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Biologics and Stem Cell-Based Therapies for Rotator Cuff Repair

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Abstract

The rotator cuff is composed of several distinct muscles and tendons that function in concert to coordinate shoulder motion. Injuries to these tendons frequently result in permanent dysfunction and persistent pain. Despite considerable advances in operation techniques, surgical repair alone still does not fully restore rotator cuff function. This review focuses on recent research in the use of biologics and stem cell-based therapies to augment repair, highlighting promising avenues for future work and remaining challenges. While a number of animal models are used for rotator cuff studies, the anatomy of the rotator cuff varies dramatically between species. Since the rodent rotator cuff shares the most anatomical features with the human, this review will focus primarily on rodent models to enable consistent interpretation of outcome measures.

Graphical abstract

Injuries to the tendons of the rotator cuff frequently result in permanent dysfunction and persistent pain. This review focuses on recent research in the use of biologics and stem cell-based therapies to augment surgical repair of the rotator cuff, highlighting promising avenues for future work and remaining challenges. Since the rodent rotator cuff shares the most anatomical features with the human, this review will focus primarily on rodent models to enable consistent interpretation of outcome measures.

Keywords

rotator cuff; tendon; injury; stem cells

Introduction

The rotator cuff is a collection of four muscles (the supraspinatus, infraspinatus, teres minor, and subscapularis) and their tendons. Each of these muscles plays a unique role in shoulder movement, but together, they provide strength and stability to the joint during motion.

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Competing interests

The authors declare no competing interests.

Common injuries to the rotator cuff typically involve the tendon; tendons can tear as a result of mechanical overuse (i.e., wear and tear) or become inflamed.[1; 2] Injury to the rotator cuff tendons represents a significant socioeconomic burden, with over 4.5 million physician visits annually.[3] With injury, shoulder function is impaired, resulting in weakness, decreased range of motion, and pain. In the absence of treatment, primary tears to tendons result in secondary degeneration of the muscle and atrophy. Of the four tendons, the supraspinatus tendon is the most frequently injured due to its unique anatomical location beneath the acromion bone, which impinges on the tendon during overhead motion (Fig. 1).[4] For such tendon tears, surgical repair is often required to reattach the tendon back to its bony insertion. Unfortunately, surgical outcomes remain variable and failure rates can be extremely high in certain at-risk populations (up to 90% in the elderly).[5] The key challenge in rotator cuff tendon healing is the failure to restore a mechanically functional attachment between the tendon and bone after injury. This critical attachment (termed the enthesis) is normally composed of a specialized transitional gradient of tendon, fibrocartilage, mineralized cartilage, and bone (Fig. 1).[6–8] This gradient integrates two tissues of highly dissimilar material properties and allows for dissipation of high stresses that arise at the interface. Although surgical repair physically reattaches the tendon to bone (thereby restoring some function), the enthesis gradient is permanently disrupted. Instead the enthesis is replaced by disorganized scar tissue with impaired mechanical properties that may lead to re-rupture over time.[9; 10]

In recent years, the clinical community has focused mainly on advancing operation and suturing technologies to better restore the native biomechanical footprint of tendon attachment to bone. However, efficacy of these methods has been low or mixed, highlighting the limitations in suture- and cell-based approaches.[11–15] Further improvements to rotator cuff repair will likely reside in the development of new cell- or biologically-based technologies to augment surgery. Advancing this area of research thus has vast potential to revolutionize orthopedic practice. In this review, we synthesize the literature on biomolecules and stem cell-based therapies in the most anatomically relevant preclinical model of rotator cuff repair, the rat, and identify current challenges and future directions for this exciting research.

Rotator cuff injury models

Animal models

To date, the most widely used animal models for rotator cuff injury and repair include the rat, sheep, and rabbit, with 276, 111, and 106 search results, respectively in Pubmed (as of April 2018). By comparison, search results for alternative models such as dog, cat, and goat totaled 64 collectively, highlighting the relative dominance of the top three models. While large animals such as sheep or goat are advantageous and necessary for testing medical devices (due to the similarity in scale to human), the anatomical positioning of the bony, tendinous/ligamentous, and muscular shoulder components is not comparable to humans, which limits their use in other contexts. A pioneering study evaluating 33 animal models revealed that only the rat possessed a shoulder anatomy similar to human, with a prominent supraspinatus tendon passing beneath an enclosed bony arch and inserting into the greater

tuberosity of the proximal humerus.[16] In humans and rats, this specific anatomy leads to impingement of the supraspinatus tendon with overhead forelimb activity, which results in biomechanically-mediated degeneration of the tendon over time. Thus, the absence of these important anatomic features limits the use of non-rat models for modeling clinically relevant rotator cuff conditions. In addition to these important differences in gross anatomy, the rotator cuff tendons in non-rat animal models are also extra-articular and the collagen structure is highly aligned, rather than interdigitated.[17] Suture-based repairs are therefore challenging to model since the repairs fail within a very short period of time due to the highly aligned nature of the tendon structure.

Although the majority of rodent rotator cuff studies have been carried out in rat, the mouse shoulder anatomy was recently shown to be comparable to rat and human. The very small size of the mouse rotator cuff precluded its use in earlier studies; however, novel microsurgical techniques have now been applied to successfully create partial and full detachment injuries with surgical repair, thereby opening new avenues for mechanistic research.[18–24] Despite the numerous advantages of rat models, limitations include scar-mediated healing (human tears do not undergo spontaneous healing), quadrupedal gait, and small scale relative to human.[17] While joint replacement or other medical devices cannot be tested in rodent models, preclinical studies testing the efficacy of cell and biomolecule-based therapies are acceptable in rodents for meeting FDA standards. Since this review will focus on the potential of biologic therapies, the advantages of rodent models for these studies outweigh their limitations; this review will therefore focus on rat studies to allow consistent comparisons across studies.

Chronic/overuse models of tendon degeneration

In humans, rotator cuff tendon tears are typically preceded by degeneration of the tendon. Tendon degeneration in turn, is thought to be initiated by repeated mechanical impingement of tendon against bone, leading to pathological changes in the tissue structure and tendon cells.[16; 25; 26] To model these degenerative changes, a number of approaches have been established that use either mechanical or chemical methods to induce tendon degeneration. To mechanically induce degeneration, initial studies in the rat rotator cuff focused on artificially creating subacromial impingement by attaching a tendon or bony piece under the acromion to constrict the supraspinatus tendon passing through the bony arch.[26–28] This constriction led to degenerative changes in the tendon, evidenced by increased cellularity, rounder cell shape, and decreased collagen organization.[16; 28] Subsequently, another model of overuse tendinopathy was developed using downhill treadmill running, which caused similar degenerative changes; this method can be applied either on its own[29–31] or in combination with an artificial subacromial impingement.[26; 28] Treadmill running is non-invasive, reproducible, and mimics the local mechanical conditions of supraspinatus impingement in humans, with decreases in tendon properties.[29] Although this model does not result in spontaneous tears, it remains the gold standard for inducing chronic rotator cuff tendon pathology. To chemically induce degeneration, collagenase can be injected directly into the supraspinatus tendon to degrade the collagen structure.[16; 32] Although collagenase treatment is experimentally less laborious and results in much more rapid changes to the tendon structure compared to the mechanical methods described above, this method is not

commonly used since it does not recapitulate the clinical condition, which is largely mechanical in etiology. The degenerative effects of chronic/overuse models are also typically resolved within a relatively short time frame, which is a significant limitation.[32]

Models of acute injury and repair

Although overuse models of tendon degeneration best capture the early sequelae of damage, spontaneous tears in the tendon do not typically develop after chronic damage in rats. Therefore, the majority of studies for rotator cuff tendon tears employ an acute laceration injury of otherwise healthy tendon. These models include detachment of the supraspinatus tendon, either alone or in tandem with the infraspinatus or other tendons, to model massive rotator cuff tears.[18–20; 33; 34] Reattachment surgeries are typically carried out immediately after detachment, and overall healing evaluated at later timepoints.[21; 22; 35–41] Because the laceration injuries and repairs are quite invasive, the environment after injury differs in significant ways from degeneration-induced tears observed in the clinic. Acute injuries result in considerable inflammation and recruitment of immune cells, which secrete numerous factors that impact the healing response and drive scar formation. Since the tendon cells are typically healthy, the response to injury may not recapitulate the response of degenerated tendon in which cells are also diseased. One way to overcome these limitations may be to combine overuse and acute injury models; in one study, tendinopathy was induced by forced treadmill running, followed by full detachment/repair surgery to model tendon tear.[31] Finally, immediate repair after laceration also does not mimic the clinical scenario in which tendon tears are usually left untreated for weeks or months prior to repair. To model delayed repair, a few groups recently carried out supraspinatus tendon detachment followed by surgical repair 3 to 16 weeks later.[34; 42–47] Prolonged unloading resulted in tendon retraction, muscle atrophy, fatty infiltration, and fibrotic scarring. Despite eventual repair, these studies generally suggested that the pathological events associated with unloading cannot be reversed by simple reattachment.[18; 20; 33; 34]

Biomolecule-based therapies for rotator cuff repair

The delivery of biologics to augment surgical repair of rotator cuff tendons has been the focus of intense interest and research. Advantages of biomolecule delivery include ease of use and the off-the-shelf nature of such products. For biomolecule-based therapies, there is no need to isolate and culture cells, thus minimizing the time required for treatment, cost, and the number of invasive procedures. The goal of this approach is to create a more regenerative healing environment after rotator cuff repair surgery through the use of exogenous factors that promote cell proliferation and appropriate differentiation, while repressing inflammation and scar formation. In this section, we briefly discuss clinical outcomes related to platelet-rich plasma, which is one of the main biologics used to improve healing in human patients. We will then focus on current findings in rodents related to the delivery of growth factors, such as transforming growth factor-β (TGF-β), bone morphogenetic protein (BMP), and fibroblast growth factor (FGF), as well as immunomodulatory factors. Together, these comprise the most commonly tested, defined biomolecules for rat rotator cuff repairs.

Platelet-Rich Plasma

Platelet-rich plasma (PRP) is an undefined mixture of growth factors, including but not limited to TGF-β, FGF, and platelet-derived growth factor (PDGF), as well as varying leukocyte, monocyte, and macrophage populations.[14; 15; 48] PRP is the most commonly tested, undefined biological adjuvant for rotator cuff repairs, and several randomized clinical trials have studied the functional effects and patient outcomes of this treatment, with the goal of increasing healing of the enthesis and reducing pain and inflammation of patients after repair.[14; 15; 48] There are several reviews that summarize patient outcomes and compare data across multiple studies, with few finding significant improvements in patient outcomes and pain levels in patients with PRP-treated rotator cuff repairs versus control repairs.[15; 49] The majority of studies fail to find significant results, as most clinical studies have shown that PRP has no significant effects on healing, re-tear rate, pain, or functional outcomes at long-term follow-up tests.[50; 51] However, interesting differences have been observed when comparing treatment subgroups. These include the finding that PRP applied directly to the enthesis site produces better outcomes than PRP injected over the tendon, and that PRP treatment better reduces re-tear rates in small and medium tears compared to large tears.[50] The undefined composition of PRP presents more inconsistencies between studies and is a likely limitation of PRP treatment. For example, leukocyte-reduced PRP may lower re-tear rates more effectively than regular PRP.[52; 53] Overall, the findings of clinical studies and reviews suggest that PRP treatments have no consistently significant benefit on functional outcomes or re-tear rates in patients. This review will omit further discussion of PRP, due to the conflicting reports of efficacy in the clinic and the undefined composition of PRP formulations.[14; 48; 54]

Delivery vehicles

While we focus this section on the efficacy of biomolecules, it is also clear that the vehicle of delivery also plays a crucial role in outcomes. The ideal vehicle would allow continuous and controlled delivery to maintain an effective concentration of therapeutic agent at the healing site. However, most studies do not test bioactivity over time and dosing studies are not frequently presented. For inhibition of systemic processes (such as inflammation), repeated intraperitoneal injections of drug is an effective way to maintain adequate levels of drug. However, for most growth factors, local application is highly desired to prevent unwanted off-target effects. Growth factors are therefore delivered via (1) implantation of pumps that release a continuous supply over time, (2) local bolus injections, (3) adenoviral recombination, (4) implantation of a biomaterial carrier, or (5) delivery of transduced cells (which will be covered in greater detail in the next section).[55–59] Although pumps are advantageous for controlled delivery, their use in rats can be challenging due to the small size of the animal and the potential for dislocation from the desired site.[60] Local bolus injections may have a short bioactive half-life; further, it can be challenging to restrict activity to the healing site. Adenoviral recombination can also be challenging clinically since permanent expression of growth factors is likely undesirable past a certain stage in healing. In contrast, biomaterial carriers with biomimetic properties are particularly attractive for their ability to exert additional therapeutic responses, as well as the ability to design important features such as controlled release of factors or degradation of biomaterial. To date, none of the commonly used delivery vehicles have the capacity to target delivery to

specific cell populations. Our review of the literature finds that the choice of vehicle can have considerable impact on healing and the efficacy of delivered growth factors. Where findings diverged, we identify the mode of delivery used to provide potential explanations for these discrepancies.

TGF-β Superfamily

The TGF-β superfamily has been implicated in the development, differentiation, and healing of all musculoskeletal tissues, such as bone, cartilage, tendon, and muscle.[61–66] Members of the superfamily are generally organized into two branches based on their downstream Smad signaling.[67] While members of the TGF-β branch (including TGF-βs, activin, myostatin, and nodal) activate the receptor Smads 2/3, members of the BMP branch activate the receptor Smads 1/5/8. After phosphorylation, receptor Smads then bind to the co-Smad Smad4, and the entire complex translocates into the nucleus to initiate transcription.[67] Although Smad-mediated signaling remains the best known pathway associated with the superfamily, TGF-β family signaling can also activate a number of non-Smad pathways.[68] Recent data also indicates that the receptor Smads can activate transcription independently of Smad4 binding.[69]

For rat rotator cuff healing, TGF-βs and BMPs are attractive targets due to their well-established roles in tendon, cartilage, and bone differentiation. Since the rotator cuff enthesis is a transition of all three tissues, *in vivo* delivery of ligands to enhance TGF-β or BMP signaling seemed promising as a therapeutic strategy to enhance tissue regeneration. For TGF-β, initial landmark studies testing this hypothesis showed that bolus delivery of TGF-βs by osmotic pump (without scaffold) is more closely associated with fibrosis (indicated by increased type III collagen deposition, cellularity, and vascularity) and a heightened inflammatory response.[57] These studies suggested that the cells responsive to TGF-β signaling may be immune or scar-forming cells rather than stem/progenitor cells with tenogenic or chondrogenic potential. In the context of function however, application of exogeneous TGF-β ligands generally improve mechanical properties, which may be due to increased scar tissue.[56; 58; 70] By contrast, inhibition of TGF-β signaling via delivery of neutralizing antibodies impaired mechanical properties, indicating that early TGF-β signaling is likely required to initiate the healing cascade.[57] Interestingly, a few studies combining TGF-β ligands with bone biomimetic carriers showed enhanced enthesis fibrocartilage and bone deposition.[55; 70] Therefore, the use of such bone-promoting scaffolds in combination with TGF-βs may alter the type of cells recruited during healing and initiate a more regenerative response.

Members of the BMP branch (including BMPs 2, 4, and 7) are typically associated with cartilage and bone formation, as well as tendon inhibition.[66; 71; 72] Of this group, only BMP2 and BMP7 have been tested in the context of rat rotator cuff healing. Although BMP2 delivery via transduced stem cells dramatically impaired healing (as evidenced by reduced mechanical properties and loss of bone), sustained delivery of BMP7 enhanced fibrocartilage deposition at the tendon-to-bone site.[59; 73] However, biomechanics were not significantly improved relative to controls.[73] These limited outcomes suggest that additional

research will be required to determine whether these chondrogenic/osteogenic BMPs have therapeutic value.

In addition to these classic BMPs, another subset of BMPs, formerly known as GDFs 5, 6, and 7 (now BMPs 14, 13, and 12, respectively), were shown to have potential roles in tendon development.[61; 74–76] Of this group, only BMP13 was tested for rat rotator cuff healing. Similar to the other BMPs, results from these studies were inconsistent; while BMP13 delivered by sutures or adenovirus improved biomechanical healing, BMP13-transduced stem cells delivered to the injury failed to show any improvement.[58; 77; 78] Collectively, the inconclusive outcomes from BMP treatments highlight several challenges and open questions, including the extent to which different modes of delivery, timing, and dosing may significantly impact BMP's biologic functions *in vivo*. Interestingly, the use of stem cells as a delivery vehicle for BMPs proved least successful.

FGFs

The FGF signaling pathway consists of 22 ligands, the majority of which are secreted factors that bind and signal through receptor tyrosine kinases. Binding of ligand to one of four receptors results in phosphorylation at the intracellular tyrosine kinase domain and recruitment of key molecules that interact with specific domains. These signaling complexes then trigger a cascade of phosphorylation events and induces downstream activation of several pathways, including the Ras/MAP kinase, PI3 kinase/Akt, PLCγ, and STAT pathways. For more details on FGF signaling, please see the excellent and comprehensive review by Ornitz and Itoh.[79] Like TGF-βs and BMPs, FGFs have been implicated in multiple aspects of musculoskeletal development, as mutations in specific FGF ligands or receptors result in severe defects in limb outgrowth, skeletal differentiation, and muscle development/regeneration.[79] For tendon, FGF was the first signaling molecule identified to regulate somitic tendon induction in chick.[80] Due to potential redundancy between multiple FGF ligands/receptors and their requirement in skeletal development, the role of FGFs in mammalian tendon or enthesis development has not been easily elucidated. It is therefore unclear whether FGF signaling molecules have any role in mammalian tendon induction, differentiation, or growth/maturation, either alone or in tandem with other signaling molecules such as TGF-β.[81]

To date, there are only three studies that have tested the potential of FGF2 to enhance rat rotator cuff healing. Although the number of studies is limited, supplementation of supraspinatus repairs with FGF2 universally improved healing outcomes, regardless of mode of delivery (hydrogels or osmotic pump). Improved healing was demonstrated by improved biomechanics, enhanced cell proliferation, upregulation of tendon-specific genes, and improved structural organization.[60; 82; 83] These results suggest that FGF2 may be a promising therapeutic molecule for follow up investigation, although additional studies are certainly required to identify mechanisms of action.

Immunomodulatory Factors

In addition to growth factors, immunomodulatory factors have also been extensively evaluated, based on their capacity to alter the inflammatory environment post-injury and

improve healing outcomes. Much attention has been focused on the use of nonsteroidal anti-inflammatory drugs (NSAIDs), since drugs in this class are routinely prescribed as part of post-operative care. NSAIDs function by inhibition of cyclooxygenase (Cox) enzymes, which are present in two forms, Cox1 and Cox2.[84] Traditional nonselective NSAIDs inhibit both Cox1 and Cox2, while selective NSAIDs only target Cox2 (Table 1). For rotator cuff repair, a number of NSAIDs, including diclofenac, meloxicam, ibuprofen, indomethacin, and celecoxib showed largely detrimental effects on mechanical properties and tendon healing after acute injury.[85–88] These effects may be somewhat time-dependent; while most studies showed impaired healing with immediate delivery post-injury, delivery at later stages of healing did not seem to affect functional properties.[87] Indeed, delayed repair coupled with licofelone injections (a dual Cox and lipoxygenase inhibitor) improved mechanical properties and fibrocartilage deposition.[89] However, despite reductions in fatty infiltration and muscle fibrosis, some degree of muscle atrophy remained, which is consistent with other studies showing that chronic unloading of tendon results in permanent changes to the rotator cuff unit. Collectively, this body of work indicates that early inflammation is critical for tendon healing and functional repair. Consistent with this concept, activation of Cox2 by treatment with statins showed improved mechanical properties and cell proliferation.[90] While most studies have focused on NSAID delivery to modulate local inflammation, one study repressed the activity of TNF-α via delivery of the receptor TNFR1 and showed a transient improvement in mechanical properties at early stages that was not sustained at later timepoints.[40] This suggests that distinct inflammatory pathways may be activated during healing.

Stem cell-based therapies for rotator cuff repair

The promise of stem cells for regenerative medicine has inspired considerable attention over the past 20+ years, due to their ability to self-renew and undergo differentiation along multiple lineages. While stem cell types such as embryonic stem cells or induced pluripotent stem cells retain the capacity to create any tissue lineage, postnatal tissue-specific stem cells are typically more restricted.[91–93] Tissue-specific stem cells can either undergo multilineage differentiation (such as mesenchymal stem/stromal cells, MSCs), or are largely restricted to a single lineage (such as muscle satellite cells).[94; 95] For musculoskeletal regeneration, the most commonly used stem cells are multilineage stem cells derived from bone marrow (bMSCs) or fat (adipose-derived stem/stromal cells, ASCs).[94; 96] Collectively, these two stem cell types have been differentiated into almost every connective tissue lineage, including bone, cartilage, tendon, and the intervertebral disc.[97–101] More recently, cells with self-renewal capacity, clonogenicity, and multi-lineage potential have been isolated from tendon tissue.[102–106] In the original characterization of these tendon stem/progenitor cells (TSPCs), it was suggested that these cells may reside within the tendon proper and comprise a sub-population of tenocytes.[102] Other studies, however, suggest that TSPCs may be located within the epitenon, which is the thin epithelial layer surrounding all tendons.[107–109] For bMSCs, ASCs, and TSPCs, definitive markers are not well established and these cells are typically derived and characterized based on plastic adherence, expansion potential, and multilineage potential. Markers that have been used to identify putative bMSCs include various cell surface markers (Sca-1, Stro-1, CD146, CD90, and CD44), PDGFRα, and

region-specific Hox expression (Table 2).[102; 110; 111] TSPCs were found to share several markers with bMSCs, with the additional expression of markers Scx, Comp, and Sox9.[102] Recently, single-cell RNA-seq screening implicated nestin as a potential marker for distinguishing TSPCs; however the *in vivo* location of these cells was not determined.[112] The use of stem cells clinically remains controversial, with few high quality studies. Two clinical studies provide insight into patient outcomes after surgical augmentation with MSCs. In one, bMSCs applied during arthroscopic single-row rotator cuff repair drastically reduced re-tear rates at a ten year follow-up.[113] A more recent study also found a significant decrease in re-tear rate after surgical augmentation with ASCs.[114] The paucity of clinical data from studies performed in the US is largely due to the high risks associated with stem cell-based therapies, including growth of tumors, administration site reactions, and other adverse effects.[115] Rigorous pre-clinical studies testing stem cell delivery and functional repair in animal models will be required to inform potential therapies and clinical translation. In this section, we therefore focus on *in vivo* applications of commonly used MSC populations (bMSCs, ASCs, TSPCs) for rat rotator cuff repair and current findings in this area. Tissue engineering efforts that focus solely on *in vitro* differentiation strategies will not be discussed.

Directed differentiation of stem cells in vivo

One strategy for stem cell-based rotator cuff repair harnesses their multipotent capacity to direct differentiation toward a tendon or fibrocartilage phenotype. Differentiation can be carried out *in vitro* to create an engineered tendon/enthesis construct prior to implantation, or naive MSCs can be delivered within a biomimetic matrix to promote the proper cell fate *in situ.*[116–119] Commonly used scaffolds for rotator cuff include electrospun fibers, xenograft tendon scaffolds, or hydrogels (typically fibrin or type I collagen gels).[43; 59; 120–123] The use of a carrier vehicle is advantageous since it ensures proper localization of delivered cells; the environment provided by the scaffolds has the additional potential to improve host cell proliferation or differentiation. While MSCs delivered within such carriers generally showed some positive indicators of improved healing (such as improved histology, fibrocartilage deposition, or expression of enthesis markers),[46; 120; 122] functional outcomes have been mixed. Although some studies reported improved mechanical properties,[120; 123] others observed no differences relative to carrier or suture controls by the final timepoint.[39; 46; 121] An alternative strategy to direct differentiation is transduction of transcription factors that may induce a tenogenic cell fate, such as the transcription factors *Scx* or *Mkx.*[71; 124; 125] For rotator cuff, bMSCs transduced with *Scx* improved fibrocartilage and mechanical outcomes after acute injury (compared to naive bMSCs), suggesting that delivery of tenogenic cells may have greater therapeutic value relative to naive stem cells.[126] Similarly, delivery of TSPCs in fibrin improved rotator cuff mechanical properties after injury,[127] however direct comparisons against bMSCs or ASCs were not determined. Though promising, additional research is required given the paucity of studies using TSPCs for rotator cuff repair.

Stem cell delivery to modulate local environment

In addition to direct differentiation, MSC delivery may have other therapeutic benefits. For example, naive MSCs are thought to be immunoprivileged and may exert

immunomodulatory effects on the local environment via secreted factors.[128] One study demonstrated a therapeutic effect of MSC-conditioned media applied to injured rotator cuff.[47] While enthesis healing was not determined, application of the MSC secretome inhibited muscle degeneration and atrophy. While still not definitively established, the immunomodulatory benefits of MSCs may be due to their effect on macrophage recruitment or polarization. Co-culture of ASCs with macrophages suggest that ASCs can shift macrophages toward an anti-inflammatory M2 phenotype and exert a protective effect on tendon fibroblasts.[56] Delivery of ASCs to augment rotator cuff repair *in vivo* generally reduces inflammation and the presence of inflammatory cells; however, functional properties do not improve.[32; 129] Similarly, delivery of bMSCs showed increased numbers of anti-inflammatory M2 macrophages at later timepoints, in parallel with upregulated enthesis markers.[122] Collectively, these studies indicate that MSCs derived from bone marrow or fat may have immunoprotective properties, but the impact on functional restoration may be minimal.

Finally, naive stem cells can also be used as a vehicle for local biomolecule production via gene transduction, which can have cell-autonomous or non-autonomous effects on healing. For rotator cuff healing, biomolecules of interest include growth factors or molecules that regulate cell proliferation, differentiation, or matrix degradation/remodeling.[58; 59; 130; 131] While stem cells transduced with BMPs had no or detrimental effects on healing,[58; 59] there have been some success with other molecules such as an shRNA for TOB1 (a protein that inhibits proliferation) and MT1-MMP (otherwise known as MMP14).[130; 131] One of the challenges for this delivery strategy is temporal control; molecules will be produced by stem cells as long as they are present in the injury site. This is in contrast to delivery strategies such as bolus injections, which have a relatively brief half-life, or biomaterials that can be engineered to degrade and disappear over time. In some cases, prolonged or excessive production of molecules can provoke undesirable responses by host cells. In the case of BMPs, which regulates important biological functions for many different cell types, uncontrolled response may explain some of the poor healing outcomes.

Conclusions

Although there is considerable interest in biological augmentation of rotator cuff repair, outcome measures have thus far been mixed. Our review of the literature suggests that the method of delivery (for biologics as well as cells) may have a considerable impact on success. To date, much of the outcome measures have focused on structural and functional recovery. While these remain critical measures, advancing biologic and cell-based therapies will also require mechanistic studies that follow cell fate. The recent adaptation of rotator cuff injury and repair in mouse will enable such studies; genetic manipulations and cell lineage tracing can be easily accomplished in mouse to directly test promising signaling pathways, identify responsive cells, distinguish host from donor cells, and identify local cell populations that are relevant for healing. Despite current limitations, biological augmentation remains a highly exciting research area and represents the next frontier in functional rotator cuff restoration. Successful strategies will likely require a combination of biomolecules, intelligent biomaterials, and stem cells (Fig. 2).

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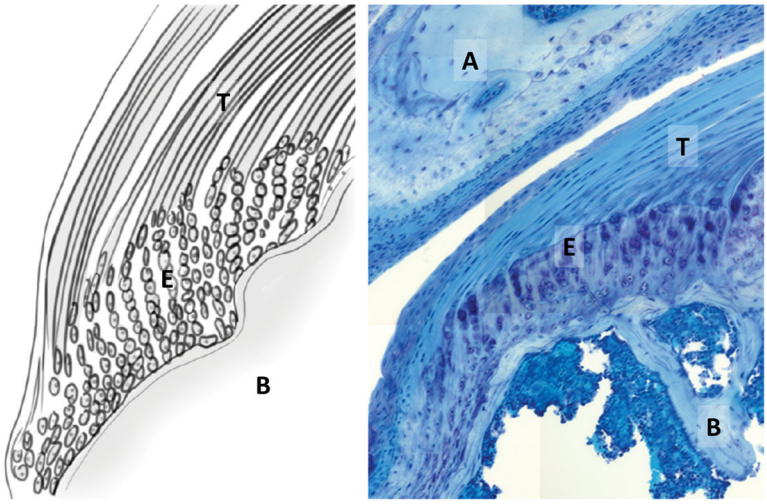

Figure 1.

Anatomy and cell morphology of the supraspinatus tendon enthesis. (Left) Schematic of the tendon-to-bone (T/B) insertion via fibrocartilage enthesis gradient. (Right) Toludine blue staining of the supraspinatus tendon inserting into the humeral head, beneath the acromion (plastic section, 20X). T: supraspinatus tendon, B: humeral bone; E: enthesis; A: acromion.

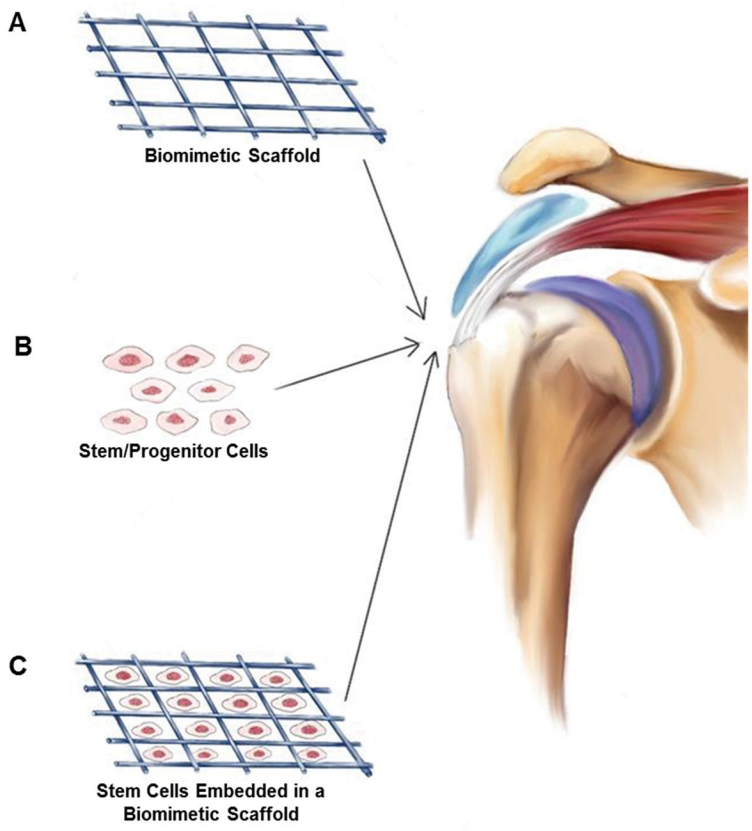

Figure 2.

Overview schematic highlighting biologic and stem cell strategies for rotator cuff repair. (A) Cell-free biomimetic scaffolds can be implanted to control release of biomolecules or exert their own beneficial proliferative or differentiating effects on local tissues. (B) Stem/progenitor cells delivered to the injury site can modulate the local environment or undergo direct cell differentiation. (**C**) The combination of biomaterial scaffold and stem/progenitor cells has been explored in recent studies to increase healing after rotator cuff injury and repair.

Table 1

Non-selective and selective NSAIDs

Nonselective NSAIDs	Selective NSAIDs
Ibuprofen	Meloxicam
Diclofenac	Celecoxib
Indomethacin	
Ketoprofen	
Aspirin	
Piroxicam	
Naproxen	

Table 2

Commonly used markers for MSC validation

Negative selection markers	Positive selection markers	
CD34 (hematopoietic cells)	Sca-1	CD146
CD45 (leukocytes)	Stro-1	CD44
CD11b (monocytes/macrophages)	PDGFRα	CD90
	Hox	CD105