Cell Therapy in Tendon Disorders

What Is the Current Evidence?

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Background: Various types of tissue-derived cells are being experimented with for the treatment of tendinopathy, tendon repair, and use in tissue engineering.

Purpose: The aim of this systematic review is to explore the current evidence with a view to evaluate the potential of this therapeutic intervention.

Study Design: Systematic review.

Methods: A review of the literature was conducted using PubMed. Search criteria included keywords “tendinopathy,” “tendinitis,” “tendinosis,” “epicondylitis,” “stem cell,” and “cell therapy.” Articles not written in English language were excluded.

Results: A total number of 379 articles were identified and a critical appraisal of the relevant articles was undertaken, which encompassed human and animal research. The review included articles related to various tissue-derived cells such as tendon progenitors, adipose tissue, synovium, muscle, bone marrow, and skin. The utility of cell therapy in tissue engineering and rotator cuff repair was also assessed.

Conclusion: With the limitation of the available evidence, the literature suggests that cell therapy is applicable and may be effective for the treatment of tendinopathy. However, further research into the precise biological mechanisms, long-term implications, and cost-effectiveness is needed.

Keywords: tendinopathy; tendinosis; tendinitis; cell therapy; stem cells; tendon repair

Tendinitis is a general term often used to describe painful conditions affecting tendons associated with repetitive strain, overuse, degeneration, or poor biomechanics. The pathogenesis of tendinopathy is often the consequence of the body's inadequate attempts to regenerate the tendon and the pathologic responses that result in tendon failure. The tendon becomes grossly thickened, disorganized, and loses its physical properties. Angiogenesis occurs and cell mediators are released. The net result is tendon fatigue leading to tearing, pain, and failure.

Scientific research has shown that inflammatory change is not a predominant finding in this condition. Histopathologically, tendinosis is characterized by degeneration and disorganization of collagen fibers, increased cellularity, and vascularity with no significant inflammatory cells present. Therefore, using the term tendinosis may be more accurate when referring to tendon disorders.

Traditionally, tendinitis was treated by controlling inflammation through conventional methods including corticosteroid and nonsteroidal anti-inflammatory medications, with no scientific evidence to support these treatment modalities. More recently, the injection of whole blood and platelet-rich plasma has been used to promote tendon healing. The proposed mechanism is that inherent growth factors released from platelets will encourage tenocyte migration and differentiation at the site of tendon injury, although the evidence for this remains weak. Cell therapy, however, has the potential for restocking the injured tendon with tissue-specific cells to regenerate the tendon without excessive morbidity to the donor site. This has brought a great deal of attention to the use of the multipotential progenitor cells such as embryonic stem cells, periosteal cells, and mesenchymal stem cells (MSCs).

The recent advances in cell therapy have paved the way for better therapeutic processes in musculoskeletal disorders. It has become technically feasible to harvest tissue cells and culture them to expand the cell population; this has enabled the process of using biocompatible carrier materials in combination with cell culture to provide optimal tissue regeneration. Pluripotential stem cells can be isolated,
TABLE 1
A Summary of Cell Therapies of Different Cell Origins

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Source</th>
<th>Advantage</th>
<th>Disadvantage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mesenchymal stem cells (MSCs)</td>
<td>Bone marrow-derived</td>
<td>1. Multilineage potential including tenocyte-like cells</td>
<td>Cannot control differentiation into undetectable tissue lineages such as bone, cartilage, and muscle</td>
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<tr>
<td></td>
<td></td>
<td>2. Hypoimmunogenicity</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Adipose tissue-derived</td>
<td>1. Increases rate of tendon healing and maturation</td>
<td>1. As with MSCs, above</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2. Improves biomechanical and histologic properties of the tendon</td>
<td>2. Cell population diminishes with age</td>
</tr>
<tr>
<td></td>
<td>Synovium-derived</td>
<td>May promote bone-tendon regeneration</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Muscle-derived</td>
<td>As with MSCs, above</td>
<td></td>
</tr>
<tr>
<td>Fibroblasts</td>
<td>Skin</td>
<td>1. Great potential in tendon engineering and tendon repair</td>
<td>1. As with MSCs, above</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2. Widely available</td>
<td>2. Limited application in tendon therapy</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3. No morbidity to donor site</td>
<td>As with MSCs, above</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4. No significant effect to the donor site</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>5. Potential source of cells for storage</td>
<td></td>
</tr>
<tr>
<td>Tendon progenitor/</td>
<td>Tendon</td>
<td>Can develop into tendonlike tissues</td>
<td>1. Morbidity to donor site</td>
</tr>
<tr>
<td>stem cells</td>
<td></td>
<td></td>
<td>2. No tenocyte markers</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3. No human studies</td>
</tr>
</tbody>
</table>

cultured, and injected into the injured tendon. The stem cells, under the influence of endogenous and exogenous factors, can subsequently differentiate into the appropriate cell type. This technology is currently being used in tendon formation and stimulating graft incorporation. There may also be a role for the concomitant administration of growth factors, that is, platelet-rich plasma.

Tissue engineering, using stem cells, has facilitated the process of generating living substitutes for tissues and organs, which has many advantages over classic tissue reconstruction such as lack of immunogenicity of allogenic grafts or loosening of prosthetic implants.

We have undertaken a systematic review of the scientific English literature to highlight the available evidence to assess both the applicability and effectiveness of stem cell therapy for tendinosis in both animal and human research.

MATERIALS AND METHODS

The available literature was reviewed using PubMed. The search was performed using the terms “tendinopathy,” “tendinitis,” “tendinosis,” “epicondylitis,” “cell therapy,” and “stem cells,” which resulted in 1172 articles. Restricting the search to English language, clinical trials, and meta-analyses resulted in 379 articles. Relevant articles were reviewed and included in this study. The studies have not been classified according to their level of evidence. The review covered all types of tissue-derived stem cell origins used in the treatment of tendinopathy, such as bone marrow, tendon, muscle, synovium, adipose tissue, and skin cells. A summary of the characteristics of each of these cell origins is presented in Table 1. The table demonstrates the advantages and potential disadvantages of each cell origin.

NATURAL HEALING PROCESS

After a tendon injury, the tendon normally heals through scar tissue formation, which may take up to 1 to 2 years to mature. During this course, the cellularity of the tendon is increased, nevertheless, the infiltrating scar tissue fibroblasts do appear morphologically different from native tenocytes. The appearances of these scar tissue fibroblasts simulate those of myofibroblasts rather than tenocytes because of their large size, basophilic staining, and large vesicular nuclei.

The types and characteristics of the collagen fibers in a reparative scar tissue are different from those of a normal tendon because of the reduced interdigitation of the collagen fibers with the presence of large quantity (20%-30%) of type III collagen (normally <1%) in the scar tissue. Although type III collagen fibers present superior elasticity to type I fibers, they have inferior strength properties. This is primarily because of the smaller diameter of type III collagen when compared with type I. The qualities of the scar tissue improve with maturation caused by better
interdigitation of the collagen fibers, increase in type I to type III collagen ratio, and enlargement of fibrillar diameter. However, the mechanical properties of the tendon remain suboptimal because of the lack of proper structural organization and poor matrix formation. Subsequently, a larger amount of fibrous tissue is needed to compensate for this mechanical insufficiency, which results in a thickened but stiffened tendon. Ultimately, the tendon loses its specificity as a spring, which in turn jeopardizes the functional activity of the animal or human. In elite athletes or racehorses this may be the difference between championship-winning performance or not.

Mesenchymal Stem Cell and Cell Progenitors

Mesenchymal stem cell (MSC) is a term used to describe cells that have the ability to proliferate and differentiate into progenitors of different mesenchymal tissues. Mesenchymal stem cells are characterized by their unique cell surface marker proteins, adhesion molecules, cytokines, growth factors and their receptors, extracellular matrix molecules, and their multilineage potential. An added value to the application of the MSCs, is their hypoimmunogenicity from the lack of the major histocompatibility complex-II molecular expression.

The exact mechanism by which stem cells demonstrate a wide range of differentiation potential is not fully understood. However, it has been speculated that stem cell transdifferentiation could be responsible for this phenomenon. Transdifferentiation is a process in which certain factors, such as signaling from local and distant tissue, play a part in determining the differentiation of stem cells into diverse tissue types such as myocytes and tenocytes. This has led researchers to undertake a number of animal studies and human clinical trials to use the properties of MSCs in the treatment of tendon disorders.

Mesenchymal stem cells and progenitor cells are abundantly available in various human tissues such as bone marrow, adipose tissue, hair follicles and scalp subcutaneous tissue, periodontal ligament, thymus, trophoplastic tissue, umbilical cord blood, tissues, and umbilical cord blood. Circulating MSCs are also present in peripheral blood. These cells were found to be able to differentiate into multiple mesenchymal lineages such as osteolines, chondrolines, and adipolines.

Bone Marrow-Derived MSCs. The hemopoietic system of the bone, by its nature, is a highly proliferative organ that contains hemopoietic stem cells but also nonhemopoietic cells widely known as MSCs or mesenchymal progenitor cells. These MSCs are able to proliferate and develop into different mesenchymal tissues such as bone, cartilage, fat, tendon, muscle, and marrow stroma, making this organ a favorable source for tissue engineering. However, it has been proven in the literature that the population of these bone marrow-derived MSCs (BM-MSCs) diminishes with age. A study by Ouyang et al. has proven that allogenic bone marrow stromal cells could survive for approximately 8 weeks after implantation into a rabbit patellar tendon and were able to differentiate into tenocyte-like cells 5 weeks after implantation. The study has also demonstrated the translateability of bone marrow stromal cells by the local delivery and preservation of their differentiation potential for the entire duration of the observation. However, the labeled cell number diminished with time, which was thought to be related to the remodeling process as the cells were replaced by collagen matrix. Additionally, as tenocytes have no cell marker it was difficult to characterize the differentiated cells. Having said that, the study suggested that the bone marrow stromal cell application for in vivo tissue engineering is reasonable and can be used as progenitor cells for neotendon formation.

A study by Hankemeier et al. performed a similar experiment using patellar tendon. The study demonstrated that autologous bone marrow MSC-mediated repair tissue showed significant increase in maximum stress, modulus, and strain energy density from improved histologic features and biomechanical properties of the tendon by increasing the number of cells and amount of mature collagen fibers. The study concluded that providing a large number of MSCs to the wound site could significantly improve its biomechanical properties. Nonetheless, there was no discernible improvement in the microstructure of the tissue compared with the control. In addition, injecting undifferentiated BM-MSCs into the tendon led to ectopic bone formation.

A study by Chong et al. performed a similar experiment using patellar tendon. White rabbits were used as the experimental animals and 7 others were used as the source of BM-MSCs. The researchers transected the midsubstance of the Achilles tendon of both limbs so that one side would receive BM-MSCs in a fibrin carrier and the other would receive fibrin glue alone (control). The tendon injury was repaired using modified Kessler technique with a running epitendinous suture and keeping the rabbits immobilized after surgery. Subsequently, tissue samples were obtained at 1, 3, 6, and 12 weeks to evaluate tissue morphology, cell tracing, and histology using immunohistochemistry and morphometric and mechanical analyses. The authors found no differences in the gross morphologic characteristics of the tendons between the 2 groups. The fibrin degraded after 3 weeks, while the labeled BM-MSCs remained active and present in the intratendinous region for a minimum of 6 weeks. Three weeks later, the organization of the collagen fibers and the morphometric nuclear parameters were better in the treatment group ($P < .05$). However, at 6 and 12 weeks, there were no differences between the groups in terms of morphometric nuclear parameters.
The biomechanical assessment demonstrated better modulus in the treatment group at 3 weeks (P < .05) but not at later time points. The authors concluded that intratendinous cell therapy with BM-MSCs could improve histologic and biomechanical parameters in the initial phases of tendon healing after primary tendon repair.

Adipose Tissue-Derived Stem Cells. Although BM-MSCs are intuitively a good source of stem cells for tissue engineering applications, MSCs can be found in almost all organs and tissues. Adipose-derived mesenchymal cells have become a favorable source for MSC therapy in the field of orthopaedics because of their wider availability and simplicity to obtain. However, their application in tendon repair is currently limited.

Synovial-Derived Stem Cells. DeBari et al were the first group to identify and isolate synovium-derived mesenchymal stem cells (SMSCs) in 2001. This represented a step forward for stem cell therapy in musculoskeletal conditions. In a systematic review by Fan et al, SMSCs were found to be successful in the treatment of a wide range of musculoskeletal disorders. As well as cartilage regeneration, SMSCs can be used in bone, tendon, and skeletal muscle regeneration.

Harvesting autologous hamstring tendon for ACL reconstruction is a popular technique for treating ACL tears. Nevertheless, one of the major weaknesses of this procedure is the lack of efficient tendon-bone healing, which can be detrimental to a patient's postoperative recovery and rehabilitation because of the instability of the tendon-bone interface. A study by Tomita et al reported that SMSCs could successfully be utilized for bone-tendon regeneration in the rat model as bones and tendons are closely placed in the knee joint. These findings were corroborated by Ju et al through a study that found SMSCs to be able to accelerate early remodeling of bone-tendon healing by inserting rat Achilles tendon grafts into a tibial bone tunnel followed by SMSC injection into the bone tunnel. It was noted that these cells were able to enhance an early remodeling of the tendon-bone healing, which consequently led to a significant increase in collagen fiber production and improved configuration of oblique collagen fibers connecting bone to tendon. Over time, the interface tissue resolved and the implanted tendon appeared to blend well to the bone. However, the authors stated 3 main limitations of their research, which included lack of biomechanical testing, postoperative limping as they used ipsilateral Achilles tendons rather than hamstring or extensor hallucis longus, and lastly the lack of reproducibility of an intra-articular environment as the study used an extra-articular model. Additionally, the authors stated that the tendon healing process took place at a faster rate in rats than in humans; therefore, the authors suggested a large-scale animal study to determine applicability of these results in humans, which would have major implications on the improved postoperative rehabilitation for ligament reconstruction.

Muscle-Derived Stem Cells. Muscles were also found to contain stem cells capable of differentiation into muscular, osseous, fatty, hematopoietic, and cartilage tissues. Williams et al used an amputated human leg and a pectoralis muscle to create cell cultures, which then underwent immunologic and histochemical staining assays. These cells included stem cells with distinctive stellate morphology (control), skeletal myotubes, smooth muscle, bone, cartilage, and fat. These experiments confirmed the presence of MSCs in the skeletal muscles that were able to differentiate into a variety of mesodermal tissues, opening the horizon to a wider application of the muscle-derived stem cell for tissue regeneration.

Dermal Fibroblasts

In 2001, Toma et al successfully isolated multipotent adult stem cells from the dermis of the mammalian skin. These cells were found to be able to differentiate into brain cells, glia cells, muscle cells, and fat cells. Additionally, in vitro experiments and animal studies have shown that dermal fibroblasts have great potential in tendon engineering and tendon repair. Connell et al performed a prospective clinical pilot study in humans using collagen-producing cells derived from the skin. The authors showed that fibroblasts could be expanded in number within the laboratory, made to stretch in a longitudinal direction, and deposit collagen similar to the behavior of the normal tenocytes. It was postulated that these skin-derived cells could be used to repopulate injured tendon and lead to tendon regeneration. The study examined the safety and potential use of skin-derived fibroblast in the treatment of 12 patients with refractory lateral epicondylitis who failed nonoperative treatment for at least 6 months. A 4-mm skin sample was harvested from the lateral aspect of the hip using a 4-mm punch biopsy needle. The sample was then placed in cell transport medium (DMEM/F12 mixed gentamicin) and cultured at 4°C to 10°C refrigeration to approximately 10 × 10⁶ collagen-producing cells that had similar features to tenocytes. Using a dual-syringe technique, these cells were embedded into the patient's own plasma and injected into the intrasubstance tears of the common extensor tendon under ultrasound guidance. This combination allows the mix to act as a gel, filling tendon tears and defects.

Patients were followed for 6 months. The authors used Patient-Rated Tennis Elbow Evaluation Scale (PRTEE) to assess pain severity and functional disability. The evaluation of the tendon was conducted using 4 sonographic criteria: tendon thickness, hypoechochogenicity, intrasubstance tears, and neovascularization. Patients reported significant improvement in the PRTEE 6 months after the injection (P < .05). Additionally, ultrasound appearances also improved, which was assessed by the restoration of the normal fibrillar appearance with reduction of the number of tears, new vessels, and tendon thickness (P < .05). The authors currently have randomized trials under way using the same technique for the treatment of patellar and Achilles tendinosis. The use of skin-derived fibroblasts in the treatment of chronic lateral epicondylitis was found to be safe and effective with no significant complications in the majority of patients, apart from 1 patient who had persistent pain and underwent surgery. The procedure also has several advantages as skin cells are abundantly available in the human body, allowing for
sampling without compromising the main source of these cells; skin cells can be harvested with a minimally invasive approach, manufactured in accordance with the regulatory guidelines, and reimplanted into the same patient in an autologous fashion. However, because of the mixture of cells with patients’ plasma, the authors were uncertain as to how much of the improved PRTEE scores were the result of the cell therapy alone. Other limitations of the study included single sonographer, lack of tenocyte markers, and histopathologic correlations. The authors recommended extensive efficacy trials to demonstrate the safety and potential of this procedure.

**Tendon Stem/Progenitor Cells**

Scientific studies have shown that multipotential stem cells do exist inherently in tendons and ligaments. This is demonstrated by the fibrocartilage and osseous changes that occur in tendons after an injury. Moreover, it has been found that tendon-derived immortalized cell lines or human tendon-derived fibroblasts have generic expression of different tissues including fat, bone, and cartilage that allows various differentiation lines. It is also known that human periodontal ligaments contain postnatal stem cells that have multipotency qualities and are capable of differentiating into adipocytes and osteoblastic cells, hence the name tendon stem/progenitor cells (TSPCs). However, it remains unclear as to where these cells actually reside, although it has been proposed that such cells are possibly located in the endotenon amid the collagen fascicles, close to the vascular structures.

In a study by Chen et al., TSPCs were found to exist in an extracellular matrix, which consists of biglycan (Bgn) and fibromodulin (Fmod). The authors performed an experimental study by creating cultured cell suspensions from mouse patellar tendon and human hamstring tendon and applied onto a plate. It was noticed that isolated TSPCs, when completely expanded in vitro, could develop into tendonlike tissues after in vivo transplantation.

In a study by Chen et al., autologous tenocytes were used with porcine-derived bioscaffolds for massive rotator tendon defect reconstruction in rabbits. The bioscaffold carriers were created from porcine small intestine submucosa and type I/III collagen bioscaffold. Tenocytes were harvested by punch biopsy from the rabbit patellar tendon and prepared for incorporation in the bioscaffolds. The authors excised the rotator cuff tendons of 50 rabbits and used 5 different methods of rotator cuff repairs, which included reimplantation of the native excised tendon as positive control (n = 10), repair with porcine small intestine submucosa alone (n = 10) and with seeded tenocytes (n = 10), and collagen bioscaffolds alone (n = 10) and with seeded tenocytes (n = 10). The histologic outcomes were compared between these groups at 4 and 8 weeks and demonstrated that both tenocyte-seeded bioscaffolds developed inferior histologic appearances to autograft tendon at 4 weeks but formed qualitative tendon tissue regeneration at 8 weeks, similar to that of the positive control. The authors concluded that the implantation of autologous tenocyte on collagen-based bioscaffolds leads to better rotator cuff tendon healing and remodeling when compared with implantation of bioscaffold alone, which might be useful treatment for massive rotator cuff tears. The authors suggested further studies to evaluate the most effective postoperative weightbearing regimen of this reconstructive technique and its effect on the biomechanical strength of regenerative tendon tissue.

**CELL THERAPY AND GROWTH FACTORS**

Growth factors present in the platelets, eye, and fibroblasts can stimulate collagen deposition and tendon regeneration. In an experiment performed by Wolfman et al., growth and differentiation factors GDF-5, GDF-6, and GDF-7 were implanted into subcutaneous and intramuscular areas, which led to an ectopic formation of neotendon. However, this resulted in type II collagen deposition only, and therefore the composition was suboptimal. It was proposed that MSCs migrated to the area of implantation and differentiated into tendonlike tissue. In another study by Aspenberg and Forslund, Achilles tendon defects in a rat model were improved after 10 μg of GDF-5 and GDF-6 injections. The authors concluded that if these findings are replicated in larger animals, the outcomes of this procedure could transform the management of Achilles tendon rupture from surgical intervention to simple injection in an outpatient setting.

The hypothesis of using adenoviral GDF-5 transfer to improve Achilles tendon healing by transitory transgene expression was examined by Rickett et al. In their study, it was shown that injection of adenovirus particles could successfully deliver the GDF-5 gene in healing tendons and leads to thicker tendon regeneration after 8 weeks. Histologically, however, greater cartilage formation in type II collagen was found in the intervention group when compared with the control.

A study by Hoffmann et al. showed that MSC differentiation into tendonlike cells was mediated by intracellular signaling factor Smad-8 expression and simultaneous stimulation with BMP2 both in vitro and in vivo. Additionally, the authors have indicated that while Smad8 promoted tendon differentiation, it inhibited the osteogenic pathway, which is normally caused by BMP2. Although it was hypothesized that Smad-8 played a major role in the signaling cascade that mediated MSC differentiation into tenocyte-like cells, the authors believed that the activation was not related to BMP2 but to another unknown factor.

**CELL THERAPY FOR TENDON REPAIR IN HORSES**

Horses are prone to develop tendon injury, particularly in the weightbearing digital flexor tendons that insert at the palmar aspect of the metacarpus, causing significant functional disability to the horse. In racing horses, this can be career-ending.

The use of cultured bone marrow MSCs for the treatment for spontaneous tendinitis in horses was suggested by Smith et al. The use of MSCs to aid regenerated tendon tissue can be expensive; therefore, bone marrow mononucleated cells (BMMNCs) could be used as a substitute.
cell source for tissue engineering. The effectiveness of these 2 cell types (cultured bone marrow MSCs and BMNMCs) in the treatment of the equine collagenase-induced tendinitis model was compared against placebo effect by Crovace et al. The study showed that both cultured bone marrow MSCs and BMNMCs led to tendon regeneration while placebo caused healing with scar tissue formation. Radiographic and scintigraphic evaluations have also been performed and showed no evidence of any ossification within the treated tendon.

Mesenchymal stem cells can be obtained in advance before tendon injury and stored for later use if the animal experiences a new injury or reinjury. It has also been shown that reculture and implantation of frozen or stored MSCs can be successfully undertaken. Bone marrow aspiration should ideally be conducted within the first month after injury so that implantation can still be performed 1 to 2 months after the injury. The concept behind this is to allow for the initial inflammatory phase to settle and granulation tissue to form, which are essential for the growth of the implanted stem cells. After this treatment method, horses undergo a rehabilitation exercise program with regular ultrasound follow-up. A large number of horses in the United Kingdom and Europe have been treated with this procedure.

A literature review to assess the stem cell therapy in equine tendon regeneration was conducted by Richardson et al. The study presented encouraging data related to the clinical use of bone marrow-derived stem cell therapy in horses. The authors found no evidence of significant adverse effects related to this treatment modality. However, the authors have highlighted various weaknesses in the literature, such as the lack of any mechanical testing and biochemical and molecular analyses to allow for comparative evaluation between the new tissues obtained using this method and ordinary scar tissue. Additionally, there were no control animals in these clinical studies, which further compromised the validity of the data. Therefore, long-term follow-up and surgical correlation are needed.

CELL THERAPY AND TISSUE ENGINEERING

MSC-Collagen Gel Complex

A study performed by Young et al. showed that MSC-seeded implants could result in significant improvement in the repair biomechanics of a tendon gap model. The authors created cell-gel composite from cultured autologous marrow-derived MSCs in a collagen gel delivery system, which was then contracted onto a pretensioned suture to produce a prosthesis. One-centimeter gaps were induced in both Achilles tendons of a rabbit, but one of them was filled with tissue prosthesis and the other with suture material only to serve as a control. A follow-up assessment of the implants was conducted using biomechanical and histologic criteria after 4, 8, and 12 weeks. It was observed that load-related structural and material properties in the tendons treated with tissue implants were twice as high when compared with the values of the control side at all times (P < .05). The modulus and maximum stress for the tissue repair were higher than normal values by 34% and 37%, respectively. In addition, there was progressive improvement in the properties of load-related material (P < .05) and significant increase in the cross-sectional area of the treated tissue (P < .05) with better alignment of the collagen fibers. The authors concluded that the biomechanics, structure, and tendon function could be significantly improved using MSC-contracted organized collagen implants into large tendon defects after injury.

Significant improvement in tendon repair was reported by using different autologous MSC concentrations (1, 4, and 8 x 10^6 cells/mL) with type I collagen gel. However, this was not in a dose-dependent manner. Additionally, there was evidence of ectopic bone formation in approximately 30% of the cases. In a study by Awad et al., maximum force and maximum stress of 20% was achieved with cell gel-suture repairs compared with normal tendon. In the same study, the authors found no additional benefit in increasing cell density in the composite structure. In fact, it was shown by another study that reducing cell-collagen ratio by 20 times improved cell viability in the culture, reduced the chance of ectopic bone formation, and improved the biomechanics and histologic appearances of the repaired tissue at 12 weeks after surgery. In the same study, the authors proposed that greater initial stability would be required for high cell-to-collagen ratios in the absence of suture. Furthermore, it was suggested that implants should exhibit physical properties comparable with those of a normal tissue but at the same time undergo gradual controlled degradation rates to protect cells in the early postoperative phases. Cell-to-collagen composite and biomaterials should be able to tolerate in vivo loads as a basic concept for successful tissue engineering.

Cell Therapy and Bioscaffolds

The development of bioreactors in the recent years was based on the fact that human tendon defects were considered larger and more complicated than experimental tissue engineering performed on small animals or small defects. Therefore, this necessitated larger tissue repairs with structural and mechanical properties similar to human normal tissues. A number of studies have illustrated that seeded constructs have better histologic appearance and biomechanical properties than implantation of scaffold alone.

Hui et al. and Ouyang and colleagues undertook a number of studies regarding the use of MSCs for tendon/ligament repair. The authors found that poly-lactide-co-glycolide (PLGA) was better than other synthetic biodegradable polymers in allowing MSCs to adhere and grow. Furthermore, they illustrated that the structure and biomechanics of tendon repair in a rabbit Achilles tendon model was improved by the composite of bone marrow stromal cells and knitted PLGA scaffold. The conclusions of these studies stated that the composite MSCs along with knitted PLGA provide a possible alternative for tendon repair, although it may not lead to full regeneration of the tendon as neotendons are not identical to the native tendon. It was also believed that tendon regeneration, following MSC and knitted PLGA implantation, was only
partial, which was attributed to the limitations of the regenerative process of the tendon and the lack of tissue-specific differentiation factors for tendon regeneration. Moreover, the regenerated tendons demonstrated inferior tensile modulus when compared with knitted PLLA and natural healing. This was partly explained by the larger volume of the cells present in the repaired tendon. Additionally, the wavelength of the collagen fibers was shorter in higher collagen type III to type I ratio when compared with a normal tendon.

Kryger et al undertook in vivo and in vitro experiments to assess the role of bone marrow-derived MSCs, adipose-derived MSCs, epitenon tenocytes, and tendon sheath fibroblasts in tendon engineering by seeding them into acellularized allogenic tendons as flexor tendon grafts. Using a rabbit model, the authors reported that all cell types exhibited morphologic features similar to those seen in a fibroblast such as elongated nuclei and spindle-shaped cytoplasm. Adipose-derived MSCs demonstrated a higher proliferation rate at later passage when compared with epitenon tenocytes. Histologically, the seeded tendon grafts were indistinguishable between the different experimental groups. Because all 4 cell types showed similar growth patterns, it was suggested that successful in vivo implantation of the reseeded acellularized tendon grafts could be achieved using these cells. All the grafts were viable after 6 weeks. Interestingly, it was observed that in vivo implantation resulted in a better scaffold repopulation of each cell type when compared with the in vitro findings. It was therefore concluded that because all results were similar and the MSCs (bone- and adipose tissue-derived) can be harvested easily, using MSCs for the purpose of tissue engineering would be more convenient than epitenon and sheath fibroblast cell types. The authors have highlighted the possible differences in healing biology between rabbits and humans. Additionally, the effect of early mobilization on tendon graft viability was not assessed in this study as the tendon was divided proximally to unload the implant. It is also uncertain as to whether the tenocytes seen in the constructs were due to proliferation of allogenic tenocytes or infiltrated autologous cells. The authors recommended further studies to assess the biomechanical status of the constructs and prevent adhesion formation.

Juncosa-Melvin et al. produced promising results for the use of MSCs in tendon repair by altering the mechanical settings of MSCs. A collagen sponge-MSC complex was implanted into rabbit patellar tendon defects. Following this, one group was subjected to mechanical stimulation and the other was kept without any alteration. It was noted that the mechanical stimulation had led to increased collagen type I and III gene expression of stem cells. The mechanical stimulation group also showed better maximum force, linear stiffness, maximum stiffness, and linear modulus 12 weeks after surgery. However, certain limitations existed in this research; the evaluation of the constructs was performed only once with one loading condition (2.4% peak strain) and the protein production was not determined.

In a systematic review by Goh et al., the applicability of cell-seeded implants in tendon repair and regeneration was assessed. However, the ideal scaffold and cell source for tissue engineering remain uncertain. The authors addressed the issue of the availability of appropriate cells for the tissue engineering, which led researchers to explore the utility of allogenic cells. Although immune rejection is a theoretical risk with allogenic cells, the authors found no cause for concern as MSCs have low inherent immunogenicity.

CELL THERAPY IN ROTATOR CUFF REPAIR
Large and massive rotator cuff tears are notoriously difficult to treat and have high retear rate after arthroscopic repair. Allogenic graft surgery was developed to help overcome this dilemma; however, one of the major obstacles of this technique is the paucity of viable cells on cryopreserved scaffolds. Researchers have successfully isolated bone marrow stromal cells from the proximal humerus, which were able to differentiate into human osteoblasts and tenocytes. Ouyang et al. conducted an experiment to revitalize nonviable dense grafts by applying a sheet of BM-MSCs to cryopreserved tendon allografts and cultured the composite. It was observed that the MSCs had successfully infiltrated the tendon after 3 weeks, and many of the MSCs exhibited a spindle-like appearance similar to that of tenocytes.

Incorporating BM-MSCs into collagen scaffolds stimulated tendon healing and improved biomechanical properties of the tendon. Therefore, it has been suggested that using MSCs in rotator cuff repairs may facilitate early postsurgical mobilization and recovery. However, the time of MSC culture and MSC sheet formation, the immunogenic potential of MSC sheets, and the survival of MSC sheets remain to be determined. It was also shown that excluding osteoprogenitors during MSC isolation might not be possible using the current techniques.

CELL THERAPY AND TENDON GRAFT OSTEOINTEGRATION
As previously mentioned, synovial MSCs have also been successfully used to improve bone-tendon regeneration when Achilles tendon grafts from rats were inserted into a bone tunnel followed by SMSC injection into the bone tunnel. A study by Lim and colleagues demonstrated that MSCs enhance the strength of tendon graft-bone healing of an ACL repair by developing an intervening zone of cartilage, resembling chondral enthesis of a normal ACL insertion. In another study by Ouyang et al., it was observed that MSCs were able to not only accelerate tendon tissue formation, but also restore the native structure at the tendon-bone interface. In their study, hallucis longus tendons were translated into a 2.5-mm calcaneal bone tunnel in a rabbit model. The tunnels were then treated with or without bone marrow stromal cells. Three samples were obtained at different time intervals and analyzed histologically and immunohistochemically. The specimens showed that the cell group had more perpendicular collagen
fiber formation and higher proliferation of cartilage-like cells (fibrocartilaginous attachment) at the tendon-bone interface while the control group demonstrated progressive fibrous tissue formation. However, the fibrocartilaginous tissue did only form at half of the tendon-bone interface. This was attributed to nonuniform distribution of the bone marrow stromal cells at the interface and lack of biochemical and biomechanical transduction signals to trigger differentiation. Moreover, the optimal number of the progenitor cells needed is yet to be determined and further biomechanical and structural studies would be necessary to test the functional properties of reparative tissue.

DISCUSSION

The aim of this review is to provide a balanced critical analysis of the current evidence of cell therapy in the English literature. Cell therapy is an attractive option for tendinopathy and tendon repair as tendon in its natural state has a relatively low resident cell population and tissue turnover rate. Although there is already considerable evidence to support the use of cell therapy for the treatment of tendinopathy, deficiencies exist with regard to controlled studies in the human population, surgical correlation, and long-term follow-up.

Whereas many studies to date have focused on bone marrow as the source of cells for tendon repair, most mesenchymal tissues are also considered to contain populations of progenitor cells. In particular, adipose tissue and the dermis are recognized as a rich supply of stem cells. These organs are attractive as a potential cell source as they are readily accessible through relatively minimally invasive methods. Skin, especially, has vast potential as it contains large number of fibroblasts. Fibroblasts have been shown to take on the characteristics of tenocytes and lay down collagen when immersed in a tendon environment. Fibroblasts or tenocytes derived from another tendon are differentiated cells, hence they are attractive as they are less likely to morph into undesirable cell lineages. They remain among the most promising cell types.

Obstacles remain with respect to cell culture and transport. Culture and expansion of cells currently take several weeks before sufficient numbers are obtained worthy of repopulating the tendon and laying down new collagen fibers. Improved culture media promises to decrease the amount of time, but there remains a frustrating wait between the time of injury, cell accrual, and implantation. Culture needs to be undertaken according to strict regulatory guidelines in a dedicated good manufacturing practices facility. This adds to the expense and raises logistical issues of transport from the culture facility back to the clinic. Freezing is feasible to facilitate transport, but more research will be necessary to ensure that there is no decline in cell viability. Freezing cells also has the advantage of potentially harvesting, culturing, and storing cells prior to injury, so they are "ready to go." This may be appealing for use in elite athletes to immediately facilitate the healing process.

Most pharmaceutical companies are wary of autologous treatments, appreciating the time and expense involved. They would prefer an off-the-shelf treatment from a solitary source that can be used to treat many persons. However, autologous treatments are appealing as they are more likely to be accepted by the recipient with a decreased risk of complications, including rejection. Moreover, our review failed to raise any serious safety concerns. The harvesting, manufacturing in specialized laboratories, and the implantation of cells are all technically demanding and contribute to cost.

Regulation currently remains the most formidable obstacle to the widespread introduction of these new technologies. Burden of proof required to satisfy regulatory bodies in both Europe and the United States is high, and there is considerable research required to reach these levels of regulatory approval. This is in contradistinction to the widespread adoption of platelet-rich plasma treatments in which the scientific evidence is rather poor but which are readily available as they are not subject to the same standards of regulation and remain relatively cheap. Regardless, further human studies are necessary before the widespread adoption of cell therapy techniques for the use in tendon disorders.

CONCLUSION

Stem cell research has evolved significantly over the last several years. The literature provides promising evidence with regard to the application and effectiveness of cell therapy in tendinopathy. Nevertheless, the evidence is limited in humans, with very few clinical trials. The results of the studies examined in this review remain encouraging; however, further studies are necessary to clarify the exact cellular, biological, and molecular mechanisms for tendon healing using this technique. Regardless, cell therapy is likely to become an important component of tendon treatment in the near future.

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