficient to fully restore shoulder function, even though it is an obvious mechanical deficiency that should be corrected. Severely weakened muscles may now contribute to new or continued impingement. Even if gross extrinsic shoulder kinematics are restored, the repaired tendon likely suffers from intrinsic deterioration exacerbated by an atrophied and relatively inactive muscle. Interestingly, even in acute traumatic tears, there is evidence that

delaying repair for even a few months may have detrimental consequences on functional outcomes, highlighting the importance of intervening in this process early, before the appearance of overt morphological changes.[52,53]

An important and poorly studied problem in this patient population is that there are multiple and conflicting adaptation pressures on the muscle when tendon pathology is present. First, pain reduces overall function and, therefore, muscle loading. Second, tendon tears alter the mechanical environment of the muscle-tendon-bone system, which may place increased demands on the muscle to achieve "normal" joint torques. Third, as tendon tears progress, the muscle begins to retract, which further impairs muscle force-generating capacity. Fourth, as the tear becomes more chronic, fibrosis and fatty infiltration may further compromise force production and mechanosensitivity. Finally, the repair procedure itself may cause muscle injury as the muscle, in its fibrotic state, is stretched to achieve repair.[54]

Patient age is another important confounding factor in the recovery of RC muscle from the atrophic degenerative pathology associated with chronic tears. Numerous studies have demonstrated that muscle size and strength, as well as the capacity for repair and hypertrophy, diminish with age (reviewed by Grounds[55] and Clark and Manini[56]). In line with these findings, age is thought to contribute to both the development of progressive tears[1,57,58] and functional recovery following repair.[59,60] Furthermore, the incidence of comorbidities that may influence muscle healing, such as type 2 diabetes and hypertension, increases with age. In the context of RC pathology, perhaps the main question is whether the response to environmental stimuli (eg, changes in loading) is fundamentally different in sarcopenic or dynapenic aged muscle. Taken together, this question suggests that in moving from the bench to the bedside, developmental biology and regenerative medicine strategies for improving muscle function following RC tears will have to contend with issues specific to the aging population.

Developmental Biology and Regenerative Medicine

The developmental biology approach to the problem of muscle atrophy can be summarized in understanding the cellular and molecular pathways that regulate atrophy and hypertrophy and the external cues that drive these pathways. Then, theoretically, carefully placed breaks in the pathway or blockage of external drivers can be used to mitigate atrophic changes, or, conversely, primed cells or signaling molecules can be delivered to activate or participate in the process of hypertrophy. This is the realm of regenerative medicine. Developmental biology and regenerative medicine go hand in hand because without proper definition of the cellular and molecular pathways, the tools of regenerative medicine would be blindly applied, possibly causing negative off-target effects or missing the target entirely. For the purposes of this discussion, what has been learned about the development of RC muscle pathophysiology will be considered separately from the tools that might be used to combat it, but the link between the 2 approaches is important.

Developmental Biology Approaches

Numerous studies have characterized the intracellular pathways regulating muscle atrophy. These pathways globally consist of gene expression changes that lead to increased protein breakdown and decreased protein production (reviewed by Glass[61]). These gene expression changes cause an overall reduction in the amount of contractile material in muscle, which reduces fiber diameter and muscle CSA. The primary players in these pathways are fairly well conserved regardless of the origin of the atrophy (eg, injury, disease, disuse),[62] and a variety of strategies are under investigation to interfere in this signaling cascade. However, ideally, therapeutic interventions would interfere minimally with basic cellular processes and instead modify the external signals driving the pathological cellular behavior. Unfortunately, piecing apart the origins of RC muscle atrophy is challenging. It is likely driven by a combination of factors whose interaction and influence change through the progression of pathology.

The most obvious and likely driver of muscle atrophy in the torn RC is chronic unloading. The link between unloading and muscle atrophy is well established in both humans and animal models, thus it is not surprising that the degree of RC muscle atrophy correlates with the size of the tendon tear and consequently with loss of force transmission.[31] Passive muscle unloading is likely furthered by a progressive reduction in muscle activity due to pain and limited function, which itself decreases mechanical load on the muscle. However, these are the atrophic drivers that are directly addressed by the current standard of care. Surgical tendon reattachment is designed to restore force transmission between the bone and muscle, whereas physical therapy and rehabilitation are designed to progressively increase muscle activity and movement. However, surprisingly, reversal of these atrophic drivers is frequently insufficient to reverse the atrophic state of the muscle. Some cases of large or massive tears involving muscle retraction can involve damage to the suprascapular nerve.[63] Numerous studies in humans and animals have demonstrated profound muscle atrophy in response to nerve damage, blockade, or transection.[64] However, there is no evidence that nerve damage is a consistent feature even of massive tears,[65] making it an unlikely candidate for driving global RC muscle pathology.

Typically, restoration of functional loading would be sufficient to reverse atrophic changes due to unloading. Significant increases in load or fiber damage activate a specialized population of muscle-resident progenitor cells called "satellite cells." These cells proliferate (divide), migrate to overloaded or damaged fibers, and fuse with existing fibers or with each other to ultimately increase muscle size and strength. Under normal conditions, this process is very efficient, and muscle size and function are generally restored. However, in some cases of advanced age or disease, the function of the SC population may be compromised. Specifically, an aged systemic environment has been shown to decrease the regenerative potential of even young SCs,[66–68] and extensive cycles of regeneration have been shown to exhaust the population.[67] When these cells are

unable to respond to hypertrophic or regenerative cues, other cells (fibroblasts and adipocytes) may be used to create adaptation. This is the primary difference between healing and regeneration. Fibrous ECM and fat might be able to maintain muscle's structural integrity, but at the expense of function.

The increased and persistent presence of these noncontractile tissues in RC muscle could explain some of the muscle's resistance to rehabilitation. In the most basic sense, the ECM and fat occupy contractile space, thereby reducing force-generating capacity. Additionally, recent data suggest that fibrosis is also a physical barrier to muscle growth and regeneration.[69,70] The infiltration of fat and matrix thickening create a microenvironment for fibers that distorts healthy signaling processes.[71] In such an environment, the mechanical signals for growth may not get through to SCs even if they are intrinsically able to proliferate, integrate, and cause muscle growth. The influence of fibrosis and fatty infiltration likely also extends beyond mechanics. The cells that occupy ECM and fat have their own signaling pathways, many of which are regulated by secreted molecules that simultaneously promote their own expansion and inhibit SC proliferation and differentiation. Thus, understanding the cellular and biochemical mechanisms that control the process of fibrosis and fatty infiltration is critical both for preventing these maladaptations and for designing strategies to reverse them.

Chronic inflammation is one of the best known drivers of fibrosis (reviewed by Serrano and Muñoz-Cánoves[40]). Inflammatory cells rapidly migrate to the site of tissue injury and secrete a variety of factors involved in tissue healing, including transforming growth factor β1 (TGF-β1) and tumor necrosis factor α (TNFα). As RC tendinopathy involves progressive damage to the tendon (and likely to the muscle, as partial muscle unloading increases shear stresses between loaded and unloaded fibers), these inflammatory cells have a significant and persistent presence in the cuff during the early stages of tendon tearing.[72,73] Although infiltration of inflammatory cells and secretion of these factors is an important early step in muscle regeneration, it is typically quickly resolved and followed by activation, proliferation, and fusion of SCs. Among other things, persistent inflammation, like that seen in the progression of RC tendinopathy, continuously stimulates fibroblasts to proliferate and secrete matrix, causing a buildup of ECM.

The consequences of fibrosis on muscle function are broad. As the ECM fully encapsulates fibers, stiffening of this matrix likely creates a substantial physical barrier to muscle growth. Muscle ECM also plays a large role in the transmission of external stresses and strains that activate mechanosensitive pathways regulating a variety of physiological processes. Theoretically, stiffening these connections could mechanically shield fibers from applied strain, shifting more of the deformation to the degenerating tendon. On the biochemical side, TGF-β1 both promotes fibrosis[74] and is a potent inhibitor of proliferation and differentiation of SCs.[75,76] Satellite cell proliferation and differentiation also are cued by the composition and stiffness of the matrix (reviewed by Thomas et al[77]), making these proinflammatory factors doubly inhibitive to muscle regeneration. These findings suggest that identifying ways to break this cycle and break down ECM might be important for growth and recovery of fibrotic muscle. This should be a particularly interesting target for physical therapists, as there are a number of therapeutic strategies to degrade or "shape" connective tissue.

Many potential mechanisms have been proposed for the formation of intramuscular fat, including changes in mechanical loading due to an increase in the separation between muscle fibers,[45] metabolic changes associated with muscle atrophy,[78] and persistent muscle injury.[43] Each of these mechanisms is plausible in the context of RC muscle atrophy and depend, in part, on which cells are responsible for fat formation: SCs, non-SC myogenic populations, or nonmyogenic fibrogenic and adipogenic progenitors. There are also a variety of hypotheses about the consequences of fatty infiltration in muscle, with most data being derived from studies of fat anatomically distant from muscle or from co-cultures of myoblasts with adipose progenitors. The relevance of these studies to fatty infiltration depends significantly on the phenotype of intramuscular fat. There are 3 major identified fat phenotypes in humans: white, brown, and an intermediate phenotype termed "beige." White fat secretes inflammatory factors, such as TNFα and TGF-β1,[79,80] that are well-characterized drivers of the muscle atrophy pathway[81] and factors that promote muscle insulin resistance in co-culture.[82] On the other hand, brown fat secretes several growth factors involved in hypertrophy, including insulin-like growth factor 1 (IGF-1).[83,84] There is some evidence suggesting that RC fat may be white,[46] brown,[44] or beige,[85] leaving its role in muscle atrophy and metabolism ambiguous. The correlation between the amount of fat in diseased muscle and functional recovery suggests it is detrimental to muscle function.[12,14,86] However, fat is a very plastic tissue, and new research points to ways to use both the fat progenitor cells and the mature fat tissue for regenerative muscle therapies.[87]

Of all the potential drivers of muscle atrophy, the fibrosis and fatty infiltration characteristic of the muscle pathology might be the best targets for new therapies aimed at supplementing current treatment protocols. Dissolving or otherwise repackaging these features, either during physical interventions or during surgical reconstruction, has the potential to open up the muscle to receive and respond to signals for healthy growth. The question then is, does the muscle lack the machinery to make new muscle, or is the pathology simply preventing regeneration? This important question extends to disease states and injuries across the body.

Regenerative Medicine Approaches

"Regenerative medicine" is a broad umbrella term encompassing strategies from genetic engineering to surgical implants. Thousands of techniques have been developed under this heading targeting every tissue type in the body. In the RC, the vast majority of regenerative

medicine research has focused on the tendon, as the tendon is at least the macroscopic location where the pathology originates and where surgical reconstructions fail. These techniques have included application of exogenous growth factors, growth factors in an engineered matrix, platelet-rich plasma, stem cell injections, and stem cells in an engineered matrix (reviewed by Randelli et al[88]). Platelet-rich plasma and several tissue engineered matrices have been used clinically with mixed results[89,90]—possibly due, in part, to the confounding effects of atrophic muscle. Although most have not been applied in the context of RC muscle atrophy, many regenerative medicine techniques have shown significant promise in promoting muscle regeneration in the general context of injury and disease. These techniques fall into 3 basic categories: (1) promoting the intrinsic mechanisms of regeneration, (2) supplementing the population of regenerative cells, and (3) targeting features of the pathology thought to be impeding regenerative potential.

Intrinsic sources of "new" muscle. Thanks to numerous studies in developmental biology, we now understand a great deal about the process of muscle growth following significant changes in loading. Although some short-term hypertrophy is possible in the absence of SCs,[91] large and long-term muscle growth relies heavily on these cells.[92] Repair of the structural damage frequently imparted to fibers during overloading also is dependent on proper SC function.[93] Although other cell types resident in muscle such as muscle-derived stem cells (MDSCs) and pericytes have been proposed to contribute to intrinsic regeneration,[94,95] they cannot independently regenerate muscle. In the absence of SCs, regeneration fails. Therefore, SCs have become a prime target for regenerative strategies aimed at promoting intrinsic growth and regeneration.

In healthy muscle, a host of signaling molecules and growth factors regulate SC behavior (reviewed by Kuang et al[96]). The effect of exogenous delivery or inhibition of many of these factors has been evaluated for promoting growth and regeneration in muscle; however, the most widely studied and promising is IGF-1. Insulin-like growth factor 1 promotes SC proliferation and differentiation,[97] and delivery of this growth factor to muscle promotes hypertrophy, helps maintain muscle mass during aging, and accelerates regeneration in animal models.[98–101] However, IGF-1 is involved in a variety of homeostatic pathways in many cell types, and there is considerable debate about the mechanism behind its effect. Its effect on SCs has been well documented, but it also has a potent anabolic effect on muscle fibers,[99] promotes inflammatory cell recruitment to sites of muscle injury,[102] and accelerates motoneuron regrowth.[103] Although these effects are likely to improve muscle function, other "off-target" effects may not. Insulin-like growth factor 1 is involved in the proliferation of fibroblasts and adipose progenitors[104,105] and thus could exacerbate pathology in an already fibrotic and fatty muscle. More research will be necessary to evaluate the multitarget potential for IGF-1 delivery. Other growth factor candidates include basic fibroblast growth factor, platelet-derived growth factor, and hepatocyte growth factor, but therapeutic development of these growth factors lags behind that of IGF-1.

Many strategies attempt to combat muscle atrophy by protecting muscle mass rather than by trying to promote recovery. These strategies are focused more on the muscle fiber than on the SC itself, as intrafiber pathways are the master regulators of muscle atrophy. One of the major targets of these strategies is myostatin, a factor produced by muscle that is highly expressed during disuse muscle atrophy in humans.[106] Myostatin likely acts both on mature fibers, decreasing protein synthesis, and on SCs, inhibiting their proliferation and differentiation.[107] Delivery of myostatin inhibitors to muscle results in significant increases in muscle mass and strength and improved muscle regeneration in animal studies.[108–112] Unlike IGF-1, muscle fibers and SCs are the main targets of myostatin, suggesting myostatin blockade could be a promising highly-specific strategy for inhibiting muscle atrophy in the torn RC. Inhibition of the myostatin pathway can be accomplished either directly using molecules that bind myostatin and prevent its action on cells or through targeted blockade of the receptors on cells that mediate its effects. Recent data suggest that blocking myostatin receptors is more effective in protection from atrophy and promotion of hypertrophy than inhibiting myostatin directly.[112] However, it is important to note that increased muscle size is not always accompanied by increased muscle strength with myostatin blockade, especially in the context of aging.[113,114]

Exogenous delivery of growth factors or inhibitors of the atrophy pathway is a promising strategy to combat the problem of muscle atrophy in the RC. They are comparatively easy to mass market and pose little threat of immune rejection. Additionally, many have already been through clinical trials and demonstrated safety.[115,116] However, efficacy in humans remains a question, and there may be disease- or muscle-specific hurdles to overcome. This strategy has primarily been evaluated in small animal models with little overt muscle pathology, and little is known about how fibrosis and fatty infiltration (which appear to be different from human) might affect delivery and efficacy of this strategy.

Extrinsic sources of "new" muscle. As SCs are the primary source of muscle growth and repair, supplementing the SC population in the torn RC is a logical strategy to promote muscle growth and functional recovery. Strategies to replace or supplement the SC population in muscle have been in development since the late 1980s.[117] These strategies have primarily focused on the treatment of Duchenne muscular dystrophy (DMD), but are broadly applicable to the muscles of the RC. Studies in animal models have demonstrated that donor SCs can indeed be injected into muscle and functionally contribute to regeneration (engraft).[117–119] However, the usefulness of cell delivery for mitigating or reversing RC muscle atrophy hinges on the resolution of several issues, namely, the state of the current SC population, the source of cells, and the current state of muscle health (residual pathology).

First, unlike in DMD, where deficits in SC number and proliferative ability have been well documented as a function of disease progression,[67,120] there is no clear consensus on the state of SCs in muscle from torn RCs. Although a decrease in SC number has been demonstrated in the later stages of pathology relative to the initiation of tendon tearing, recent data suggest that this decrease may be due to a transient increase in SC numbers in the early stages of pathology, rather than a depletion following full-thickness tears.[37,87] Elevated SC numbers also are seen in the early stages of DMD and neurogenic atrophy and are thought to reflect increased injury response and compensatory hypertrophy of unaffected fibers.[121] The presence of a relatively sizeable population of functionally capable SCs in cases of large and chronic tears suggests the problem for RC muscle may not be a lack of regenerative capacity, but rather a lack of appropriate regenerative signaling.

Second, although SCs are the primary natural source for muscle regeneration, they are not abundant in muscle, making them difficult to isolate in sufficient numbers from donors unwilling to part with large pieces of muscle. To get around this issue, researchers have recently turned investigations toward other, more abundant cell types with myogenic potential. These cell types include other muscle-resident populations such as MDSCs or pericytes,[94,95] as well as marrow-derived mesenchymal stem cells (MSCs)[122] and adipose-derived stem cells (ASCs).[123] Although MDSCs and pericytes likely have the best intrinsic capacity for myogenesis, they also require a substantial muscle biopsy for isolation. Likewise, MSC isolation is typically costly and invasive. Adipose-derived stem cells, which can be efficiently isolated from large stores of subcutaneous fat, are currently the most abundant and easily accessible population of stem cells with myogenic capacity.

Finally, muscle pathology in the advanced stages of DMD closely resembles RC muscle pathology in cases of large and chronic tears, where fibrosis and fatty infiltration have replaced the majority of the muscle volume. These features are thought to be inhibitive to SC proliferation and fusion and thus are likely responsible, at least in part, for the poor donor engraftment seen in clinical trials for SC injection in the treatment of DMD (reviewed by Cossu and Mavilio[124]). Expecting SCs to help regenerate muscle in such an environment may be akin to expecting them to form new muscle in tendon or in subcutaneous fat. Without the physical and biochemical cues for muscle regeneration, a primarily muscle-committed cell such as the SC may simply die. However, stem cells with multilineage potential, such as MSCs and ASCs, can quickly adapt to different tissue environments and are likely to respond to fibrogenic and adipogenic cues by contributing to fibrosis and fatty infiltration, which would be counterproductive. Furthermore, systemic environmental factors, such as the age of the recipient, the presence of comorbidities, and lifestyle habits (eg, smoking), may influence the functional capacity of the injected cells.

Although these issues paint a somewhat bleak picture for the potential of cell delivery in the torn RC, it may still be a viable therapeutic strategy. Even if the native SC population is fully present and capable, the regenerative demand on the muscle following surgical tendon reattachment will be very high. If the appropriate signals for myogenesis can bypass the pathological environment, increasing the population of myogenic cells may help promote quick regeneration before fibrotic healing can take place. Additionally, surgical repair of the torn RC provides access to a unique source for autologous ASCs, bypassing the complication of immune rejection, which is another hypothesis for the poor efficacy in the DMD trials. New research suggests that the fat that surrounds the muscles of the RC is beige rather than white, with an ASC population of higher myogenic potential than subcutaneous ASCs.[85] Additionally, beige fat is associated with growth factor secretion and a host of metabolic benefits and thus has the potential to be a significantly more potent cell source than the white ASCs typically considered for stem cell injections. Finally, stem cell delivery in combination with other approaches, such as those targeting fibrosis, is likely to be most successful because it simultaneously promotes growth internally and breaks down external barriers.

How do we handle fibrosis? The pathological muscle environment likely provides a significant physical and biochemical barrier to muscle growth. Strategies aimed at true regeneration (restoration of normal muscle structure and function) will need to resolve the fibrosis and fatty infiltration that occupy much of the muscle volume in the torn RC. Unfortunately, the majority of this pathology is currently considered to be irreversible. There are muscle-intrinsic mechanisms for remodeling the ECM to meet the structural needs of the muscle, but it appears that past a certain point, the fibrosis is either too pervasive to remodel or shields the muscle from the signals for remodeling. So little is known about the origin or process of fatty infiltration that it is currently unclear what pathway to target. However, understanding the signals that drive the processes of ECM remodeling and fibrosis has recently led to development of several targeted interventions that have shown promising results in animal models. Interestingly, many of these interventions show improvements in muscle regeneration and partial reversal of fibrosis.

Inhibition of myostatin, in addition to promoting muscle growth, has been shown to result in significant reduction of fibrosis.[107,125] This reduction of fibrosis is thought to trigger the death of fibroblasts residing in, and contributing to, the fibrotic ECM. Similarly, direct inhibition of TGF-β1 or connective tissue growth factor (CTGF), major regulators of fibroblast proliferation and matrix secretion, reduces ECM accumulation, partially reverses fibrosis, and improves muscle regeneration in animal models.[126–128] Similar to modulation of the myostatin pathway, inhibition of TGF-β1 or CTGF action can be accomplished either through delivery of molecules that bind TGF-β1 or CTGF or through their cellular receptors. Another potential mediator of muscle fibrosis is halofuginone, an inhibitor of downstream targets of TGF-β1. Halofuginone treatment in

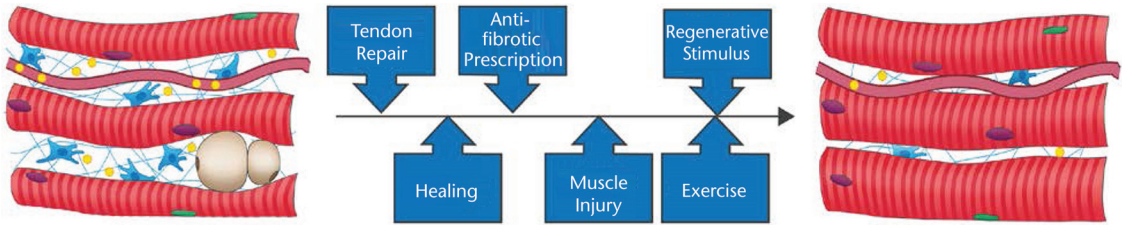

Figure 2.
Theoretical time course of combinatorial therapy to combat muscle atrophy, fibrosis, and fatty infiltration associated with chronic rotator cuff tears.

mice dramatically reduced ECM accumulation and enhanced myogenesis[129,130] and, importantly, has demonstrated safety in humans in phase I clinical trials.[131] Additionally, exogenous delivery of the natural enzymes (ie, matrix metalloproteinases) that muscle secretes to break down ECM has shown promise for reducing fibrosis and improving muscle regeneration.[132] However, care must be taken when applying such a therapy to the torn or repaired RC, as matrix metalloproteinase degradation of collagen is not tissue specific, and these molecules could potentially degrade tendon matrix as well, exacerbating the pathology.

In addition to strategies directly targeting the fibrotic pathway, strategies focusing on targeting inflammation also hold potential for reducing fibrosis and preventing atrophy. Inhibition of the TNF-α pathway has been shown to reduce fibrosis in other organ systems[133,134] in a mechanism that is likely conserved across tissue types. Although the effects of TNF-α pathway modulation on fibrosis have not been directly investigated in muscle, targeted inhibition of the pathway has been shown to improve muscle regeneration.[135] Thus, downstream modulation of inflammation could have a significant effect on muscle pathology in the RC, both during conservative treatment in the early stages of tendon tearing and following surgical repair. However, it is important to note that inflammation is a critical feature of muscle healing, and complete removal of inflammatory signals results in significant muscle pathology. Thus, these therapies will have to be carefully tailored to the specific atrophic and fibrotic pathways. A few of these strategies have been applied with some success to animal models of RC injury.[136,137]

In addition to pharmacological and molecular approaches to mitigating fibrosis, physical or mechanical strategies, such as exercise, manual therapy, and a number of modalities, have been proposed to alter fibrosis. For example, lithotripsy,[138] ultrasound,[139] deep tissue massage,[140,141] and strengthening exercises[142–145] have all been proposed to stimulate collagen remodeling in skeletal muscle and other tissues. These strategies also should be tested in the context of chronic RC injury, as they may be important components of a comprehensive strategy for recovery.

Combined Approaches (Rehabilitation, Surgery, Biologics, and Pharmacology)

There is mounting evidence that early intervention to restore tendon-bone connectivity is an important strategy for maximizing recovery after RC tears. Although conservative care combined with physical therapy is an effective strategy for pain relief and functional improvement, recent data suggest that tendon tears will progress over time. However, early intervention is difficult in this patient population, at least in part due to the insidious onset of disease. Therefore, a comprehensive strategy to deal with the chronically injured RC is necessary.

Although speculative, we believe the sum of the literature supports the following framework for managing these patients (Fig. 2). Physical therapy will likely be an important presurgical step, as exercise creates an environment that is conducive to regeneration.[146,147] Restoration of the mechanical environment will almost certainly require surgery, at least for the foreseeable future. The surgery also affords the medical team the opportunity to capture patient-specific tissues, which can be used for further treatment. The next phase of treatment is likely to be clearance of tissue damage (ie, breakdown of ECM and probably death or atrophy of adipocytes). This phase is likely to be necessary for healthy, new muscle formation. This phase may generally be accomplished with physical therapies and chemical agents to induce tissue breakdown, but the precise strategies will need to be defined by further research. Finally, the muscle regeneration phase is likely to be some combination of physical therapy (to promote growth) and a cellular or molecular therapy to promote muscle growth. There is mounting evidence that the forces applied to muscle during physical manipulations increase the effectiveness of regenerative medicine strategies.[147,148] Importantly, the ability to closely monitor mechanical forces and strains on the regenerating tissue will likely be the burden of the physical therapist. Regulating these mechanical features of exercise over time ultimately will determine the success or failure of regenerative approaches.

The future of interventions targeting RC muscle atrophy, regardless of whether they be physical, surgical, regenerative, or a combination, is likely going to have to contend with a poor starting environment. We have focused here primarily on the fibrosis and fatty infiltration associated with the pathology, but aging, comorbidities, and lifestyle also present their own unique hurdles. Aging, in particular, can play a significant role in the relationship between muscle mass and strength, where mass may increase without improved strength. True functional recovery will likely depend on address-

ing the interaction among multiple factors.

Summary and Conclusion

Developmental biology and regenerative medicine approaches to disease modification hold tremendous promise. An understanding of disease biology and recovery is opening new avenues for treatment. The physical therapy profession is already involved in these discoveries, but the frontline clinician needs to become aware of these treatment approaches because the interaction between biologic therapies and mechanical and physiologic stresses has already been established as critical for success. In patients with chronic RC injury, exercise, surgery, and biologic and cellular agents will likely be a comprehensive treatment choice in the next several years.

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