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Biologic approaches to enhance rotator cuff healing after injury

Christian Isaac, MS, MD[a,b], Burhan Gharaibeh, PhD[a,b], Michelle Witt, MS[a], Vonda J. Wright, MD[b], Johnny Huard, PhD[a,b,*]

[a]*Stem Cell Research Center, University of Pittsburgh, Pittsburgh, PA, USA*
[b]*Department of Orthopaedic Surgery, University of Pittsburgh, Pittsburgh, PA, USA*

Background: Despite the advances in surgical procedures to repair the rotator cuff, there is a high incidence of failure. Biologic approaches, such as growth factor delivery and stem cell and gene therapy, are potential targets for optimization to improve the outcome of rotator cuff therapies and reduce rates of reinjury. This article outlines the current evidence for growth factor and stem cell therapy in tendon healing and the augmentation of rotator cuff repair.

Methods: Literature on the PubMed—National Center for Biotechnology Information database was searched using the keywords *growth factor, factor, gene therapy, stem cell, mesenchymal,* or *bone marrow* in combination with *rotator cuff, supraspinatus,* or *infraspinatus*. Articles that studied growth factors or stem cells alone in rotator cuff repair were selected. Only 3 records showed use of stem cells in rotator cuff repair; thus, we expanded our search to include selected studies on stem cells and Achilles or patellar tendon repairs. Bibliographies and proceedings of meetings were searched to include additional applicable studies. We also included hitherto unpublished data by our group on the use of stem cell transplantation for rotator cuff therapy.

Results: More than 70 articles are summarized, with focus on recent original research papers and significant reviews that summarized earlier records.

Conclusions: Use of growth factors, stem cell therapy, and other tissue-engineering means serve to augment classical surgical rotator cuff repair procedures. The combination of stem cells and growth factors resulted in enhanced repair that emulated uninjured tissue, but the literature search reflected paucity of research in this field. Preclinical evidence from gene therapy and stem cell studies can be used as a start to move therapy from the experimental phase to clinical translation in patients.

Level of evidence: Review Article.

Keywords: Rotator cuff; gene therapy; stem cell therapy; biological augmentation; tissue engineering; mesenchymal stem cells; muscle-derived stem cells

Tendon degeneration and rupture is a common disorder that affects athletes and workers alike. The rotator cuff (RC) is formed about the proximal humerus by the tendinous insertions of a group of muscles that dynamically stabilize the glenohumeral joint. Partial-thickness and full-thickness RC tears can be found in 30% to 50% of the population aged older than 50 years.[47] Surgical procedures

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*Reprint requests: Johnny Huard, PhD, Stem Cell Research Center, Bridgeside Point II, 450 Technology Dr, Suite 206, Pittsburgh, PA 15219, USA.

E-mail address: jhuard@pitt.edu (J. Huard).

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to repair RC tears have shown great improvement over the years, but failure rates can range from 30% to 94%.[28]

Tendon is thought to heal in 3 phases. Initially, there is inflammation with subsequent removal of tissue debris by macrophages, followed by fibroblast infiltration and the deposition of collagen type III to form a callous, before the final remodeling phase that causes contraction of the collagen-rich scar.[22] The resultant scar tissue has a higher ratio of collagen type III to collagen type I, a property that renders scar tissue weaker than adjacent normal tissue and increases the risk of rerupture.[34]

In humans, the RC often ruptures at the enthesis or tendon−bone insertion site that is transitioned by a well-defined zone of fibrocartilage. Studies in animal models have shown that a normal tendon−bone insertion site is not regenerated after repair; instead, the injured tissue is replaced with reactive scar formation rich in collagen III, which is mechanically weaker than the original zone of calcified cartilage formed during embryogenesis.[31] Failure to recapitulate the normal tendon−bone interface may be due to the absence of appropriate molecular signaling and, ultimately, the failure of cellular differentiation at the zone of injury.[31] Therefore, the idea of redirecting the healing process away from scar formation and toward the regeneration of a native tendon−bone insertion site is an attractive strategy for improving the outcome of RC repairs.

Growth factor and stem cell therapy may serve to augment RC repairs by targeting tissue regeneration, similar to embryonic development versus the traditional scarring process, resulting in the formation of a tissue that more closely resembles the uninjured, native tissue. The use of tissue-engineering techniques to manipulate the healing process may allow us to improve clinical outcomes. This review will outline the current evidence for growth factor and stem cell therapy in tendon healing and the augmentation of RC repair.

Methods

We used the PubMed and Google Scholar search engines to search the literature using the following search terms: *growth factor, factor, gene therapy, stem cell, mesenchymal,* or *bone marrow* combined with *rotator cuff, supraspinatus,* or *infraspinatus*, and included all permutations. We selected the papers that studied growth factors or stem cells alone in RC repair. Only 3 reports were found that used stem cells in RC therapy, and we thus expanded our stem cell search to include selected studies on stem cell and Achilles or patellar tendon repairs. Bibliographies were hand searched to include any applicable studies that were not captured by our search.

Growth factors

Numerous growth factors, including the bone morphogenetic proteins (BMPs), basic fibroblast growth factor (bFGF), platelet-derived growth factor (PDGF), and transforming growth factor-β (TGF-β), can be found in the RC in predictable expression patterns during the early healing process. Many of these growth factors have a unique temporal expression profile and are thought to play an important role in directing tissue formation during the acute phase of RC healing.[29,60] Therefore, the ability of these growth factors to augment tendon−tendon or tendon−bone healing, or both, is currently under investigation. Most commonly, growth factors are being delivered locally to the tendon repair site using gene therapy via tissue-engineered scaffolding, coated sutures, or dissolved in a fibrin sealant.[19] Most growth factors, however, are short-lived and thus repeated administration is needed. Genetic engineering of stem cells to express the needed factors can provide a solution.

Gene therapy was investigated to allow for continuous delivery of a gene product over time. In vivo gene therapy has used recombinant viruses or plasmid DNA that code for growth factors of interest to be delivered by direct injection. This technique allows for accurate delivery of genetic material but does not control for the efficiency of transduction and, ultimately, expression of factors.[19] Alternatively, ex vivo gene therapy involves the harvest and transduction of autologous cells before they are reintroduced at the zone of injury. Cells are expanded in culture, transduced with virus to express the growth factor of interest, and selected on the basis of antibiotic resistance. This is a more laborious process but confirms that growth factor expression is functional and allows the detection of any genetic abnormalities or transformation before cell delivery.[44]

A number of bioscaffolds are being incorporated into animal models of tendon repair. These include porcine small intestine submucosa (SIS),[62] chitin,[15] or collagen matrix chitosan-based hyaluronan hybrid scaffolds, and various nanofibers constructed of polyglycolic acid or poly(lactide-co-glycolide).[14] The scaffolds appear to be safe and can localize growth factors or cells to the repair site. In addition, some bioscaffolds may even have the capacity to increase scar formation and strengthen tendon−tendon RC repair.

Various animal models mimic RC repair in humans and emphasize tendon−bone healing. As mentioned, growth factors can be delivered through a variety of vehicles. Most studies have used a bioscaffold material to deliver growth factor to the injury site. Studies that use ex vivo gene therapy employ cells to express a growth factor locally. Therefore, such studies were considered a ''combined therapy'' and are beyond the scope of this review.

Bone morphogenetic proteins

Bony in-growth into a neotendon bridged by a transition zone of fibrocartilage is required to recapitulate the normal tendon−bone insertion site. The BMPs belong to the TGF-β superfamily and are known for their ability to stimulate bone formation. Certain BMPs, namely BMP-1, BMP-12, BMP-13, and BMP-14, are expressed in the acute

phase of healing after RC tears.[38,60] BMP-12 and BMP-13 are thought to be important regulators of fibrocartilage, neotendon, and ligament formation.[33] BMP-13 and BMP-14 can increase the tensile strength of the regenerated tendon, and BMP-14 is found at the tendon edges on the bursal side of the torn human RC.[1] Thus, these BMPs, in particular, are thought to modulate tendon healing. More recently, other BMP family members, BMP-2 and BMP-7, were shown to induce collagen production when added to cultured tenocyte-like cells derived from samples of human RC.[42] For these reasons, the ability of BMPs to augment RC repair is currently under investigation.

Studies in sheep and rat models have shown BMP therapy alone is beneficial for RC repair. Rodeo et al[48] found that a mixture of growth factors within an osteoinductive bone protein extract placed on the tendon−bone interface via a collagen sponge resulted in stronger repairs, illustrated by greater load-to-failure at 6 and 12 weeks after repair. Similarly, application of recombinant human (rh-) BMP-12 alone in a collagen or hyaluronic acid sponge strengthened the repair and accelerated healing in an ovine model.[52] Treatment with BMP-13 can also strengthen RC repair and structure, as BMP-12 and BMP-13 repairs both demonstrated less cellularity and vascularity, with more fiber alignment and cellular organization.[37,52] Dip-coating of sutures with recombinant growth/differentiation factor-5 (rh-BMP-14) accelerated tendon healing and enhanced strength.[12]

These results, however, need to be taken with caution. Little or no difference was observed in studies when normalized or compared with controls. Although Dines et al[12] showed accelerated healing using suture coated with BMP-14, no biomechanical or histologic difference was found when compared with controls at 6 weeks.[12]

Basic fibroblast growth factor

Basic FGF is known to effect proliferation and collagen secretion of RC tendon cells in vitro. After RC injury, bFGF is expressed in vivo, peaking between 5 and 9 days after tendon rupture.[29,55,60] Furthermore, bFGF increased tendon healing strength and accelerated tendon−bone remodeling in vivo.[24,25,56] Ide et al[25] repaired rat RC with acellular dermal matrix grafts and supplemented bone tunnels with bFGF via a fibrin sealant carrier. Repairs had significantly higher tendon maturation scores at 6 and 12 weeks that corresponded with greater ultimate load-to-failure. These results indicate that acellular dermal matrix graft remodeling is accelerated by local administration of bFGF. Thus far, augmentation with bFGF appears promising, but further animal studies are needed to evaluate the effect of bFGF in RC repair.

Platelet-derived growth factor

PDGF is another important growth factor in RC healing. It has been found to act as a mitogen and chemokine and is temporally expressed in the acute phase of RC healing, peaking between 7 and 14 days after injury.[18,19,29,60] However, treatment of rat RC repairs with PDGF-BB did not improve histology despite inducing proliferation and angiogenesis in a dose-dependent fashion at 5 days after repair.[19]

Since that initial report, two PDGF-BB studies have been reported in a sheep model of RC repair [21,57] using suture coated with a collagen−PDGF-BB solution[57] and interpositional grafting using a collagen matrix coated with different quantities of rh-PDGF-BB.[21] Both ovine models have showed improvement in histology and limited biomechanical force differences between experimental and control groups. More importantly, the response was dependent on the dose, timing, and the delivery vehicle used for PDGF delivery.

Transforming growth factor-β

TGF-β signaling is essential developmentally for tendon formation.[45] TGF-β is also intimately associated with scar formation and is expressed almost ubiquitously within the RC throughout the acute phase of healing in 3 isoforms: TGF-β1, TGF-β2, and TGF-β3.[29,60] Adult wounds characteristically heal with an abundance of scar tissue and TGF-β1, whereas, fetal wounds are scarless and lack TGF-β1 expression but express TGF-β2, and TGF-β3.[45,54] Thus, studies are currently attempting to manipulate the expression of TGF-β isoforms to favor TGF-β3 during cuff healing to decrease scar formation and strengthen the resultant repairs.

Current literature on TGF-β is complicated and even conflicting at times. In one study, overexpression of TGF-β3 in isolation failed to improve rat supraspinatus tendon−bone healing, despite sustained delivery to the subacromial space in the absence of TGF-β1 or TGF-β2.[28] In contrast, sustained delivery of TGF-β3 to the tendon−bone insertion site using a heparin/fibrin-based delivery system resulted in significant improvements in structural properties at earlier times and material properties at later times.[35] However, the collagen III content was not evaluated, and fibrocartilage did not form at the insertion site.

In comparison, another study used calcium phosphate matrix to deliver TGF-β3 to the tendon−bone interface and reported greater fibrocartilage formation, increased collagen organization, more favorable collagen I/III ratios, and improved load-to-failure.[30] These histologic improvements, however, were also seen in calcium phosphate−treated controls, making the actual effect of TGF-β3 difficult to interpret. Thus, results are encouraging but more work is necessary to determine the optimal dose, timing, and effect of individual isoforms in the application of RC repair.

Stem cell augmentation of tendon healing and RC repair

In this section, literature records on the use of stem cells for RC repair are reviewed, and significant findings are listed for

each population. It is worth mentioning that tendon-derived stem/progenitor cells (TSPCs) have been isolated and cultured from human, mouse, rabbit, and rat tendons.[3,49,50,63] Data obtained thus far has shown that TSPCs can differentiate, form tendon-like tissue, and have the ability to synthesize tendon extracellular matrix. However, no studies using TSPCs to augment RC repair have been reported to date. Nonetheless, the origin of tendon-derived stem cells is interesting, given that our group and other researchers have reported that blood vessel walls harbor pericytes, myoendothelial cells, and other multipotent cells.[11,65]

Mesenchymal stem cells

Mesenchymal stem cells (MSCs) can differentiate into several mesenchymal tissues, including bone, fat, muscle, and tendon.[5] Bone marrow (BM) has been identified as an excellent source of MSCs and can be accessed readily by surgeons intraoperatively to provide cells in a convenient and timely fashion.[59] For these reasons, BM-derived MSCs (BMSCs) may have valid clinical utility, and various strategies have been used to coax these cells down a tenogenic lineage, including application of growth factors,[32] ectopic expression of transcription factors, exposure to tensile loads, and high-density coculture with tenocytes.[51] Thus, there is plenty of evidence to show that BMSCs can be manipulated to differentiate into a tenogenic lineage and produce tendon tissue when exposed to the appropriate environmental cues.

During the last 15 years, a number of studies in animal models have used a variety of different vehicles, including type I collagen gels, collagen sponges, and fibrin as a carrier to deliver MSCs to the Achilles, patellar, or RC tendons to evaluate the effect of MSCs on the histologic appearance, microstructure, biomechanics, and strength of tendon healing after repair. Overall, the results have been promising and are reviewed in Table I. There is a general trend toward modest improvements in histology and strength of repair.

A number of groups have shown improved histology and biomechanics in rabbit tendons after repair with BMSC treatment compared with controls.[2,8,27,61] BMSCs contracted onto pretensioned suture resulted in greater load-related structural and material properties when used to repair Achilles tendon defects.[61] Similar improvements were seen when a BMSC-collagen mixture was implanted into surgically created patellar tendon defects.[2] One group examined histology and biomechanics 12 weeks after delivering MSCs via collagen gel-sponge composites into full-thickness, full-length, defects in the central third of rabbit patellar tendons. Maximum force, maximum stress, linear stiffness, and linear modulus of repaired patellar tendons were significantly improved with MSC treatment compared with controls but were still inferior to uninjured, normal, central-third patella tendons. Histologically the cellular repairs showed improved cellular alignment that was comparable to normal tendon.[27]

Another study by Chong et al[8] delivered BMSCs to rabbit Achilles tendon defects using fibrin as a carrier and noted improvements in biomechanics and histology 3 weeks after implantation but no appreciable differences at later intervals. The authors conclude that improvements in primary tendon repair with intratendinous cell therapy are restricted to the early stages of tendon healing.[8]

Two different studies recently used a rat Achilles tendon model to compare MSC therapy versus other cell therapy, with important results. In one report, the Achilles was transected, including the enthesis, before surgical repair without cell injection, with chondrocyte injection, or with MSC injection. The healing rates were studied macroscopically at 15, 30, and 45 days. Histologic means were used to score the production and organization of the new enthesis. Biomechanical tests were used to determine the load required to rerupture the new bone−tendon junction. Both cell therapies significantly improved healing rates and the load-to-failure after 45 days compared with classical surgery without cells. In addition, a new enthesis formed with both cell therapies but not in controls. However, only the MSC group showed an organized enthesis with columnar chondrocytes that resembled the native enthesis.[39]

In the other study, Okamoto et al,[40] isolated whole BM cells (BMC) and cultured BMCs from 9 Fisher rats. They compared BMCs, MSCs, and no cells in an Achilles tendon defect. Ultimate failure load in the BMC group was significantly greater than in the MSC and control groups at 7 and 14 days. After 28 days, the ultimate failure load in the BMC group was the same as normal tendon. Histologically, these results correlated with more intense collagen III staining after 7 days and a switch to more intense type I collagen after 28 days in the BMC group compared with the MSC group. In addition, the expression of TGF-β and vascular endothelial growth factor (VEGF) appeared to be greater in the BMC group at 4 days compared with the MSC and control groups.[40]

Taken together, these two studies are very promising for cell therapy in tendon repair; the former to improve regeneration of the enthesis, an application that may prove especially useful for RC repair and the latter for using whole BMCs to improve the strength and quality of tissue formed with tendon repairs. The idea of using BMCs is especially provocative because they do not require prolonged expansion times in culture and are thus more practical in surgical applications than cultured MSCs.

There is a lack of literature that looks specifically at stem cell augmentation alone in RC repair. Excluding studies that used combinations of stem cells and growth factors, our search found only three studies that used tenocytes, MSCs, and BM mononuclear cells (BMMCs), respectively, as a monotherapy to augment cuff repair. Tenocytes were shown to improve the histologic appearance when seeded on collagen-based scaffolds and

Table I Augmentation of tendon repair with cell monotherapy

First author, year	Cell type (vehicle)	Animal model	Major findings
Young,[61] 1998	BMSC (collagen gel)	Rabbit AT	Improved biomechanics & histology
Awad,[5] 1999	BMSC (collagen gel)	Rabbit PT	Improved biomechanics & histology; no change in microstructure; heterotopic ossification
Juncosa-Melvin,[27] 2006	BMSC (collagen sponge)	Rabbit PT	Improved biomechanics & histology
Chong,[8] 2007	BMSC (fibrin)	Rabbit AT	Improved biomechanics & histology (at 3 weeks only)
Gulotta,[18] 2009	BMSC (fibrin)	Rat RC	No change in biomechanics or histology (at 2 or 4 weeks)
Chen,[6] 2010	hESC-MSC (collagen sponge)	Rat AT	Improved biomechanics & histology
Cohen,[9] 2010	hESC-CTP-TG	Mouse AT	Graft remodels & strengthens in vivo
Nourissat,[39] 2010	MSC/chondrocytes	Rat AT	Improved biomechanics, healing, enthesis (MSC > chondrocytes)
Okamoto,[40] 2010	BMC/MSC (injected)	Rat AT	Improved biomechanics; increased col I/III, TGF, VEGF (BMC > MSC)
Ellera Gomes,[13] 2011	BMMC (injected)	Human RC	All cases had + outcomes after 1 year; appears safe
Uysal,[58] 2011	ASC (injected)	Rat AT	Improved biomechanics + tendon/endothelial differentiation
Ju,[26] 2008	SMSC (injected in bone tunnel)	Rat ACL	Improved histology, accelerate tendon—bone healing
Connell,[10] 2009	SDF	Human epi	SDF produce collagen; safe for human injections
Chen,[7] 2007	Tenocytes (SIS/collagen)	Rabbit RC	Tenocytes improved healing, remodeling > scaffold alone

ACL, anterior cruciate ligament; *ASC,* adipose-derived stem cells; *AT,* Achilles tendon; *BMMC,* bone marrow mononuclear cells; *BMC,* whole bone marrow cells; *BMSC,* bone marrow—derived mesenchymal stem cells; *epi,* epicondylitis; *hESC-CTP-TG,* tendon grafts produced in vitro by hESC-derived connective tissue progenitors; *hESC-MSC,* human embryonic stem cell mesenchymal stem cells; *PT,* patellar tendon; *RC,* rotator cuff; *SDF,* skin-derived fibroblasts; *SIS,* small intestine submucosa (porcine); *SMSC,* synovial mesenchymal stem cells; *TGF,* transforming growth factor; *VEGF,* vascular endothelial growth factor; +, positive; >, greater than.

implanted as interposition grafts to repair massive RC defects in rabbits.[7] However, whether the tenocyte-collagen scaffold increases the strength of the repair is unclear because the study did not include any biomechanical data. MSCs did not change structure, composition, or strength of the healing tendon attachment site, despite being present and metabolically active, in a study that used fibrin to deliver MSCs to the RC of Lewis rats.[18]

Despite the paucity of animal studies, a pilot study was able to enroll 14 patients with complete RC tears repaired with transosseous stitches through miniopen incisions. Prior to cuff repairs, autologous BMMCs were harvested from the iliac crest and subsequently injected into the repaired tendon borders.[13] The BMMC fractions were obtained by cell sorting and resuspended in saline enriched with 10% autologous serum. These patients were monitored for a minimum of 12 months, and University of California, Los Angeles scores improved on average from 12 ± 3.0 to 31 ± 3.2, and tendon integrity was demonstrated by magnetic resonance imaging in all 14 patients. No control group was included in this study, but historically for this procedure, overall rates of rerupture during the first post-operative year range from 25% to 65%, depending on lesion extent. Unfortunately, only 14 patients were enrolled in this study, making it difficult to determine the efficacy of BMMCs as an adjunct to cuff repair at this time. However, implantation of BMMCs in RC tendon borders appears to be a safe and promising approach to enhance the healing of tendon repairs. Further research will be critical to better investigate the use of this biologic approach.

Most of the studies to date have looked at the ability of MSCs derived from BM to augment tendon repair. Research using other cell types is also ongoing. A few studies have shown that MSCs and connective tissue progenitor cells can be isolated from human embryonic stem cells (hESCs).[6,9] For example, hESC-derived MSCs improve biomechanics and histologic appearance when used adjunctively to repair a rat Achilles tendon defect.[6] Alternatively, tendon grafts can be generated from connective tissue progenitor cells in culture and restore ankle joint extension when implanted in a mouse to bridge an Achilles tendon defect. Interestingly, these tendon grafts appear to remodel in vivo over time, showing increased strength and vascular in-growth.[9] Adipose-derived stem cells and synovial MSCs have also shown efficacy in animal models of tendon repair.[26,58] In addition to stem cells, tenocyte-like fibroblasts, readily cultured from a human skin biopsy specimen, have been shown to be safe for patient injection.[10] The authors suggest that collagen-producing cells may be used in therapies for refractory epicondylitis. Lastly, stem cells have also been isolated from skeletal muscle and the remainder of this review will focus on the application of muscle-derived stem cells.

Muscle-derived stem cells

We have isolated a population of muscle-derived stem cells (MDSCs) using a modification of a method known as the preplate technique[17,46] that uses the adhesion characteristics of the cells to collagen-coated flasks. Specific

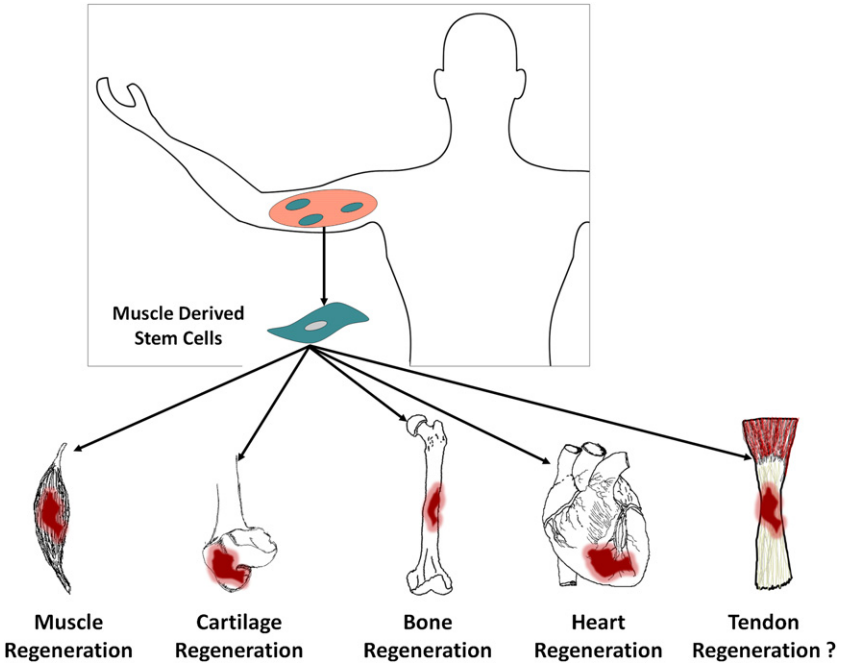

Figure 1 Schematic shows the multi-potentiality of muscle-derived stem cells and their utility for regenerative therapeutic purposes.

details of the methods and materials used for the preplate technique are reported by Gharaibeh et al.[17] Like satellite cells, MDSCs have myogenic abilities. However, MDSCs are a separate population of cells that express distinct markers and phenotypes and are superior to satellite cells in their regeneration abilities.[23] In vivo studies showed that MDSCs differentiate into multiple lineages, self-renew, and regenerate bone, cartilage, muscle, blood, and cardiac tissue[4,41,53] (Fig. 1).

RC repair using MDSCs

Our laboratory has previously shown that the injection of MDSCs into the supraspinatus tendon of athymic rats resulted in the engraftment of transplanted cells into a continuous, longitudinal pattern with a morphology comparable to resident tendon fibers.[44] The ability of MDSCs to improve tendon healing has not been fully shown thus far and remains a goal of future studies, especially when injected into a strain-injured muscle where both the tendon and muscle undergo injury.

Treatment of MDSCS to differentiate into tenocytes

Recently, we have treated MDSCs with FGF at a low dose of 3 ng/mL, similar to that of Hankemeier et al.[20] Gene expression of various matrix markers—collagen I, collagen III, fibronectin, vimentin, scleraxis, and tenomodulin—were observed at 2, 7, 14, and 21 days of continuous FGF treatment. Overall, MDSC treatment with FGF increased proliferation but showed no effect on differentiation towards tenogenic lineage at all intervals. Data were collected from various cell lines and quantitated and normalized to untreated MDSCs. MDSCs were injected into models of chronic supraspinatus and acute Achilles injury. FGF pretreatment does seem to improve engraftment of MDSCs in a model of acute collagenase injury (Fig. 2). Cell engraftment is limited at the injection site. A low percentage of donor cells should not detract from their potential paracrine activity.[16] Factors secreted by the injected cells may play an important role in the regeneration process.

Discussion

Augmentation of RC repair by growth factors and stem cells is intended to enhance the repair process, improve the mechanical force of the tendon, and reduce the rates of rerupture. This review has focused on research that investigated the influence of individual growth factors or stem cells from a single source to augment tendon healing and RC repair. Overall, data presented by several investigators indicate only a modest improvement in structure and biomechanics of tendon in RC repair after therapies with growth factors or stem cells. These therapies individually have yet to prove the ability to regenerate native tissue as seen histologically or rival uninjured tissue biomechanically. Combination therapies using several growth factors and different populations of stem cells may

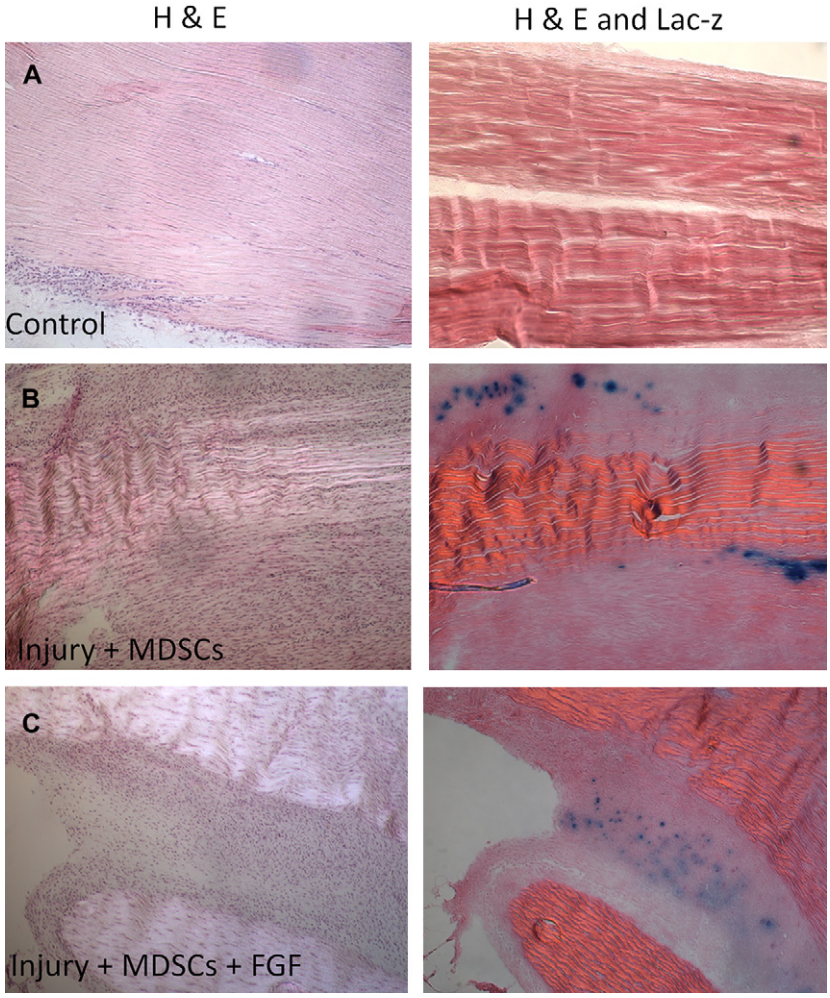

Figure 2 Engraftment of muscle-derived stem cells (*MDSCs*) in collagenase-induced tendon injury model. All tissues were harvested 1 week after cell injection. Injury was performed by injecting 12.5 U of collagenase (Sigma-Aldrich; Catalog no. C5894) into Achilles tendon. The MDSC injections were performed 3 days after injury, and cells were injected in the injury site. **(A)** Control tendon with no collagenase injury and no MDSC injection. **(B)** Injured tendon injected with 3.0×10^5 murine MDSCs transduced with Lac-z. **(C)** Injured tendon injected with 3.0×10^5 murine MDSCs transduced with Lac-z after being pretreated with basic fibroblast growth factor (*FGF;* 3 ng/mL for 2 days). Images are shown at original magnification ×100. *H & E,* hematoxylin and eosin.

prove to be more effective for RC therapy. In addition, a caveat of augmentation thus far has been the overproduction of scar tissue at the injury site.[19] There is also some concern for heterotopic ossification with stem cell therapy.[2]

Tissue-engineering strategies that use stem cells to express growth factors via ex vivo gene therapy are currently being investigated. It is possible that ''combined therapies,'' using stem cells expressing one or more growth factors may improve the outcome of RC repair by recruiting host cells via paracrine signaling and modulating the healing process toward formation of more normal tissue while reducing scar tissue. More work is needed to determine the optimal combinations, timing, and dosing of growth factors. In addition, more studies that compare stem cells from different compartments, including the recently discovered tendon stem cells,[3,63] are needed to determine which stem cell population has the greatest ability to influence tendon repair.

Combining growth factor treatment with BM aspirate during arthroscopic RC procedure was shown to enhance tendon-like phenotype in the injury site.[36] BMCs treated with growth factors or platelet-rich plasma could also become a potential a standard treatment for RC injury.[64]

In our experience using MDSCs, we found that most cells at the injury sites were host-derived, but the exact source of these host cells is not fully elucidated. The transplanted cells improve the function of injured organs by mobilizing the host's repair cells to the injury site by releasing certain factors (eg, VEGF) that indirectly help in the repair process.[16,41,43] This paracrine effect is not necessarily localized at the injury site, and cell recruitment

can be from other parts of the body and through the blood stream. This observation has also been reported by others using a variety of different stem cell types in different tissues.[16]

Conclusions

Few studies exist documenting the role of stem cells, MDSCs in particular, in tendon tissue engineering; however, our laboratory has previously shown that the injection of MDSCs into the supraspinatus tendon of athymic rats resulted in the engraftment of transplanted cells into a continuous, longitudinal pattern, with a morphology comparable to resident tendon fibers.[44] The ability of MDSCs to improve tendon healing has not been fully demonstrated thus far and remains a goal of future studies, especially when injected into a strain-injured muscle where both the tendon and muscle undergo injury. Rigorous basic science and preclinical translational studies using MDSCs need to be performed to ensure safety before such therapies become part of the standard orthopedic care.

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