PM R 7 (2015) S33-S40

www.pmrjournal.org

Regenerative Medicine

# Where Do Injectable Stem Cell Treatments Apply in Treatment of Muscle, Tendon, and Ligament Injuries?

**Kenneth Mautner, MD, Joseph Blazuk, MD**

**Abstract**

Treatment options for muscle, tendon, and ligament injuries span a constantly evolving spectrum. For years, treatments focused on symptomatic relief. Closer scrutiny of symptomatic treatment suggests that the provision of transient relief of symptoms may have caused more harm than good. Cortisone injections provide a trade-off of short-term relief for poorer long-term outcomes. When conventional treatment failed, patients have faced limited options including surgery, which has increased risk and limited efficacy. Regenerative injections offer a more robust option for soft tissue disease. Basic science and clinical studies show conflicting results to support the use of platelet-rich plasma injections for soft tissue disorders, and even fewer trials have focused on injectable stem cells with limited findings. Additional studies are needed to determine the potential benefits of this regenerative therapy.

## Introduction

The algorithm for treatment of muscle, tendon, and ligament injuries continues to evolve. At its core, the pathology of these injuries boils down to an increase in physiologic demand that overwhelms structural integrity. Traditional treatment paradigms focus on trials of symptomatic management with a reduction in the offending activity [1]. As musculoskeletal tissue injuries become chronic, the effectiveness of current therapies wanes. Previously, many patients were left with limited options beyond coping with persistent pain and reduced function or a possible surgery with modest outcomes.

Advances in our understanding of the pathophysiology of tendon, muscle, and ligament injuries, together with evolving research in regenerative therapies, may alter our treatment algorithm. The role of orthobiologics in the treatment of musculoskeletal tissue disease could potentially fill a void in the spectrum of treatment options. Currently, however, data are conflicting regarding the use of platelet-rich plasma (PRP) injections to treat soft tissue disorders, and no stem cell technologies have been approved by the Food and Drug Administration (FDA) for the treatment of degenerative musculoskeletal conditions [2]. It is clear that further studies are needed to explore the effectiveness of stem cell therapy and its role as the next generation of orthobiologics.

## Injection Therapies for Soft Tissue Injury

Current injection therapy options for soft tissue injuries include corticosteroids, prolotherapy, percutaneous needle tenotomy, PRP, and variations on injectable stem cells.

### *Corticosteroids*

Corticosteroids have historically been the most widely used injection therapy for muscle, tendon, and ligament injury [3]. Although they provide some short-term analgesia, corticosteroids may inhibit collagen synthesis and decrease tendon strength [4]. A systematic review of more than 40 randomized, controlled trials for corticosteroid injections published in *Lancet* in 2010 noted that these injections provide short-term pain relief but that these effects were reversed at intermediate and long terms. It was also noted that patients treated with corticosteroid injections had worse outcomes than did persons who did not receive an injection [5]. In a placebo-controlled study published in *JAMA* in 2013, 165 patients with at least a 6-week

1934-1482/$ - see front matter 
http://dx.doi.org/10.1016/j.pmrj.2014.12.012

history of lateral epicondylosis were randomized into 4 groups—2 involving corticosteroid injection, with or without physical therapy and 2 involving placebo injection, with or without physical therapy. Participants who received a corticosteroid injection had worse clinical outcomes and higher rates of recurrence at 1 year compared with participants who received a placebo [6]. One randomized controlled trial and one cohort study examining the effects of corticosteroid injections on Achilles tendonitis showed no benefit compared with placebo with regard to cure rate or healing times. Furthermore, a review of animal studies has demonstrated that these injections temporarily weaken the tendon if given intratendinously but have no effect on tendon strength if injected into the paratenon [7]. At best, corticosteroid injections provide short-term relief for soft tissue injuries. At worst, they lead to lower functional outcomes and higher recurrence of disease.

### *Prolotherapy*

The use of prolotherapy dates to the 1930s [8], when it was developed for pain associated with presumed ligament laxity. Sclerosing agents such as hyperosmolar dextrose are the most popular and best-studied prolotherapy agents [9]. The theoretical basis of their efficacy is that irritant solutions along with needling of soft tissues stimulate a low-grade inflammatory reaction that initiates a healing cascade of the injured soft tissues. Multiple uncontrolled studies and case series have demonstrated effectiveness of prolotherapy in the treatment of musculoskeletal pain, including low back [10,11] , neck, and whiplash injuries [12]; chronic sprains and/or strains; tennis and golfer's elbow [13]; plantar fasciitis [14]; knee [15], ankle, and shoulder pain; and chronic tendinosis, including Achilles tendinosis [16,17]. Prolotherapy today remains an often-used treatment for chronic soft tissue injuries.

### *PRP*

The first use of PRP dates back to 1987 following open-heart surgery [18]. Periodontal and wound healing were early successful clinical applications. By the 1990s, the use of PRP to accelerate healing gained acceptance in surgical circles. However, the machines were large, expensive, and only used in hospital operating rooms. By the 2000s, the machines were smaller and available for use in an office setting [19].

Platelets contain a significant number of key signal proteins, growth factors, chemokines, cytokines, and other bioactive factors that initiate and regulate the inflammatory cascade [20]. Elevated platelet concentrations are known to stimulate proliferation and differentiation of mesenchymal stem cells at an injury site [21], resulting in natural wound healing [22].

The role of PRP in sports medicine increased after Mishra and Pavelko [23] published a study noting its effectiveness in treatment of recalcitrant chronic lateral elbow tendinosis. Further studies have corroborated the efficacy of PRP in treatment of tendinosis [24,25] and ligament injury [26]. However, studies refuting the improved efficacy of PRP versus injection of saline solution have also been published. Most notably, a study by de Jonge et al in 2010 and their follow-up in 2011 demonstrated no added benefit of PRP versus saline solution for chronic mid-portion Achilles tendinopathy [27]. In a randomized controlled trial, an experimental group of 16 patients with Achilles tendon rupture who were injected with PRP was compared with a group of 14 patients who did not receive the PRP injection. Both groups underwent surgical repair, and no significant differences in functionality were found between the 2 groups as measured by a heel raise index and an Achilles Tendon Total Rupture Score [28]. A prospective randomized trial evaluating the use of PRP in anterior cruciate ligament (ACL) reconstruction showed no added benefit to ACL reconstruction alone with respect to inflammatory parameters (C-reactive protein), magnetic resonance imaging appearance of the graft, and clinical evaluation scores (KT-1000 arthrometer and visual analog scale) [29]. As treatment protocols become standardized, future research should elucidate the optimal use of PRP in soft tissue injures, whether chronic or acute.

### *Stem Cells*

Whereas PRP acts to provide a favorable environment to recruit progenitor cells and stimulate healing of musculoskeletal tissues, stem cell therapy offers the possibility of directly injecting progenitor cells to the area of damage. By definition, stem cells are able to self-renew and exist in an undifferentiated or unspecialized state, and they are capable of differentiation or specialization along multiple lineages. Nascent stem cells exist within various adult tissues including bone marrow, brain, dermis, periosteum, skeletal muscle, synovium, trabecular bone, and vasculature [30].

Adult stem cells consist of 2 general classifications: hematopoietic stem cells, which are responsible for the formation of blood products, and mesenchymal stem cells (MSCs). In the early 1990s, adult MSCs were discovered to have an active role in connective tissue repair [31]. Since that time, impressive progress toward the development of safe clinical applications for MSC-mediated therapy has been achieved. It is now technically feasible to harvest tissue cells, culture them (if needed) to expand the cell population, and then inject these cells directly into areas of injury.

Several injectable stem cell therapies with differing cell origins now abound, including MSCs, tenocyte-derived stem cells, adipose-derived stem cells,

amniotic-derived cells, and dermal fibroblasts. The most well-studied sources of MSCs include bone marrow—derived MSCs and adipose-derived stem cells. Distinguishing characteristics of cell origin currently boil down to ease of obtaining the cells, autologous versus allogenic source, availability and concentration, lineage differentiation ability, morbidity to the donor site, and the need of culturing ex-vivo. Current FDA restrictions prevent the manipulation or culture expansion of stem cells. No head-to-head human clinical studies have been conducted to compare the efficacy of stem cell therapies from different cell origins. Indeed, there is a general paucity of human clinical studies of stem cells on soft tissue injuries.

## Basic Pathophysiology of Tendon, Muscle, and Ligament Disease

Soft tissue injuries to tendons, muscles, and ligaments occur when forces overwhelm the structural strength of that tissue [1]. This phenomenon can happen on a clinical or subclinical level. Most of the research examining the role of stem cells for soft tissue disease has focused on chronic injuries either from acute trauma that fails to heal over its expected time course or injuries caused by repetitive microtrauma.

After tendon injury, the cellularity of the tendon is increased; nevertheless, infiltrating scar tissue fibroblasts are morphologically different from native tenocytes, and the types of collagen fibers laid down in reparative scar tissue are different from those of normal tendon. A large quantity (20%-30%) of type III collagen is present (normally, $<$1% of type III collagen is present). Although type III collagen fibers have superior elasticity compared with type I fibers, they have inferior strength properties [32].

Similarly, in a muscle injury, trauma disrupts normal cellular architecture. An inflammatory response is triggered whereby macrophages promote muscle damage through the release of free radicals and potentially through growth factors and cytokine-mediated signaling [33]. Growth factors regulate satellite cell recruitment, proliferation, differentiation, and fusion. In dysfunctional muscle repair, such as in muscular dystrophies, inflammatory fibroblast activation persists and the reparative capacity of stem cells (satellite cells) is attenuated, resulting in muscular fibrosis and the laying down of excessive type I collagen [34]. Ultimately, fibrosis contributes to a decline in muscle contractility and range of motion. Fibrosis is also a complication of athletes who have sustained a muscle injury that often can lead to decreased performance and reinjury.

Ligament healing involves retraction of the disrupted ligament ends and formation of a blood clot that is subsequently resorbed and replaced with a heavy cellular infiltrate [35]. There is increased vascularity and blood flow to the area of damage. Fibroblasts produce a dense, cellular, collagenous connective tissue matrix that bridges torn ligament ends. Over time, the collagenous material aligns; however, the collagen ratios remain abnormal compared with the preinjured ligament. Collagen cross-linking, innervation, fibril diameters, and vascularity all remain altered [35].

The common thread among all these areas is that after injury, an inflammation cascade is an integral part of the natural healing process [36]. An orchestrated interaction of cytokines, growth factors, free radicals, and matrix metalloproteases occurs with the goal of producing the same structural framework after the injury that existed before the injury. The exact mechanisms behind disease and remodeling are still being investigated. The success of healing is measured histopathologically by the composition of the remodeled structural framework and clinically by the resolution of pain and the return of normal strength, range of motion, and function.

## Evidence for Stem Cell Use in Soft Tissue Injury

Theoretically, stem cells can support and serve as a cell reservoir for musculoskeletal tissue repair. Stem cells can be isolated and then injected into damaged tissue. Once the stem cells are in the desired location, either local signaling or the addition of exogenous factors can drive the stem cells to differentiate into the target cell line [37,38]. A study by Ouyang et al [39] has demonstrated that bone marrow stromal cells can survive for 8 weeks after implantation into a rabbit patellar tendon and that they were able to differentiate into tenocyte-like cells 5 weeks after implantation.

The use of autologous adult bone marrow—derived stem cells in research can be segregated into 3 main areas: nucleated cell isolates, isolated MSCs without culture expansion, and isolated MSCs with culture expansions. Most adult bone marrow consists of blood cells in various stages of differentiation. These components can be divided into plasma, red blood cells, platelets, and nucleated cells. The adult stem cell fraction is present in the nucleated cells of the marrow [40]. The population of bone marrow—derived MSCs declines significantly with age (Figure 1) [41,42].

To improve yields, tissue expansion is sometimes used, although this practice is currently restricted in the United States. Allowing the MSCs to adhere to plastic in tissue culture facilitates this process [43]. Once a pure MSC population is isolated, the tissue culture is transferred to monolayer, where the cells can continue to grow adherent to the plastic flask. As the cells cover the surfaces of the plastic flasks, they are periodically removed from those surfaces and reseeded in a greater number of flasks. This one cycle of cell collection,

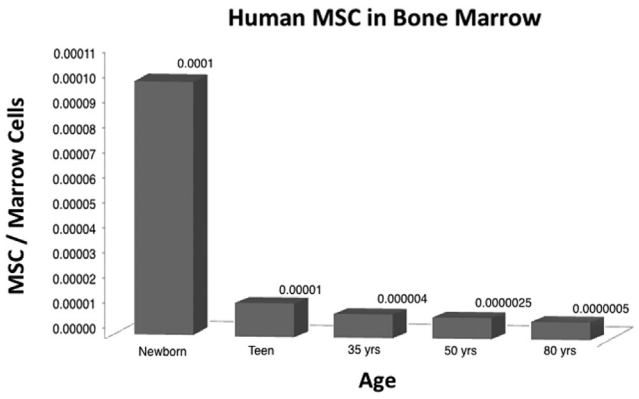


**Figure 1.** The concentration of mesenchymal stem cells (MSCs) declines precipitously with age. Adapted from Alison MR. Stem cells in pathobiology and regenerative medicine. J Pathol 2009;217:141-143 [42].

seeding, and feeding is known as a "passage." MSCs are usually harvested between the third and fifth passage and at that point can increase to between 100-10,000 times more cells than originally harvested [44]. Once cells are ready for reimplantation, they are engrafted into damaged tissue, where they begin the process of differentiation. At times, a scaffolding material is used to allow the MSCs to attach and engraft [45]. In addition to current FDA restrictions, the downside to tissue expansion is the time requirement, transport, and manufacturing processes, which also increase costs and potential risks.

Like bone marrow, adipose tissue is derived from embryonic mesodermal tissue. Adipose stroma contains relatively large numbers of undifferentiated cells capable of producing cartilage, ligament, tendon, muscle, and bone [46]. The argument for using adipose-derived stem cells as opposed to bone marrow—derived MSCs is that adipose tissue has a higher number of MSCs [47]. Adipose-derived stem cells also appear to have increased angiogenic capability compared with bone marrow [48]. However, isolating the heterogeneous stromal vascular fraction, which is the fraction containing adipose-derived stem/progenitor cells, requires enzymatic digestion of aspirated adipose. This process is considered "more than minimal" cell manipulation and is currently prohibited by the FDA.

Moreover, bone marrow—derived MSCs may have more immunomodulatory effect than adipose-derived stem cells. In a recent study in mice comparing the use of adipose-derived stem cell and bone marrow—derived MSC injections after induction of endotoxic shock, mice receiving bone marrow—derived MSCs had a significantly higher survival rate compared with adipose-derived stem cells and control subjects [49]. Additionally, in one study comparing processed bone marrow aspirate with lipoaspirate, no significant differences were found in adherent stromal cell yield, growth kinetics, and differentiation capacity [50]. More research is needed in this area.

The use of allograft micronized dehydrated human amniotic/chorionic membrane (mDHACM) in a saline solution suspension for injection has now been made possible by the PURION (MiMedx, Marietta, GA) process of cleaning, sterilizing, and drying human amniotic/chorionic membrane obtained from donors [51]. In vivo and in vitro studies have shown that the biochemical properties of amniotic membrane help to reduce inflammation and enhance soft tissue healing [52]. In its natural form, human amniotic membrane has also been shown to have antibacterial and pain reduction properties. These tissues contain growth factors that stimulate general protein and collagen synthesis, collagenase activity, and chemotaxis of fibroblasts and of smooth muscle cells [53]. Furthermore, the amnion possesses all 3 germ layers and has low immunogenicity [54]. Unfortunately, very few studies using these products for orthopedic conditions such as muscle, tendon, or ligament injuries have been published.

Tenocyte-like fibroblast cells have been used in tendon regeneration [55]. Skin represents an abundant and accessible source of cells that can be used for cell therapy. In vitro, these skin-derived fibroblasts can be driven toward tenocyte differentiation by being immersed in a tendon environment and subjected to mechanical forces [56]. Fibroblasts or tenocytes derived from another tendon are differentiated, and hence they are attractive because they are less likely to morph into undesirable cell lineages. However, the necessity of tissue culture and transport currently precludes their use in the United States.

There is a general paucity of clinical studies of stem cell treatment of soft tissue injuries. Several relevant studies that have emerged are highlighted in the following sections.

### *Ligament*

Ligament injuries are common in the field of sports medicine. Some common ligament injuries include ankle sprains with a disrupted anterior talofibular ligament, elbow injuries with involvement of the ulnar collateral ligament, and knee injuries involving the ACL.

Very good outcomes with modern-day ACL reconstruction are reported; however, several complications including ligament laxity, donor site morbidity, and pathogen transfer are associated with the use of an autograft or allograft [57]. Injectable stem cell therapy would offer an attractive alternative to surgical reconstruction, avoiding some of these complications and perhaps offering faster return to play/prior level of function. Currently, no studies on stem cell therapy for ligament injury in humans have been published. A key impediment is the basic science demonstrating capabilities of differentiation toward ligamentous tissue.

A few researchers have examined this issue. The potential use of adipose-derived stem cells in ligament

tissue engineering was initially explored by examining their ability to express several ligament markers under growth factor treatment. Adipose-derived stem cell populations treated for up to 4 weeks with transforming growth factor−β1 or insulin-like growth factor−1 did not show any significant and consistent upregulation in the expression of ligament markers [58].

However, researchers using the co-culture process have had better success. Co-culture refers to the culturing of stem cells together with the targeted mature cells. ACL fibroblasts were isolated from New Zealand white rabbits after ACL resection and enzymatic digestion. Cells were co-cultured with MSCs on a bioscaffold. Immunohistochemical staining after 2 weeks showed that MSCs co-cultured on a bioscaffold were able to differentiate into ligament fibroblasts [59].

### *Skeletal Muscle*

Just as injectable stem cells for ligamentous injury still largely remains at the basic science level, there is a void of clinical studies regarding stem cell use for muscle injuries. Chronic muscle strain and sequelae can manifest in various ways. Current treatments focus on restoring muscle balance, stretching, strengthening, and symptomatic pain management [60]. Some patients are left with chronic pain or fibrosis that can lead to reduced function. Orthobiologics hold promise in this area.

In one clinical study, 3-month-old white rabbits had cardiotoxin injected into their tibialis anterior muscles to cause cell death and atrophy 3 days before cultured perirenal adipose tissue stromal cells were transferred. The anterior tibialis muscles were studied 2 months after cell transfer and were found to be heavier, with a larger fiber cross-sectional area and higher maximal force compared with damaged control subjects [61].

Presently, most reports on research using stem cell therapies for skeletal muscle regeneration are limited to animal models. Successful results have been reported for Duchenne muscular dystrophy models in which dystrophin can be restored after systemic delivery of various stem cells [62].

A study by Winkler et al [63] in 2009 showed a dose-response correlation between the number of transplanted bone marrow−aspirated mesenchymal stem cells and the amount of functional recovery resulting from treatment by intramuscular injection within a skeletal muscle crush injury in rats. The clinical implications of these data for human muscle recovery are difficult to extrapolate.

### *Tendon*

Chronic tendon injuries are commonplace in the field of musculoskeletal medicine, and some of their treatment options have already been discussed. Often these problems are self-limited, although many involve substantial time and disability before improvement. Furthermore, lost training opportunities can have an impact on peak performance and the career of elite-level athletes.

Compared with the number of clinical trials that have been performed for stem cell treatment of ligament and muscle disorders, clinical trials for tendon disorders are relatively numerous. Several animal studies have been published, and several are reviewed here.

In one study, MSCs were implanted with a collagen gel delivery vehicle into 1-cm gap defects in rabbit Achilles tendons and compared with contralateral Achilles tendons that only had suture material implanted as controls. Analysis of the tissue revealed an improvement of biomechanical properties, tissue architecture, and functionality of the tendon after injury. Treated tissues had a significantly larger cross-sectional area and more physiologic alignment of collagen fibers [64].

Chong et al [65] studied the effect of inoculation of bone marrow−derived MSCs in the Achilles tendon defects of 57 rabbits. A transection in the Achilles tendon was performed and either treated with Kessler suture with or without the addition of MSCs. The tendons injected with bone marrow−derived MSCs had improved mechanical and histological parameters only at the early stages (3 weeks), but not at 6 and 12 weeks. The lack of significance at later periods could be explained by the study design and power of the study [65].

Bone marrow−derived MSC injections into the equine superficial digital flexor tendon (which is similar to the human Achilles tendon) of 12 horses who had sustained naturally occurring tendon injury showed a high initial cell loss (75% over 24 hours) but retained small numbers of labeled cells for up to 5 months in a controlled experiment. Compared with control subjects treated with saline solution, tendons treated with bone marrow−derived MSCs had greater elasticity, reduced cross-sectional area, and improved collagen organization [66].

Several human studies have been performed as well. In a prospective pilot study conducted in the United Kingdom, collagen-producing tenocyte-like cells derived from skin fibroblasts were prepared, cultured, and injected under ultrasound guidance in 12 patients with the clinical diagnosis of refractory lateral epicondylitis. At 6 months after the injection, 11 of 12 patients had a satisfactory outcome with a decrease in the number of tears, new vessels, and tendon thickness seen on ultrasound. No adverse events occurred [30]. In a prospective, randomized, double-blinded controlled trial, 46 patients with a total of 60 patellar tendinopathies received ultrasound-guided injections of autologous skin-derived tendonlike cells. Dermal fibroblast cells that underwent tissue culture over 4 weeks were then suspended in autologous plasma and injected under ultrasound guidance to the patellar tendon. Compared with patellas injected with autologous plasma only, the

cell group had statistically significant and faster improvement in pain and reduced functional disability as measured up to 6 months after the procedure [55].

Over the course of 5 years, Pascual-Garrido et al [67] followed up on 8 patients with refractory patellar tendinopathy who were injected with autologous, nonexpanded, bone marrow—derived MSCs. Of the 8 patients, 7 said they would have the procedure again and were completely satisfied. Statistically significant improvement was seen for most clinical scores including the Tegner, Knee Injury and Osteoarthritis Outcome Score (KOOS) activity of daily living, KOOS symptom, and KOOS sport scores [67]. Lastly, a prospective, double-blind, randomized controlled trial evaluating 45 patients with clinical plantar fasciitis refractory to conservative therapies for 2-12 months were randomly assigned to receive injections of saline solution or 2 different concentrations of mDHACM. Each week over an 8-week postinjection follow-up, patients in the treatment groups had statistically improved pain and function scores as measured by the American Orthopedic Foot and Ankle Society Hindfoot score compared with control groups, although no difference was observed between patients receiving 0.5 mL or 1.25 mL of mDHACM injection [68].

## Conclusion

The paradigm of healing soft tissue injuries of ligament, muscle, and tendons continues to evolve. Very little evidence supports use of the traditional treatment model of rest, nonsteroidal anti-inflammatory drugs, and corticosteroid injections, and these treatments are often ineffective at reversing the condition. Surgery is invasive and noted for having potential adverse effects with mixed results. Injectable stem cell therapy is an attractive alternative because these cells can potentially reconstruct and orchestrate healing of the original tissue architecture. However, injectable stem cell therapy is currently considered experimental because evidence is sparse. Many basic questions are still unanswered, including the best source for stem cells in the orthopedic arena. The best source for stem cells may vary with the type of disease encountered. Additional questions are related to the optimal number of cells to inject, the optimal number and timing of injections, and patient selection. It is apparent that large-scale, multiyear studies comparing stem cell treatments with conventional therapies are still needed to determine the role of these therapies in the treatment of muscle, tendon, and ligament injuries commonly seen in the arena of sports medicine.

## References


1. Hess GP, Cappiello WL, Poole RM, Hunter SC. Prevention and treatment of overuse tendon injuries. Sports Med 1989;8:371-384.
2. Lysaght T, Campbell AV. Regulating autologous adult stem cells: The FDA steps up. Cell Stem Cell 2011;9:393-396.
3. Nichols AW. Complications associated with the use of corticosteroids in treatment of athletic injuries. Clin J Sports Med 2005;15:E370.
4. Kapetanos G. The effect of the local corticosteroids on the healing and biomechanical properties of the partially injured tendon. Clin Orthop Relat Res 1982;163:170-179.
5. Coombes BK, Bisset L, Vicenzino B. Efficacy and safety of corticosteroid injections and other injections for management of tendinopathy: A systematic review of randomized controlled trials. Lancet 2010;376:1751-1767.
6. Coombes BK, Bisset L, Brooks P, Khan A, Vicenzino B. Effect of corticosteroid injection, physiotherapy, or both on clinical outcomes in patients with unilateral lateral epicondylalgia: A randomized controlled trial. JAMA 2013;309:461-469.
7. Shrier I, Matheson GO, Kohl HW. Achilles tendonitis: Are corticosteroid injections useful or harmful? Clin J Sport Med 1996;6:245-250.
8. Schultz L. A treatment for subluxation of the temporomandibular joint. JAMA 1937;109:1032-1035.
9. Rabago D, Best TM, Zgierska AE, Zeisig E, Ryan M, Crane D. A systematic review of four injection therapies for lateral epicondylosis: Prolotherapy, polidocanol, whole blood and platelet-rich plasma. Br J Sports Med 2009;43:471-481.
10. Ongley MJ, Klein RG, Dorman TA, Eek BC, Hubert LJ. A new approach to the treatment of chronic low back pain. Lancet 1987;2:143-146.
11. Cusi M, Saunders J, Hungerford B, Wisbey-Roth T, Lucas P, Wilson S. The use of prolotherapy in the sacroiliac joint. Br J Sports Med 2010;44:100-104.
12. Hauser RA, Hauser MA. Dextrose prolotherapy for unresolved neck pain. Pract Pain Manage 2007;7:56-60.
13. Scarpone M, Rabago DP, Zgierska A, Arbogast G, Snell E. The efficacy of prolotherapy for lateral epicondylosis: A pilot study. Clin J Sport Med 2008;18:248-254.
14. Ryan MB, Wong AD, Gillies JH, Wong J, Traunton JE. Sonographically guided intratendinous injections of hyperosmolar dextrose/lidocaine: A pilot study for the treatment of chronic plantar fasciitis. Br J Sports Med 2009;43:303-306.
15. Reeves KD, Hassanein K. Randomized prospective double-blind placebo-controlled study of dextrose prolotherapy for knee osteoarthritis with or without ACL laxity. Altern Ther Health Med 2000;6:68-80.
16. Topol GA, Reeves KD. Regenerative injection of elite athletes with career altering chronic groin pain who fail conservative treatment: A consecutive case series. Am J Phys Med Rehabil 2008;87:890-902.
17. Ryan M, Wong A, Taunton J. Favorable outcomes after sonographically guided intratendinous injection of hyperosmolar dextrose for chronic insertional and midportion Achilles tendinosis. Am J Roentgenol 2010;194:1047-1053.
18. Ferrari M, Zia S, Valbonesi M. A new technique for hemodilution, preparation of autologous platelet-rich plasma and intraoperative blood salvage in cardiac surgery. Int J Artif Organs 1987;10:47-50.
19. Alderman D, Alexander RW. Advances in regenerative medicine: High-density platelet-rich plasma and stem cell prolotherapy for musculoskeletal pain. Pract Pain Manage 2011;11:49-63.
20. Sampson S, Gerhardt M, Mandelbaum B. Platelet rich plasma injection grafts for musculoskeletal injuries: A review. Curr Rev Musculoskelet Med 2008;1:165-174.
21. Mishra A, Tummala P, King A, et al. Buffered platelet-rich plasma enhances mesenchymal cell proliferation and chondrogenic differentiation. Tissue Eng Part C Methods 2009;15:431-435.
22. Foster TE, Puskas BL, Mandelbaum BR, Gerhardt MB, Rodeo SA. Platelet-rich plasma: From basic science to clinical applications. Am J Sports Med 2009;37:2259-2272.

23. Mishra A, Pavelko T. Treatment of chronic elbow tendinosis with buffered platelet-rich plasma. Am J Sports Med 2006;34:1774-1778.
24. Mautner K, Colberg RE, Malanga G, et al. Outcomes after ultrasound-guided platelet-rich plasma injections for chronic tendinopathy: A multicenter, retrospective review. PM R 2013;5:169-175.
25. Mishra AK, Skrepnik NV, Edwards SG, et al. Platelet-rich plasma significantly improves clinical outcomes in patients with chronic tennis elbow. Am J Sports Med 2014;42:463-471.
26. Podesta L, Crow SA, Volkmer D, Bert T, Yocum LA. Treatment of partial ulnar collateral ligament tears in the elbow with platelet-rich plasma. Am J Sports Med 2013;41:1689-1694.
27. de Jonge S, de Vos RJ, Weir A, et al. One-year follow-up of platelet-rich plasma treatment in chronic Achilles tendinopathy: A double-blind randomized placebo-controlled trial. Am J Sports Med 2011;39:1623-1629.
28. Schepull T, Kvist J, Norman H, Trinks M, Berlin G, Aspenberg P. Autologous platelets have no effect on the healing of human Achilles tendon ruptures. Am J Sports Med 2011;39:38-47.
29. Nin JR, Gasque GM, Azcarate AV, Beola JD, Gonzalez MH. Has platelet-rich plasma any role in anterior cruciate ligament allograft healing? Arthroscopy 2009;25:1206-1213.
30. Connell D, Datir A, Curtis M. Treatment of lateral epicondylitis using skin-derived tenocyte-like cells. Br J Sports Med 2009;43:293-298.
31. Bruder SP, Fink DJ, Caplan A. Mesenchymal stem cells in bone development, bone repair, and skeletal regeneration therapy. J Cell Biochem 1994;56:283-294.
32. Williams IF, Heaton A, McCullagh KG. Cell morphology and collagen types in equine tendon scar. Res Vet Sci 1980;28:302-310.
33. Tidball J. Inflammatory processes in muscle injury and repair. Am J Physiol Regul Integr Comp Physiol 2005;288:R345-R353.
34. Mann C, Perdiguero E, Kharraz Y, et al. Aberrant repair and fibrosis development in skeletal muscle. Skelet Muscle 2011;1:21.
35. Frank CB. Ligament structure, physiology and function. J Musculoskelet Neuronal Interact 2004;4:199-201.
36. Liu SH, Yang RS, Al-Shaikh RBS, Lane JM. Collagen in tendon, ligament and bone healing: A current review. Clin Orthop Rel Res 1995 Sep;(318):265-278.
37. Bianco P, Robey PG, Simmons PJ. Mesenchymal stem cells: Revisiting history, concepts, and assays. Cell Stem Cell 2008;2:313-319.
38. Chamberlain G, Fox J, Ashton B, Middleton J. Concise review: Mesenchymal stem cells: Their phenotype, differentiation capacity, immunological features, and potential for homing. Stem Cells 2007;25:2739-2749.
39. Ouyang HW, Cao T, Zou XH, et al. Mesenchymal stem cell sheets revitalize nonviable dense grafts: Implications for repair of large-bone and tendon defects. Transplantation 2006;82:170-174.
40. Kryger GS, Chong AK, Costa M, Pham H, Bates SJ, Chang J. A comparison of tenocytes and mesenchymal stem cells for use in flexor tendon tissue engineering. J Hand Surg Am 2007;32:597-605.
41. Stolzing A, Jones E, McGonagle D, Scutt A. Age-related changes in human bone marrow-derived mesenchymal stem cells: Consequences for cell therapies. Mech Ageing Dev 2008;129:163-173.
42. Alison MR. Stem cells in pathobiology and regenerative medicine. J Pathol 2009;217:141-143.
43. Pereira RF, Halford KW, O'Hara MD, et al. Cultured adherent cells from marrow can serve as long-lasting precursor cells for bone, cartilage, and lung in irradiated mice. Proc Natl Acad Sci 1995;92:4857-4861.
44. Crisostomo PR, Wang M, Wairluko GM, et al. High passage number of stem cells adversely affects stem cell activation and myocardial protection. Shock 2006;26:575-580.
45. Cul JH, Park K, Park SR, Min BH. Effects of low-intensity ultrasound on chondrogenic differentiation of mesenchymal stem cells embedded in polyglycolic acid: An in vivo study. Tissue Eng 2006;12:75-82.
46. Zuk PA, Zhu M, Ashjian P, et al. Human adipose tissue is a source of multipotent stem cells. Mol Biol Cell 2002;13:4279-4295.
47. Valina C, Pinkernell K, Song YH, et al. Intracoronary administration of autologous adipose tissue-derived stem cells improves left ventricular function, perfusion, and remodeling after acute myocardial infarction. Eur Heart J 2007;28:2667-2677.
48. Casteilla L, Planat-Benard V, Laharrague P, Cousin B. Adipose-derived stromal cells: Their identity and uses in clinical trials, an update. World J Stem Cells 2011;3:25-33.
49. Elman JS, Li M, Wang F, Gimble JM, Parekkadan B. A comparison of adipose and bone marrow-derived mesenchymal stromal cell secreted factors in the treatment of systemic inflammation. J Inflamm (Lond) 2014;11:1.
50. De Ugarte DA, Morizono K, Elbarbary A, et al. Comparison of multi-lineage cells from human adipose tissue and bone marrow. Cells Tissues Organs 2003;174:101-109.
51. Fetterolf DE, Snyder RJ. Scientific and clinical support for the use of dehydrated amniotic membrane in wound management. Wounds 2012;24:299-307.
52. Niknejad H, Peirovi H, Jorjani M, Ahmadiani A, Ghanavi J, Seifalian AM. Properties of the amniotic cell membrane for potential use in tissue engineering. Eur Cell Mater 2008;15:88-99.
53. Parolini O, Solomon A, Evangelista M, Soncini M. Human term placenta as a therapeutic agent: From the first clinical applications to future perspectives. In: Berven E, ed. Human Placenta: Structure and Development. Hauppauge, NY: Nova Science; 2010, 1-48.
54. Toda A, Okabe M, Yoshida T, Nikaido T. The potential of amniotic membrane/amnion-derived cells for regeneration of various tissues. J Pharmacol Sci 2007;105:215-228.
55. Clarke AW, Alyas F, Morris T, Robertson CJ, Bell J, Connell DA. Skin-derived tenocyte-like cells for the treatment of patellar tendinopathy. Am J Sports Med 2011;39:614-623.
56. Ge Z, Goh JC, Lee EH. Selection of cell source for ligament tissue engineering. Cell Transplant 2005;14:573-583.
57. Miller SL, Gladstone JN. Graft selection in anterior cruciate ligament reconstruction. Orthop Clin North Am 2002;33:675-683.
58. Eagan MJ, Zuk PA, Zhao KW, et al. The suitability of human adipose-derived stem cells for the engineering of ligament tissue. J Tissue Eng Regen Med 2012;6:702-709.
59. Fan H, Liu H, Toh S, Goh JCH. Enhanced differentiation of mesenchymal stem cells co-cultured with ligament fibroblasts on gelatin/silk fibroin hybrid scaffold. Biomaterials 2008;29:1017-1027.
60. Noonan TJ, Garrett WE. Muscle strain injury: Diagnosis and treatment. J Am Acad Orthop Surg 1999;7:262-269.
61. Bacou F, el Andalousi RB, Daussin PA, et al. Transplantation of adipose tissue-derived stromal cells increases mass and functional capacity of damaged skeletal muscle. Cell Transplant 2004;13:103-111.
62. Quintero AJ, Wright VJ, Fu FH, Huard J. Stem cells for the treatment of skeletal muscle injury. Clin Sports Med 2009;28:1-11.
63. Winkler T, von Roth P, Matziolis G, Metha M, Perka C, Duda GN. Dose-response relationship of mesenchymal stem cell transplantation and functional regeneration after severe skeletal muscle injury in rats. Tissue Eng Part A 2009;15:487-492.
64. Young RG, Butler DL, Weber W, Caplan AI, Gordon SL, Fink DJ. Use of mesenchymal stem cells in a collagen matrix for Achilles tendon repair. J Orthop Res 1998;16:406-413.
65. Chong AK, Ang AD, Goh JC, et al. Bone marrow-derived mesenchymal stem cells influence early tendon-healing in a rabbit Achilles tendon model. J Bone Joint Surg 2007;89:74-81.
66. Smith RKW. Stem cell therapy for tendinopathy: Lessons learned from a large animal model. Br J Sports Med 2013;47:e2.
67. Pascual-Garrido C, Rolon A, Makino A. Treatment of chronic patellar tendinopathy with autologous bone marrow stem cells: A 5-year-followup. Stem Cells Int 2012;2012:953510.
68. Zelen CM, Poka A, Andrews J. Prospective, randomized, blinded, comparative study of injectiable micronized dehydrated amniotic/chorionic membrane allograft for plantar fasciitis: A feasibility study. Foot Ankle Int 2013;20:1-8.

## Disclosure

**K.M.** Emory University, Emory Orthopedic & Spine Center, 59 Executive Park South Suite 2000, Atlanta, GA 30329. Address correspondence to: K.M.; e-mail: kmautne@emory.edu
Disclosures outside this publication: Consultancy, Harvest Technologies; payment for lectures including service on speakers' bureaus, Sonosite; stock/stock options, Tenex

**J.B.** Emory University, Emory Orthopedic & Spine Center, 59 Executive Park South Suite 2000, Atlanta, GA 30329
Disclosure: nothing to disclose

Submitted for publication August 15, 2014; accepted December 4, 2014.