Cell- and gene-based approaches to tendon regeneration

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Repair of rotator cuff tears in experimental models has been significantly improved by the use of enhanced biologic approaches, including platelet-rich plasma, bone marrow aspirate, growth factor supplements, and cell- and gene-modified cell therapy. Despite added complexity, cell-based therapies form an important part of enhanced repair, and combinations of carrier vehicles, growth factors, and implanted cells provide the best opportunity for robust repair. Bone marrow–derived mesenchymal stem cells provide a stimulus for repair in flexor tendons, but application in rotator cuff repair has not shown universally positive results. The use of scaffolds such as platelet-rich plasma, fibrin, and synthetic vehicles and the use of gene priming for stem cell differentiation and local anabolic and anti-inflammatory impact have both provided essential components for enhanced tendon and tendon-to-bone repair in rotator cuff disruption. Application of these research techniques in human rotator cuff injury has generally been limited to autologous platelet-rich plasma, fibrin, and synthetic scaffold materials. Cultured mesenchymal progenitor therapy and gene-enhanced function have not yet reached clinical trials in humans. Research in several animal species indicates that the concept of gene-primed stem cells, particularly embryonic stem cells, combined with effective culture conditions, transduction with long-term integrating vectors carrying anabolic growth factors, and development of cells conditioned by use of RNA interference gene therapy to resist matrix metalloproteinase degradation, may constitute potential advances in rotator cuff repair. This review summarizes cell- and gene-enhanced cell research for tendon repair and provides future directions for rotator cuff repair using biologic composites.

Level of evidence: Review Article.

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Rotator cuff disease is the most common upper extremity disability.41 Shoulder pain in general ranks third only to back and neck pain among musculoskeletal disorders requiring medical consultation. Similarly, the incidence of rotator cuff tear after shoulder trauma is very high. Some reports indicate that 58% of shoulder injuries have a rotator cuff component,119 and other reports from cadaveric dissections suggest a total prevalence rate in humans of approximately 30%.102 Epidemiologic reports indicate that over 4.4 million patient visits annually in the United States are driven by shoulder disorders.12,99 The complex anatomic arrangement forming the shoulder rotator cuff, combined with the need for high mobility to allow normal daily function, frequently leads to significant morbidity after injury.41 Moreover, the significant number of tendons forming the rotator cuff, as well as the often slow and inadequate healing of the tendon-bone junction, lead to difficulties in treatment choice and follow-up rehabilitation programs. This review will focus specifically on the use of cell- and gene-enhanced cell-based methods to
improve tendon function as it applies to the rotator cuff tendon group and, more broadly, to flexor tendons.

Tendon healing

Healing of the rotator cuff or flexor tendons in most locations goes through the traditional phases of an initial inflammatory response, characterized by acute inflammation, with accumulation of hemorrhage and leukocytes, local synthesis of bioactive and chemotactic factors, and the stimulation of angiogenesis. These culminate in the initiation of tenocyte proliferation, migration of tenocytes into the wound, and an increased synthesis of collagen and vascular structures to form immature fibrovascular tissue. After the 4- to 7-day inflammatory phase, the proliferative or reparative phase begins. Synthesis of type I and type III collagen is profound, and studies in flexor tendons suggest that increased formation of type III collagen continues for weeks to months after injury.27,34 The more ideal type I collagen architecture starts to predominate in the third and final phase, the remodeling phase, which commences 6 to 8 weeks after injury. This phase is characterized by a decrease in cellularity in the fibrovascular tissue and emergence of an ordered collagen fibril structure with more linear orientation, with fibril crimping and co-crimping. Vascularity remains increased until late in the remodeling phase, and the tenocyte density is similarly increased. The return of strength to the organizing tendon repair comes as the cell-to-collagen matrix begins to decline with maturation. Markers of organized tendon structure such as cartilage oligomeric matrix protein and decorin increase as the organization matures. The ultimate organized collagen architecture of the healed tendon rarely shows the pristine fiber pattern of the uninjured structure; rather, it shows a more disorganized architecture with increased randomly oriented tendon fibers and fiber cross-linking, forming scar tissue. Ultimately, this results in reduced elasticity, reduced mobility, and increased propensity for recurrence of injury.66

Treatment methods

Depending on the results of physical examination, radiographs, and magnetic resonance imaging (MRI) examination, initial therapy is often nonsurgical, including rest and modifications to shoulder action, and use of nonsteroidal anti-inflammatory drugs. This is often followed by corticosteroid injections, physical therapy, and surface-acting agents, such as extracorporeal shockwave therapy, pulsed magnetic therapy, laser phototherapy, deep ultrasound therapy, and muscle stimulation. Failures in conservative therapy often lead to surgical repair using open or arthroscopic approaches for subacromial decompression, tendon debridement, tendon suture or anchor supplementation, allograft reconstruction, injection of platelet-rich plasma (PRP) and other blood-derived biologic preparations rich in growth factors, enhanced tendon repair with patches and scaffold grafting, and more recently, the use of stem cell injections.62,110,127

Current strategies for rotator cuff repair

Primary repair of rotator cuff injury often results in inadequate strength of the repair or limited mobility. Cell implantation, growth factor injections or depot composites, and gene-enhanced cell therapy aim to improve the quality and mechanical function of rotator cuff repair. Relying on native intrinsic and extrinsic repair systems lends itself well to augmented repair with biologic scaffolds with or without the addition of growth factors.53,61,62

Growth factors

Growth factors are small peptide signaling molecules that control cell proliferation and differentiation, matrix synthesis, and local chemotactic recruitment of inflammatory and stem cells at various phases of tendon repair. All growth factors exert their effect by binding to cell surface receptors, which commences a sequence of intracellular signaling cascades that ultimately facilitate DNA transcriptional activity or DNA replication. Most growth factor effects are anabolic or stimulate cell division. However, exuberant growth factor formation or activity can occasionally be deleterious. Damaged flexor tendons including the Achilles tendon and supraspinatus insertion have been found to overexpress transforming growth factor (TGF) β1 and TGF-β2, and the abundance of these TGF-β isoforms results in fibrosis and scar tissue formation, suggesting that TGF-β overexpression may be a disadvantage in tendon healing. Indeed, antibodies targeting TGF-β in flexor tendon repairs improve functional tendon mobility. Several other anabolic growth factors, including insulin-like growth factor 1 (IGF-1) and bone morphogenetic protein (BMP) 12, result in a higher rate of collagen synthesis in various experimental models, and BMP-12 and IGF-1 have been found to improve the biomechanical integrity of flexor tendon repairs after in vivo injection. There are no reports of IGF-1 application in human flexor tendon conditions. However, clinical application of recombinant IGF-1 in flexor tendon disruption in racehorses has improved the rate of return to sustained athletic activity.138

Application of growth factor mixtures in the form of PRP provides an autologous source of useful anabolic agents. However, the increased levels of platelet-derived growth factor (PDGF) and TGF-β are not well controlled, and excessive exposure to TGF-β with the potential for exuberant fibrosis is a real possibility, depending on platelet concentration. Outcome after PRP application in rotator cuff repair, in a double-blind controlled trial, also does
not support PRP application in repair of a mildly or moderately torn rotator cuff. The use of recombinant cytokines and growth factors to enhance tendon healing provides better control over dosing. However, application of single growth factors remains largely experimental, and the technique suffers from the limited residence time of the peptide forms of growth factors. This limitation may be overcome to a large extent by the use of gene therapy to enhance the duration of growth factor presence, potentially increasing growth factor–facilitated healing from a matter of days to weeks or even months. Growth factor–enhanced rotator cuff repair has recently been reviewed\(^{92}\) and is covered elsewhere in this symposium. Growth factor gene delivery by use of stem cell vehicles will be discussed in more detail later.

**Mesenchymal stem cells**

**Definition**

The study of adult marrow-derived stem cells commenced over 40 years ago, and the application of cultured stromal cells in musculoskeletal repair has accelerated in the past decade.\(^{39,139}\) Early studies in rodents and humans identified a population of clonogenic marrow stromal cells, termed colony-forming unit fibroblasts, that were considered to be the precursor cell population for all connective tissues.\(^{35}\) Later studies confirmed that stromal populations are derived from multipotent bone marrow stromal cells or subsets of these, which have been variously referred to as bone marrow stromal stem cells, mesenchymal stem cells (MSCs)/marrow stromal cells,\(^{16}\) multipotent adult progenitor cells, or mesenchymal adult stem cells. “Mesenchymal stem cells” or “bone marrow stromal cells” seem to be the terms currently favored when referring to these marrow-derived stem cells. MSCs are capable of undergoing differentiation into a variety of specialized musculoskeletal tissues, including tendon, bone, cartilage, ligament, and fat (Fig. 1).\(^{15,16,108}\) Although the numbers of MSCs decline with age, bone marrow still provides a ready source of cells for culture propagation, differentiation, and implantation. In addition, MSCs have also been derived from fat, dermis, dental pulp, periosteum, synovium, tendons, umbilical cord blood, muscle, and blood. However, differences in culture morphology, growth rates, proliferation potential, and differentiation capacity have been described for the various sources of MSC populations. Nevertheless, stem cells from different tissues display many characteristics common to their bone marrow counterparts, suggesting that MSC-like populations share a similar ontogeny. The perivascular niche containing the pericyte is thought to be the most likely source of these stem cells from various tissues.\(^{25}\)

**Isolation and propagation**

Techniques for isolation of MSCs vary, but most include adherence to plastic, with preliminary cell separation techniques, including density gradients (Ficoll, Percoll, dextran, and sucrose), or more sophisticated approaches using fluorescence- or magnetic-activated cell sorting, based on antibody reaction to cell surface markers.\(^{29}\) No specific single cell surface marker defines an MSC, so several features are combined to delineate an MSC, including a high proliferative potential, ability to generate primary cell colonies (colony-forming unit fibroblasts), and the ability to differentiate into bone, fat, and cartilage. Given that different laboratories have differing culture techniques and use MSCs from different sources, there has been little consensus on the markers that are critical to establish a defined MSC population. The Mesenchymal and Tissue Stem Cell Committee within the International Society for Cellular Therapy has attempted to formalize these criteria and proposed that an MSC population has (1) plastic adherence of the isolated cells in culture; (2) positive expression of CD105, CD90, and CD73 in greater than 95% of cells in the culture, while negative for markers of other marrow populations including CD34, CD45, CD14 or CD11b, CD79a or CD19, and HLA-DR; and (3) the capability to differentiate into osteoblasts, adipocytes, and chondrocytes in vitro.\(^{32}\) Other markers may be useful, including Stro-1, LGNF-R, CD29, CD13, and CD166. However, it is clear that despite positive expression of these constitutive markers, many MSC cultures are not phenotypically homogeneous. Worse still, markers that are used to identify and better isolate MSCs for culture are rapidly lost in subsequent passages.\(^{122}\) Better characterization through genomic and proteomic profiling has helped identify true pluripotent stem cells and those with inherent lineage priming.\(^{78,101,105}\)

**Cell sources for rotator cuff repair**

The principal source of cells for stem cell–enhanced healing of the rotator cuff has been autologous bone marrow.\(^{44,46,65,69,75,127}\) Culture techniques and conditions for propagation of bone marrow–derived stem cells have been well defined.\(^{29}\) Mechanisms to increase the proportion of stem cells isolated from bone marrow aspirates have included density gradient centrifugation through Ficoll columns, separation cell sorting using CD105-positive/CD45-negative cells, and more complex multistage cell sorting processes using additional CD markers in magnetic or flow cytometric applications.\(^{59}\) To be clinically successful, patient-side preparation of stem cell–enriched mixtures from autologous bone marrow will be required, and these can be purified in the operating room by density gradient centrifugation or simple CD antibody sorting through magnetic columns.\(^{29,59,75}\)

Other autologous cell sources, including adipose tissue, muscle, synovium, periosteum, tendon-derived stem cells, dermal fibroblasts, and stem cells derived from umbilical cord or peripheral blood, have all been evaluated as sources of multipotent and pluripotent cells.\(^{19,97,120,129,141,144}\) Adipose-derived stem cells are relatively easy to harvest, and selection of adherent cells over the course of several
weeks in monolayer culture provides a cell type that improves the healing potential of flexor tendons. Similarly, stem cells derived from skeletal muscle have been used in a variety of musculoskeletal repair indications and are readily harvested and propagated before reimplantation. The principal focus of muscle-derived stem cells has been implantation of genetically engineered cells for the treatment of muscular dystrophy. However, there have been other applications, including injection of muscle-derived stem cells into the supraspinatus tendon/rotator cuff of rats. Muscle-derived stem cells were labeled with the lacZ gene and transduced cells enriched in culture by selective antibiotic resistance. Injected tendons contained abundant transplanted cells at harvest 1 week later. Implanted cells had adopted a tenocyte-like morphology and were surrounded by an organized collagen matrix.

Separating the vascular/stromal fraction of the cells in adipose tissue, by use of an overnight enzymatic digestion, minimizes the delay from harvest to application of autologous adipose tissue. The isolated cells are separated from lipid by centrifugation and are available for injection into tendinous defects within 24 hours of harvest. Injection of the vascular-stromal cell fraction in an equine collagenase-induced tendonitis model indicated that the cell injections improved the histologic characteristics and minimized inflammatory reaction in the treated tendon 6 weeks after cell injection (Fig. 2). Like many other sources of adult MSCs, the stem cells from adipose tissue are thought to be derived from the pericyte, the stem cell associated with blood vessels in fat. A direct correlation of MSC harvest yield and blood vessel density has been identified in equine adipose tissue.

**MSC effects in musculoskeletal tissues**

Constitutive multipotent capabilities, synthesis of anabolic and antiapoptotic paracrine factors, and inherent immunomodulatory properties of MSCs make them ideal stem cells for tissue-engineering and regenerative purposes. Application of MSCs for bone repair is well established, both in osteoporosis and in delayed union/nonunion. Use in cartilage and tendon regeneration is not as well supported by results in the literature. Recent studies indicate that regeneration of any 3-dimensional tissue is a complex process, requiring much more than just supplementing precursor cells. Many elements are required to coordinate the generation of a functional tertiary
structure in orthopedic systems, including a vehicle to support cells, stimulatory and coordinating paracrine factors, malleability to change during tissue regeneration, and a sense of volume limitation. Many of these factors are present in fracture repair but less robust in cartilage regeneration, where oxygen tension plays a vital part in chondrogenesis without bony overgrowth.81 However, they are not well developed in tendon laceration or strain injury, where the local environment is often inundated with fibrovascular and later disorganized fibrous tissue. Stem cell therapy in tendon repair likely has more of a role in coordinating regeneration rather than supplementing cell numbers to bridge the void in tendon architecture.

MSC effects in tendon
Implantation of autologous MSCs has improved flexor tendon repair in most studies (Table I).32,69,112 Bone marrow–derived MSCs implanted in a fibrin vehicle improved the histologic and mechanical characteristics of Achilles repair in rabbits,24 and MSCs injected at the site of Achilles reinsertion to bone also improved morphologic and biomechanical properties.89 Other studies in rats suggest that uncultured bone marrow cells may be better than MSCs in the Achilles rupture model.91 Bone marrow–derived MSCs have improved the healing of tendonitis lesions in the equine collagenase model.109 Clinical application of cultured bone marrow–derived MSCs in clinical tendonitis in racehorses has also resulted in improved return to athletic activity in long-term studies.2,40 These data suggest real benefit to the implantation of cultured autologous MSCs. Implanted cell survival over the long term is known to be low, however, and the majority of cells are lost from active participation in stem cell tenogenesis and tendon regeneration.43,108

Figure 2  Histologic appearance of superficial digital flexor tendon from horses injected with adipose-derived vascular-stromal nucleated cell fraction (hematoxylin-eosin [H&E] stain; original magnification x25). Fat was digested overnight and nucleated cells retrieved by centrifugation before implantation. The more normal histologic appearance under bright-field and polarized illumination shows that the adipose-derived nucleated cell fraction (ADNC) improved healing compared with placebo-injected control (phosphate-buffered saline solution [PBS]). Examination under polarized light shows the improved ordered crimp pattern in ADNC-injected tendon. Bars = 100 microns. (Reprinted with permission.87)

Application of MSCs in biologic matrices generally improves retention of cells at target sites and may improve tendon repair.3,73,107 A combination of MSCs in collagen, fibrin, or PRP and the effect of local mechanical forces simulates the natural healing environment of the tendon and may provide a more robust repair.55 This becomes vital in massive rotator cuff repair.86 Similar enhancement of tendon or tendon-to-bone repair has been reported with MSCs derived from other tissues, including synovium and dermis.55,67

One of the consistent findings in the use of bone marrow–derived MSCs and adipose-derived vascular-stromal fraction cells in the equine model has been their anti-inflammatory impact. This has resulted in reduced tendon fiber degeneration after the cell injection compared with vehicle-injected controls.87,109

It has become increasingly apparent that the primary role of implanted MSCs in tendon repair, and possibly in most applications for connective tissue regeneration, is in their indirect effects on tissue homeostasis. These include trophic anabolic effects through paracrine and autocrine activity, direct anti-inflammatory effects of the cells, chemotraction of additional stem cells from surrounding tendon, and a significant anti-apoptotic effect.1,17,77 Many of these effects diminish the ongoing degradation associated with tendon fiber rupture, whether it be through
induction of tendonitis in experimental models or the insidious degeneration thought to precede most clinically apparent tendon injuries.\textsuperscript{15,17,131}

Cultured bone marrow–derived stem cells have strong anti-inflammatory impact.\textsuperscript{1,8,77} Several mechanisms compound the anti-inflammatory effects of stem cells in tendon repair, including upregulation of chemokines,\textsuperscript{71} suppression of cytokine secretion from dendritic cells, and reduced naive and effector T cells (T helper 1 and T helper 2 cells) and natural killer cells, all resulting in a potent anti-inflammatory or immune-tolerant cell phenotype.\textsuperscript{1,115,117} Given these immunomodulatory effects, it should come as no surprise that active clinical trials are under way for the systemic administration of allogeneic MSCs for the control of graft-versus-host disease, a generally fatal condition after organ transplantation.\textsuperscript{39,60,126,139}

Despite the abundance of literature supporting the use of bone marrow–derived stromal cells for tendon repair (Table I), their effectiveness in experimental models of rotator cuff injuries has been less rigorously evaluated.\textsuperscript{44} In the few experimental studies assessing the impact of cultured MSCs on rotator cuff tears, tissue histology and mechanical integrity were not improved. The potential for better repair may lie in scaffolds to secure cells and improve exposure to local cues to drive organized tenogenesis. Cell dose and vehicle are also far from established in most MSC applications, and these may be vital parameters in stem cell–based tendon repairs.

Simple autologous products such as bone marrow aspirate or PRP provide composite biologics that seem to enhance repair. Clinical trials of the local application of bone marrow aspirate derived from the proximal humerus at the time of arthroscopic rotator cuff surgery have yielded positive results.\textsuperscript{75} Bone marrow was aspirated from the proximal humerus anchor holes during arthroscopic rotator cuff surgery and stem cells isolated by several protocols performed directly in the operating room, including centrifugation over a sucrose gradient or a Histopaque gradient (Accuspin System Histopaque-1077; Sigma-Aldrich Corp., St. Louis, MO, USA) and selective draw-off of the MSC-rich layer. The extraction of progenitor cells appeared similar in both simplified techniques and did not increase patient

### Table I Summary of cell-based tendon repair in experimental models

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Vehicle</th>
<th>Type of study</th>
<th>Model</th>
<th>Reference</th>
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<td>BM-MSCs</td>
<td>Decellularized tendon</td>
<td>In vitro</td>
<td>Dog</td>
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<tr>
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<td>Fibroblasts</td>
<td>Chitosan-HA hybrid</td>
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<td>PEGDA with PEG–BMP-2 hydrogel</td>
<td>In vivo</td>
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<td>BM-MSCs</td>
<td>Collagen gel</td>
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<tr>
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<td>Collagen gel</td>
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<td>BM-MSCs</td>
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<td>Horse</td>
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</table>

\textit{SFD} indicates superficial digital flexor tendon; long DF, long digital flexor tendon; BM-MSC, bone marrow-derived mesenchymal stem cells; syn-MSC, synovial-derived mesenchymal stem cells; HA, hyaluronic acid; PEGDA, polyethylene glycol; MEM, minimal essential medium; DMEM, Dulbecco’s minimal essential medium; PGA, polyglycolic acid; SIS, small intestinal submucosa.
morbidity. Outcome data after reinjection of these cells have not been reported. However, an uncontrolled patient study using intraoperative Ficoll (Ficoll-Paque; GE Healthcare, Piscataway, NJ, USA) centrifugation of bone marrow aspirate from the iliac crest and reinjection of the autologous mononuclear cell fraction into the sutured repair in 14 patients with complete rotator cuff tears showed improved outcome compared with previous experiences with suture repair alone.33 Tendon integrity on MRI after 12 months was good in all patients, and only 1 of 14 had reinjury in the long-term analysis. However, the lack of any randomized assignment to a concurrent suture repair–only group limits the strength of the study.33

Stem cell homing and local differentiation

Cell labeling and tracking studies indicate that implanted bone marrow–derived MSCs remain at the injected tendon-to-bone tunnel region for as long as 8 weeks after surgery.65 Super-paramagnetic iron oxide and fluorescent lipophilic carbocyanine DiI (diododecyl tetracyanobiphenyl cyanine)-labeled cells persisted at the injection site in the rotator cuff and resulted in improved mechanical pullout strength at 4 and 8 weeks after surgery.65 Other studies show that the conventional rotator cuff repair can be bolstered by the addition of separated bone marrow mononuclear autologous stem cells.35 Intraoperative aspiration of bone marrow from the iliac crest immediately before arthroscopic repair of the rotator cuff tear allowed the marrow to be separated by Ficoll-Hypaque (Ficoll-Paque Plus; GE Healthcare) density gradient centrifugation and the mononuclear cell layer to be resuspended in autologous serum for injection. The results in a small clinical trial suggested improved mobility scores, and 100% of the patients had tendon integrity re-established, based on follow-up MRI examinations. This procedure supplements rotator cuff sutures and may possibly induce improved healing compared with suture repair alone.

Stem cells are known to be mobilized after injury and are increased in circulation,106 which leads to the concept of homing to sites of tissue damage within the body.5,68 Once there, the abundant local cues, predominantly growth factors and associated signaling peptides, participate in MSC differentiation and contribution to local repair. Culture additives and gene transduction techniques have been used to channel MSCs down a phenotypic pathway in vitro, and this has been used on numerous occasions to “prime” MSCs toward a specific lineage before implantation.30 This has not always been successful. For muscle, the master regulatory gene appears to be the muscle transcription factor MyoD, whereas in cartilage, Sox9 appears to be a key transcription factor controlling differentiation of stem cells toward chondrocytes.9,81 MSC differentiation down tenocyte lineages appears to lack a master controller, although scleraxis has occasionally been considered to function in that capacity.81 However, the transcriptional profile of naïve MSCs, particularly the abundant expression of type I collagen, lends itself better to tendon and ligament formation than it does to muscle and cartilage.

Gene-enhanced stem cell combinations

Increasing numbers of experimental studies describe improved outcome after use of a combination of stem cells and integrated genes to foster stem cell function in the regenerating tendon (Table II).79,98 The principal growth factors evaluated include BMP-12, -13, and -14; PDGF-B; basic fibroblast growth factor (bFGF); IGF-1; and vascular endothelial growth factor (VEGF). Delivery of the appropriate transgene to the transplantable stem cell has predominantly used viral vectors, including adenovirus, adeno-associated virus (AAV), lentivirus, and retrovirus. Direct in vivo transfer by injection of naked DNA, by electroporation in situ, or by intra-articular injection also resulted in effective, albeit transient, gene expression.54,96 Proof-of-principle studies generally relied on transfection using adenovirus, which provides high transduction efficiency, but limited duration of transgene expression, with detectable messenger RNA levels often for only 2 to 4 weeks. Adenoviral vectors also stimulate a significant immune response, which shuts down expression and prevents re-dosing. Use of the AAV vector provides several months of transgene expression and a significantly reduced immune response to the vector itself. Studies of growth factor gene therapy for flexor tendon repair included use of basic fibroblast growth factor, which enhanced tendon healing without additional adhesions in a chicken model123 and stimulated substantial type I collagen expression during in vitro culture periods.135 Similarly, PDGF-B resulted in improved type I collagen formation in vitro134 and improved angiogenesis and collagen deposition after direct injection of PDGF-B complementary DNA in an HVJ-liposome suspension to the patellar tendon of rats.85 A major limitation in PDGF therapy for tendon repair has been a concurrent downregulation of other anabolic growth factors such as IGF-1 and TGF-β.50 Exposure of MSCs and tendon cells to AAV overexpressing VEGF consistently drives up expression of TGF-β isoforms133 but does little to enhance collagen synthesis. Assessment of the tendon response after implantation of stem cells transduced with adenovirus carrying growth and differentiation factor 5, using a transected Achilles tendonitis model in rats, showed a significantly thicker repair site with minor improvement in tensile strength.104 Similarly, local administration of adenovirus overexpressing BMP-14 in transected rat Achilles tendons stimulated an increase in both tenocyte proliferation and repair-site tensile strength.10

MSCs have improved healing in equine tendonitis models.109 Better structural repair was attained by use of gene-enhanced MSCs overexpressing IGF-1 in this same model. Type I collagen was more abundant in the healing tissue (Fig. 3), markers of mature tendon tissue such as cartilage oligomeric matrix protein were increased, and
tenocyte morphology, inflammatory cell numbers, and tendon fibril crimping and co-crimping were all improved in adenovirus–IGF-1–transduced MSCs. The 8-week study found gene-enhanced MSCs to be superior to naive MSCs, with tendon architecture more like normal tendon, and improved biomechanical capabilities (Fig. 4). Combinations of growth factors such as PDGF-B– and IGF-1–transduced fibroblasts combined in synthetic scaffolds improved tenocyte proliferation and collagen formation in vitro and improved histologic repair in the rat rotator cuff model.31,128

<table>
<thead>
<tr>
<th>Table II</th>
<th>Summary of gene therapy or gene tracking studies in experimental tendon models</th>
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<td>Achilles</td>
<td>BMP-14</td>
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<td>Flexor tendon (LDF)</td>
<td>Luciferase</td>
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<td>IGF-1</td>
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<td>Flexor tendons (DDF)</td>
<td>bFGF</td>
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<td>Supraspinatus</td>
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<td>Supraspinatus</td>
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<td>PDGF</td>
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<td>Tenocytes</td>
<td>BMP-12</td>
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*bFGF*, basic fibroblast growth factor; *GDF-5*, growth and differentiation factor 5; *VEGF*, vascular endothelial growth factor; *Semitend*, semitendinosus tendon; *DDF*, deep digital flexor tendon; *LDF*, long digital flexor tendon; *SDF*, superficial digital flexor tendon; *Retro*, retrovirus; *Adeno*, adenovirus; *Inj*, injection.
Various genes known to impact tenogenesis are recognized as markers of tendon formation and have been evaluated in an effort to enhance MSC differentiation to tenocytes. These include scleraxis, which has been shown to improve early rotator cuff healing in a rat model.\textsuperscript{46,48} Bone marrow–derived MSCs were transduced with scleraxis, using an adenovirus vector, which effectively transduced cells at high efficiency, before implantation in a supraspinatus laceration model. Fibrin was used as the carrier, and evaluation up to 4 weeks after implantation indicated higher ultimate load to failure and stiffness in the groups implanted with MSCs overexpressing scleraxis. In addition, more fibrocartilage and higher ultimate stress to failure were evident 4 weeks after MSC-Scx implantation. These short-term studies suggest that scleraxis may play an important role in enhanced early regeneration of the tendon-to-bone transition in the tendon detachment model.\textsuperscript{44}

Previous studies using MSCs labeled with adenovirus overexpressing the lacZ gene indicated that MSCs populated the tendon damage region in the supraspinatus detachment model in rats. However, they did not contribute to increased or appropriate transition zone development including new fibrocartilage formation or collagen fiber organization 2 or 4 weeks after MSC implantation.\textsuperscript{44} Accordingly, there was no difference in the biomechanical strength of the repair, the cross-sectional area, or the peak stress to failure.

The addition of MSCs overexpressing BMPs has also been evaluated. The addition of stem cells overexpressing BMP-12 has been shown to improve tenocyte differentiation and tendon repair.\textsuperscript{64,130,132} Achilles tendon repair and mechanical strength were improved after adenovirus–BMP-12...
transduction of muscle cell bridging overlays in a rat transplanted tendon model. More recent studies in the rat rotator cuff model indicate that MSCs overexpressing BMP-13 do not improve the mechanical or morphologic aspects of healing at 2 or 4 weeks after implantation. Previous studies in rabbit flexor tendons suggested that adenovirus–BMP-13 injection resulted in transgene persistence, although higher-dose adenovirus particle injections elicited substantial inflammatory reaction in the tendon and surrounding tissue. Likewise, adenovirus–BMP-14 increased tensile strength in treated Achilles injuries in the rat. These data suggest that studies of flexor tendon repair do not necessarily translate to repair in the fibrocartilaginous transition zone from tendon-to-bone insertion. However, studies involving periosteal progenitor cells admixed with BMP-2 in a slow-release vehicle found enhanced rotator cuff tendon–bone tunnel healing in rabbits examined at 4 to 8 weeks. The morphologic characteristics of the healing tendon-to-bone interface were improved. Fibrocartilage formation and bone formation appeared structurally more organized than controls. Tendon–to–bone tunnel techniques for reinsertion of rotator cuff and Achilles tendons also facilitates gene transfer by containing local deposition of vector and transgene. A combination of polyethylene glycol as a vehicle for transduction of muscle cell bridging overlays in a rat transplanted tendon model. No containing anabolic transgenes, seems to be an appropriate technique for enhanced rotator cuff repair. Combination of tissue-engineering techniques

Clearly, the challenges of flexor tendon injury and rotator cuff tears in humans need aggressive supplemental therapy. Combination of stem cells, either primed by stem cell exposure to recombinant growth factors or containing anabolic transgenes, seems to be an appropriate technique for enhanced rotator cuff repair. No results of clinical trials in humans have been published.

Scaffold-based carriers for cell repair

Given the variable results associated with direct MSC injection in rotator cuff repair, a growing trend for a combination of repair systems, including the use of single or double rows of suture anchors, supplemented by cell therapy and other biologics, potentially retained in collagen-based or synthetic scaffolds, has emerged as a possible treatment. A combination tissue-engineered approach to develop a functional supplement to the suture-repaired rotator cuff tendons has been coined “functional tissue engineering.” This relies on the normal constituents for a tissue-engineered construct such as an appropriate scaffold material, seeded with stem cells and potentially laden with growth factors or other bioactive peptides, to stimulate a robust repair. The role of scaffolds for augmented rotator cuff repair is covered elsewhere in this special issue (see Ricchetti et al, pp 251-265).

Fetal-derived embryonic stem cells

Fetal-derived embryonic stem cell (ESC)–like cells have recently been evaluated for tendon and ligament repair. Early-stage fetal tissue has been used to develop an ESC-like cell line that expresses all the markers of ESCs but without the teratogenic potential, providing a better safety profile. Evaluation of these ESCs in a new equine enzymatic/physical defect tendonitis model indicates advantages to the use of ESCs compared with MSCs. Culture of fetal-derived cells uses similar culture medium additives
to ESC culture, and cell proliferation continues without apparent senescence. These cells express the typical ESC markers, including nanog, SSEA 4, Tra-1-81, Tra-1-60, and Oct-4, and lack expression of P53 and major histo-compatability complex (MHC) antigenic motifs. In addition, they express other pluripotency markers such as telomerase. Direct implantation of these fetal-derived ESCs into experimental tendonitis lesions, developed by use of a combination of collagenase gel and a needle inserted parallel to the long axis of the tendon, resulted in improved ultrasonographic echodensity within 4 weeks of injection, and at tissue harvest 8 weeks after ESC injection, the ultrasonographic, MRI, and histologic scores and gene expression profile indicated improved healing compared with placebo-injected controls (Fig. 5). These data indicated, for the first time, a sequential improvement in healing parameters within weeks of cell injection compared with studies in similar equine models where MSCs and adipose-derived vascular-stromal cells were injected. Use of ESCs appears to add a new option for tissue healing. Sex-determining region Y (SRY) genetically labeled ESCs were identified in the healing tendonitis lesions 8 weeks after implantation (Fig. 6). In addition, these cells are readily available as cultured cell lines, induce no apparent rejection phenomenon, and can be stored frozen and ready for use as clinical cases are recruited. There are no published data documenting the outcome in clinical trials in animals, but anecdotal data after ESC injection of flexor tendon injury in several hundred horses used in a variety of athletic pursuits are very supportive of future application in humans.

**Induced pluripotent stem cells**

Induced pluripotent stem (iPS) cells, developed by genetically reprogramming adult-sourced cells, may be particularly beneficial in the challenging environment of rotator cuff injury. iPS cells have a bolder indication in regenerative therapies for conditions that today are not possible to cure, including spinal cord injury, diabetes, and neurodegenerative disorders. However, given the benefits of fetal-derived ESCs, as well as the concurrent social and moral dilemma regarding harvesting of embryonal or fetal tissues, iPS cells potentially solve many current concerns in stem cell therapy for nonfatal disease such as musculoskeletal injury. Like fetal-derived ESC lines for tendon repair, iPS cells can be developed as allogeneic cell lines for immediate distribution and use. iPS cells have been established in an increasing number of species, including humans. Large animals such as the horse, which comprises a major subset of experimental models of tendon disease and repair, have also recently seen the development of iPS lines. The horse has several well-known models of tendon injury, and iPS cell therapy holds considerable promise as the adult-derived version of fetal-derived ESC-like cells.

Generation of iPS cells can use viral or, more recently, nonviral vector delivery of reprogramming genes. The equine iPS cell line was developed by use of a piggyBac transposon–based method to deliver transgenes containing the reprogramming factors Oct4, Sox2, Klf4, and c-Myc, expressed in a temporally regulated fashion. The established iPS cell lines express hallmark pluripotency markers, display a stable karyotype even during long-term culture, and readily form complex teratomas containing all 3 embryonic germ layer–derived tissues upon in vivo grafting into immunocompromised mice. For clinical application in musculoskeletal disorders, lineage differentiation through gene priming should eliminate the risk of teratoma formation. However, this field is still evolving and faces significant obstacles regarding the safety of oncogenic genes vital in the development of iPS cells, as well as the issue of incipient teratoma emergence in the iPS cell lines. Lastly, incomplete reprogramming in some iPS lines, including the equine iPS cells, is evident through the necessity to maintain doxycycline in the culture medium to drive the tet-on switch of reprogramming gene expression. Without constant expression of these genes, the iPS cells differentiate, indicating incomplete pluripotency.
Endothelial progenitor cells

Endothelial progenitor cells (EPCs) are abundant in circulation, mostly originate from bone marrow sources, and play an important role in populating the stem cell niche in vascular and parenchymatous organs.\textsuperscript{125} Cultured EPCs represent an adult-derived stem cell source, isolated from marrow or the circulation, that has been applied in retinal degeneration, and peripheral and cardiac vascular disease.\textsuperscript{100,125} They also may play a role in musculoskeletal repair.\textsuperscript{88,143} In vitro culture of PRP from horses identified EPC-derived neovascular structures that formed short vascular segments. Previous studies indicate that EPCs may have a therapeutic role in bone repair\textsuperscript{143}; however, chronically damaged tendons may also benefit from the drive to revascularization.\textsuperscript{88}

Future directions

Repair of Achilles tendon rupture and rotator cuff tears in experimental models has been significantly improved by the use of enhanced biologic approaches. Most evidence indicates that cultured bone marrow–derived MSCs will not be particularly useful as a standalone supplement to debridement and suture repair of the affected tendon at the tendon-bone interface. The use of a scaffold as a carrier vehicle, as well as the use of stem cells as transport vectors for local anabolic and anti-inflammatory impact, seems to have more potential. Supplementation of the scaffold in a tissue-engineering concept may also be useful to contain recombinant growth factors and stem cell grafts to the area of damaged rotator cuff tendons. It seems that the concept of gene-primed stem cells, particularly ESCs, combined with effective culture conditions, long-term integrating vectors carrying anabolic growth factors, and development of cells conditioned by RNA interference gene therapy to resist MMP degradation, may constitute potential advances in rotator cuff repair.

The concept that MSCs contribute significantly to the pool of tenocytes engaged in active tendon regeneration seems to be increasingly unlikely as further studies of cell tracking and persistence emerge. Conversely, the newly recognized anti-inflammatory and antiapoptotic impact of MSCs on tissue healing may provide more profound potential for functional restoration. Growth factor supplementation, in the form of integrating transgenes, may allow longer-term tendon repair and potentially permanent return of function. Integrating nonviral vectors, such as Sleeping Beauty and piggyBac, are emerging as suitable vectors for stem cell transduction. The advantages are long-term gene expression in the poorly healing environment of the rotator cuff. The disadvantage of transposon/transposase-based systems has been relatively poor efficiency of cell transduction. This has been overcome to some extent by the use of hyperactive transposases (SB100X)\textsuperscript{74} that may improve the ability of Sleeping Beauty to integrate its cargo to the host cell genome.
Ultimately, successful surgical application will require a balance between patient-side simplicity and long-term efficacy. The use of complex gene-enhanced stem cell therapies will only reach clinical practice if there are significant tissue healing advantages. Further research and controlled studies will be required as a follow-up to the experimental evidence of enhanced healing using a combination of stem cells, gene therapy, and formation of a minimally hostile environment through cytokine and degradatory enzyme suppression.

**Disclaimer**

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