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Cell-based approaches for augmentation of tendon repair

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Abstract

Cell-based approaches are among the principal interventions in orthobiologics to improve tendon and ligament healing and to combat degenerative processes. The number of options available for investigation are expanding rapidly and investigators have an increasing number of cell types to choose from for research purposes. However, in part due to the current regulatory environment, the list of available cells at clinicians' disposal for therapeutic purposes is still rather limited. In this review, we present an overview of the main cellular categories in current use. Notable recent developments in cell-based approaches include the introduction of diverse sources of mesenchymal stem cells, pluripotent cells of extra-embryonic origin, and the emerging popularity of fully differentiated cells such as tenocytes and endothelial cells. Delivery strategies are discussed and a succinct discussion of the current regulatory environment in the United States is presented.

Keywords

cell-based; biologics; tendon; orthopedics; augmentation; repair

Overview of cell types and classifications

Cell-based therapies, which involve injection/inoculation/implantation of living cellular elements to patients, are becoming an increasingly prominent therapeutic option in orthopaedics. Paul Niehans (1882–1971), a Swiss physician, is probably the father of cell-based therapy. In 1931 he injected calf embryonic material to a patient, although there are no data available on the efficacy of his cell-injection practice. In 1953, it was found that rejection of organ transplants in laboratory animals could be prevented or diminished by pre-inoculating them with cells from donor animals. This preliminary finding finally led to the first successful human bone marrow transplantation in 1968[1]. Since then, orthopedic diseases have been one of the earliest targets for cellular therapy, and cartilage repair was the first indication for this therapeutic approach[2]. In 1994, Lars Peterson's group designed a study to regenerate cartilage tissue with autologous cartilage cells and showed evidence of regeneration in both animals and humans[3].

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Conflict of Interest

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Although adult mesenchymal stem cells have been the most widely used cellular element in orthopedics[4], a number of other cell-based approaches have been used. Cells used in orthobiologics can be of embryonic, fetal, or adult origin and in turn, can be either autologous or allogeneic. Cells can also be categorized based on differentiation potential. Embryonic cells within the first few divisions after fertilization are totipotent, i.e., they are capable of creating another embryo and differentiate into any cell type, including extra-embryonic cells, and have the highest therapeutic potential. After the first few divisions, embryonic cells become pluripotent and can differentiate to all cells types, except for extra-embryonic cells. Pluripotent cells can be procured directly from human embryos (embryonic stem cells [ESCs]) or from a process known as somatic cell nuclear transfer (SCNT)[5, 6].

The work of Takahashi and Yamanaka in 2006 opened a new avenue in cell-based approaches by introducing the third method for creating pluripotent stem cells: reprogramming adult cells back to a pluripotent state (induced pluripotent stem [iPS] cells). Their group was the first to demonstrate successful de-differentiation of somatic cells into a pluripotent ESC-like status by transfection with four embryonic transcription factors[7].

Because of serious ethical issues, including the possibility of human embryo creation/destruction and risk of cloning an entire organism, the use of pluripotent stem cells is heavily regulated, and therefore, is not readily accessible for research and therapeutic purposes. Accordingly, in the United States these cells can only be used in an FDA-approved trial after a stringent review process[5, 8]. A serious consideration is that ESC and iPS cells have oncogenic potential. If they are injected in an undifferentiated state, they can potentially cause teratomas, and mice generated from iPS cells show high rates of neoplasia. This oncogenicity may be due to the transcription factors used for de-differentiation which are known to be oncogenes, insufficient epigenetic remodeling, or the oncogenic retroviruses used for transfection[9, 10].

After the first 8 weeks of the embryonic stage, there is the next level of undifferentiated state where cells are “multipotent”. These cells, commonly referred to as “stem cells,” are able to differentiate into a limited number of cell types and reside in almost all tissues even after full development and are named based on their tissue of origin, such marrow- or adipose-derived stem cells. They pose less ethical and political controversy, tend to carry less risk, and cause fewer technical challenges than their pluripotent counterparts. Mesenchymal stem cells (MSCs) are by far the most widely used adult stem cells. Although bone marrow and adipose tissues are currently the most popular sources of MSCs because of less elaborate procurement methods, these cells can be obtained from almost all tissue types[11], including tendons and ligaments. Bone marrow-derived mesenchymal stem cells can differentiate into several types of connective tissue including cartilage, bone, tendon, ligament, adipose, and muscle[6, 12, 13].

All the above-mentioned cells can be considered some type of stem cell, although if not otherwise specified, current common usage in the literature mainly refers to undifferentiated adult stem cells. The term “adult” essentially refers to any stem cell beyond the first 8 weeks of embryonic life, where differential potential is confined to a limited number of cell types.

Fully differentiated cells, such as tenocytes, chondrocytes, and tissue-specific endothelial cells, constitute another category of cells with growing popularity in cell-based studies.

Delivery Strategies

Selection of an effective delivery technique is a crucial step in cell-based therapeutics. Currently, there is no consensus about the ideal carrier construct as few clinical data are available for cell-based approaches in tendon repair[10].

The two major delivery categories are direct injection of a cell suspension alone and implantation of cells that are placed in a matrix carrier vehicle. Generally, selection of a delivery category depends on the goal of treatment. Replacement of a lost part will most probably require matrix-based deliveries. Application of a cell suspension is more appropriate in cases where the overall integrity of the tissue is maintained or restored, e.g., degenerative conditions or surgical repairs. An important obstacle in the use of matrix-based approaches is the insufficiency of diffusion of substances into the matrix to nourish and sustain the embedded cells beyond a certain thickness of matrix and, therefore, the need for vascularization, which is still an unsolved clinical problem[14].

A cell suspension can be injected either locally or systemically. Successful systemic injection relies on homing of the injected cells to the target tissue/organ[15, 16]. Homing of stem cells subsequent to systemic injection has been shown in a variety of highly vascular tissues, including bone marrow, myocardium[17], and liver[18]. Systemic administration of cells entails passage through pulmonary capillaries with potential risk of entrapment in the microvasculature[19]. Although MSC homing to fracture site has been documented in the literature[20], direct delivery of cells appear to be a more plausible approach for tendons, regardless of the delivery category.

The majority of studies on tendons have used some form of a scaffold for local delivery of cells. Scaffold application techniques for tendons can be divided into gel suspensions, 3D scaffolds of solid tissue, and combination methods. Gel suspensions offer a favorable 3D filling of the defect, but the lower structural integrity and consequent reduced stability in comparison to other matrix materials may result in loss of the gel at the repair site due to erosion or resorption[10]. Fibrin sealant is a widely used gel scaffold[21, 22] with several advantages including FDA approval, viability of suspended cells, and absence of an adverse effect on tendon healing[23]. Other scaffolds included collagen gel[24], gel-collagen sponge composites[25], and de-cellularized slices of tendon[26].

Other important issues are timing and frequency of delivery and the rate of release when a scaffold is used as the vehicle[27]. A single, one time administration of any cell-based therapeutic may not be sufficient to harness the full potential biologic effects because injected/implanted cells may have limited longevity. Also, the dynamic nature of the cellular composition in the recipient environment may lead to absence of desirable cell-cell interactions at the right micro-anatomical location in a timely fashion. On the other hand, repeated administration is challenging even in animal models and, in case of human patients, may subject the process to greater regulatory restrictions.

Current cell-based approaches

Fetal and Embryonic cells

Culture of ESCs is inherently difficult because of the very small number of cells available to initiate the culture and the complex mixture of growth factors required for induction of differentiation[6]. Almost all investigations regarding the use of fetal and embryonic cells in tendon have been either in vitro or animal studies.

Stepwise differentiation of human ESCs promotes tendon regeneration by secreting fetal tendon matrix and differentiation factors. Chen et al showed MSCs that were derived subsequent to differentiating human ESCs are capable of regenerating patellar tendon in a rat model without formation of teratomas.[28] In another report, Watts et al reported that the use of intra-lesional injection of male, fetal derived embryonic-like stem cells in an equine flexor tendon injury model led to improved tissue architecture, tendon size, tendon lesion size, and tendon linear fiber pattern[29].

In vitro differentiation of ESCs to produce tendinous structures is an active area in tissue engineering. Cohen et al[30] described the efficient derivation of connective tissue progenitors (CTPs) from human ESC lines and fetal tissues. CTPs were induced to generate tendon tissues in vitro, with ultrastructural characteristics and biomechanical properties typical of mature tendons. They also interposed rolled sheets of cultured CTPs in nude rat Achilles tendon full-thickness defects. The group found restoration of plantar flexion compared to control rats.

Amniotic and placenta-derived cells

MSCs derived from extra-embryonic tissues such as umbilical cord and placental tissues are emerging as an attractive source because of relatively easy availability as these tissues are normally discarded at birth, eliminating many ethical concerns[3132, 33]. Accordingly, and also as an alternative to conventional pluripotent cells, amnion-derived stem cells, especially amniotic epithelial cells (AECs), are promising sources for cell-based therapy and have been a focus of active investigation[34].

The amnion derives from the epiblast before gastrulation, the event prior to which cells are still pluripotent. Therefore, amnion-derived cells can potentially differentiate into all cell types[3536]. Interestingly, MSCs isolated from amniotic fluid, umbilical cord blood[37] and Wharton's jelly in the horse show similar biological characteristics: all cell lines expand rapidly in culture, exhibit multi-differentiation potential, are positive for CD90, CD44, CD105, and negative for CD34, CD14 and CD45[38].

Amniotic fluid is another source of AECs. These cells demonstrate a typical epithelial appearance[39] and their pluripotency has been shown by the expression of molecular markers of pluripotent stem cells[35, 36]. Other very interesting characteristics displayed by amniotic cells are absence of tumorigenicty[35], low immunogenicity, and the ability to induce immune-tolerance[40]. The latter two features make these cells promising candidates for allogeneic scenarios. Liu et al have also shown that ovine AECs can differentiate into bone and tendon tissue, both *in vitro* and when implanted into live animals[40–4243]. Furthermore,

stemness and a favorable effect of ovine AECs in a large animal model of Achilles tendon injury have been reported by Muttini[44] and Barboni[40].

An alternative to amnion-derived cells, human amniotic membrane (HAM) is a more conveniently obtainable source which has been used in burn patients and to prevent peritendinous adhesions[45]. Dogramaci et al have recently demonstrated favorable results following application of HAM in an ovine flexor tendon reinforced tendon repair.

More recently, using extra-embryonic tissue, Park et al[46] demonstrated the effects of human umbilical cord blood-derived MSC injection to a full-thickness subscapularis tendon tear in a rabbit model without surgical repair, which were evaluated by gross morphology, histology, and motion analysis of the rabbit activity. The group reported partial healing of tendon tears with histologic evidence of regenerated tendon tissue predominantly composed of type I collagens. Of note, the group did not detect any teratoma formation in study samples.

Mesenchymal stem cells

Mesenchymal stem cells (MSCs) appear to be excellent candidates in cell-based orthobiologics due to their long-term proliferation, high self-renewal rates, and ability of differentiation toward specific cell lineages. Although the exact origin of MSCs is still unclear, it is being increasingly recognized that many tissues harbor an intrinsic stem cell “niche” that could potentially be exploited or stimulated in different ways[47]. One of the more recent hypotheses states that their origin is associated with a unique population of cells lining blood vessel walls, namely endothelial and/or perivascular cells[48].

MSCs have been a focus of intense in vitro and preclinical/animal research. Their ability to differentiate into specific lineages, including a tenogenic lineage, makes them a promising cell source for tendon and tendon-to-bone repair. This section will review several pre-clinical and some recent clinical studies that used MSCs for rotator cuff repair and elbow conditions.

Bone-marrow derived cells

Bone-marrow was first described as a viable source of MSCs (BM-MSCs) in 1970 by Friedenstein et al[49]. Bone marrow represents the standard and the most common source of autologous MSCs with the ability to biologically augment various tendon healing sites.

There are several reports on different methods of obtaining BM-MSCs safely and efficiently. McLain et al[50] were able to isolate autologous BM-MSCs from iliac crest aspirate. Later, several groups showed that concentration of autologous bone marrow aspirate enhances the numbers of progenitor cells[51, 52]. Although the techniques of obtaining and concentrating bone marrow aspirate are evolving[53], fewer than 0.01% of isolated cells are true multipotent stem cells based on the standard criteria described by Dominici et al[54]. In addition to iliac crest, BM-MSCs have also been successfully harvested from vertebrae[50], femur, tibia, and humerus[55–57], obviating the need for iliac crest harvesting.

Animal studies that used MSCs to improve tendon-bone healing in rotator cuff repair have shown encouraging results (Table 1). Gulotta et al demonstrated a positive effect using allogeneic BM-MSCs transduced with the gene for matrix type-I matrix metalloproteinase (MT1-MMP) and scleraxis (SCX) in a rat rotator cuff model[5859]. They showed that SCX led to a significance increase in the strength of repair and the amount of fibrocartilage at 4 weeks[21]. These effects were not seen when BM-MSCs transduced with BMP-13 were used[60].

Another approach to access bone marrow is the creation of multiple transosseous channels in the greater tuberosity, a technique known as microfracture. This has been evaluated in rats to stimulate autologous BM-MSCs into rotator cuff defects[61, 62]. In rabbits, the cells from bone marrow aspirate were either seeded on a polyglycolic acid (PGA) sheet[63] or in an open-cell polylactic acid (OPLA) scaffold[64]. The outcomes of these cell-based scaffold studies have shown improved histological and biomechanical tendon properties but incomplete repair of the tendon-bone insertion.

More recently, human BM-MSCs have been implanted in an athymic rat supraspinatus tendon detachment and repair model. The results showed improved fibrocartilage formation, collagen orientation, and biomechanical strength 2 weeks following repair[65]. Further analysis of the data demonstrate that Indian hedgehog (Ihh) and Sox9 signaling play an important role in the tendon-to-bone healing mechanism[66].

Recently, autologous bone marrow has been used to augment rotator cuff repair in the clinical setting, both through multiple bone channels to promote local infiltration of MSCs[67686970] and application of non-concentrated[71] and concentrated[72, 73] aspirate obtained from the iliac crest (Table 2). Although the results have shown significantly reduced re-tear rates based on tendon imaging, even when BM-MSCs were recruited rather than implanted, the clinical outcomes have shown only minor improvement. To further elucidate the influence of BM-MSCs, it is critical to identify the optimal number, concentration, and characteristics of multipotent stem cells that can be isolated and transplanted to the patient. A fundamental limitation is the fact that the number of defined stem cells by formal molecular criteria in either bone marrow or adipose tissue is very small.

The application of MSCs in lateral epicondylitis has shown encouraging results. Singh et al have showed that a single injection of bone marrow aspirate from iliac crest improved the Patient-rated Tennis Elbow Evaluation (PRTEE) score after a short term follow up (maximum 12 weeks)[74]. More recently, Lee et al injected allogeneic adipose-derived MSCs mixed with fibrin sealant under ultrasound guidance. They demonstrated that the procedure was safe and effective in improving elbow pain, performance, and structural defects after approximately 1 year follow up[75].

Adipose-derived stem cells

Another important source of multipotent stem cells (ADSCs) is the adipose tissue. Zuk et al have shown a favorable potential for augmenting rotator cuff repair with ADSCs[76]. Oh et al were among the first to report the use of injected ADSCs in a rabbit subscapularis rotator cuff model and found better healing properties and histologically decreased fatty infiltration

of the muscle[77]. Mora et al. used ADSCs with a collagen carrier in a rat supraspinatus repair model and demonstrated no improvement in the biomechanical properties of the tendon-to-bone healing, but the ADSC group showed less inflammation based on histologic analysis of the healing tissue[78]. These results suggest that ADSCs could be a promising source. However, more studies are necessary to clarify the roles of these cells in the tendon-bone healing and their effect on muscle degeneration in rotator cuff tears.

Synovium-derived stem cells

Synovium-derived MSCs are reported to exhibit the greatest chondrogenic potential among mesenchymal tissue-derived cells[79, 80] and thus could be a desirable source for enthesis restoration.MSCs from human subacromial bursa were recently characterized to be a potential synovial tissue for biological augmentation of rotator cuff repair[81, 82]. In a cell-based tendon tissue engineering approach, Song et al[83] isolated bursa-MSCs (B-MSCs) from patients undergoing rotator cuff repair and demonstrated that when these cells are pretreated with BMP-12 and seeded in a ceramic scaffold, they expressed tenocyte markers and formed extensive bone, tendon-like tissue, as well as fibrocartilagenous tissue, confirming their substantial potential for application in tendon-to-bone repair.

Tendon derived stem cells (TDSCs)

TDSCs have been identified as an additional cell population in tendons[84] and could be considered one of the newest types of MSCs. The multipotency of TDSCs were also characterized in torn human rotator cuff tendons[85]. TDSCs can be isolated from supraspinatus tendon and long head of biceps tendon during arthroscopic rotator cuff repair[86].

Tao et al demonstrated that early growth response 1 (EGR1) transcription factor plays a key role in TDSC tenogenic differentiation and tendon formation and healing through the BMP12/Smad1/5/8 signaling pathway[87]. Shen et al have shown that allogeneic TDSCs seeded in silk-collagen scaffold enhanced the histological and biomechanical parameters of the rotator cuff tendon. They also demonstrated increased secretion of anti-inflammatory cytokines that prevent immunological rejection[88].

Interestingly, a rare CD146+ tendon-resident stem cell population was identified in a rat patellar tendon. Subsequent to enrichment by connective tissue growth factor (CTGF), these cells demonstrated tenogenic differentiation. Application of these cells in a patellar tendon repair model successfully led to tendon regeneration and functional restoration. These data support the concept of stimulating endogenous progenitor cells, which could potentially overcome the limitations associated with transplantation of exogenous cells[89].

Endothelial cells

It has been demonstrated that local endothelial cells (ECs) are a source of developmental cues for hepatic[90] and pancreatic[91] tissues. It was later realized that local ECs are also a source of regenerative signals in fully developed tissues. Subsequent investigations further elucidated that EC-derived growth factors play critical roles in repair and regeneration of adult bone marrow[92], lung[93], and liver[47] in a tissue-specific fashion. These discoveries were

results of transplantation of tissue-specific ECs in transgenic/mutant animals incapable of visceral tissue healing. Despite promising results from these studies on visceral tissues, hypovascularity and avascularity (in case of cartilage and the avascular zone of the meniscus) of orthopedic soft tissues renders EC-based approaches a challenging endeavor and call for special experimental designs to elucidate the potential role of ECs in these tissues.

Regulatory aspects

In the United States, the growing enthusiasm for using adult stem cell therapies in sports medicine is coupled with significant legal and regulatory obstacles. It is therefore important for the clinician to understand how adult stem cells are regulated in the United States, and how these complex rules are likely to affect what can and cannot be done in clinical practice[94]. In fact, a principal reason why cellular therapies have not been implemented more rapidly in the clinical setting is because of the complex and evolving regulatory requirements that have surrounded cellular products in recent years.[8]

Cell and cell products are regulated under both Public Health Service Act (PHSA) and Food, Drug, and Cosmetics Act (FDCA)[95]. Stem cells can also be considered a medical device, and therefore can be considered as combination products[96]. The definition of cell-based therapies used by regulatory agencies is that the essential feature of these products is the intention for use in diagnosis, treatment, or prevention of disease or affecting the structure or function of the body. The FDA oversees cell therapies through its Center for Biologics Evaluation and Research (CBER) and Center for Devices and Radiological Health (CDRH)[96–98].

FDA categorizes cell-based interventions as human cells, tissues, and cellular and tissue-based products (HCT/Ps) and uses a three-tiered structure to regulate their application. Current good tissue practices (cGTPs) applies throughout[99]. cGTPa are the requirements in subparts C and D of 21 CFR part 1271 that govern the methods used in, and the facilities and controls used for, the manufacture of HCT/Ps, including but not limited to all steps in recovery, donor screening, donor testing, processing, storage, labeling, packaging, and distribution. Table 3 outlines this regulatory structure[100].

The critical term to define in practice is “minimal manipulation.” For cells or nonstructural tissues, minimal manipulation is “processing of the HCT/P [that] does not alter the relevant biological characteristics of cells or tissues.” The degree of cell manipulation is critical in determining where an HCT/P will fall in the following three-tiered framework. Certain methods *have* been expressly regarded as minimal manipulation[101]:

1. Centrifugation
2. Cutting, grinding, or shaping
3. Soaking in antibiotic solution
4. Sterilization by ethylene oxide treatment or irradiation
5. Cell separation

6. Lyophilization
7. Cryopreservation or freezing

Combining HCT/Ps with other “articles” can increase safety concerns. Therefore, the regulations exempt combining the HCT/P with water, crystalloids, or a sterilizing, preserving, or storage agent, provided that their addition poses no additional concerns regarding clinical safety. Other key criteria for classification as low risk are summarized in Table 3.

To comply with FDA regulations, it is of utmost importance that a physician understand what regulatory category a particular procedure involves. Specifically, review of previous classifications of similar products as well as an understanding of the FDA regulatory framework surrounding orthobiologics is important. A dialogue with the FDA is important during the planning of trials and prior to the initiation of new therapeutics. Failing to satisfy any of these requirements will expose the physician and clinic to increasingly stiff sanctions, ranging from site inspections and warning letters to a permanent injunction of the procedure or a shutdown of the entire establishment[102]. It is important to note that in determining the regulatory category and occurrence of non-compliance, it is FDA’s interpretation that counts.

Off-shore establishments have been increasingly used in an attempt to circumvent FDA regulations. Physicians should be mindful that FDA’s oversight holds true even if a single seemingly minor or irrelevant step (e.g., obtaining blood or marrow sample) of the overall application of HCT/P takes place in the U.S., with the rest of steps carried out offshore. It is possible to perform the entire process of harvesting, culturing, expanding, and injecting the patient’s own stem cells overseas, which is apparently outside FDA jurisdiction. However, through its formal Global Initiative, the FDA is currently forging collaborations with countries around the world to harmonize regulations. The central goal of this effort is to build regulatory capacity and develop international standards so that all the countries will employ similar approaches when regulating medical drugs and devices, including adult stem cell therapies[94].

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Table 1

Preclinical studies of MSC therapy in rotator cuff repair.

Study	Year	Animal	Repair Model	Cell Type	N MSCs	Delivery Method
Gulotta et al [20]	2009	Rat	SST	Allogenic BM-MSCs	10^6/animal	Fibrin Glue Carrier
Gulotta et al [54]	2010	Rat	SST	Allogenic BM-MSCs transduced with MT1-MMP	10^6/animal	Fibrin Glue Carrier
Gulotta et al [56]	2011	Rat	SST	Allogenic BM-MSCs transduced with BMP-13	10^6/animal	Fibrin Glue Carrier
Gulotta et al [55]	2011	Rat	SST	Allogenic BM-MSCs transduced with SCX	10^6/animal	Fibrin Glue Carrier
Shen et al [84]	2012	Rabbit	SST	Allogenic TDSCs	6×10^5/animal	Seeded in Silk Collagen scaffold
Yokoya et al [59]	2012	Rabbit	IST	Autologous BM-MSCs	n/a	Seeded in polyglycolic acid (PGA) sheet
Kida et al [57]	2013	Rat	SST	Autologous BM-MSCs	n/a	Transosseous drilling
Kim et al [60]	2013	Rabbit	SST	Autologous BM-MSCs	n/a	Seeded in open-cell polylactic acid (OPLA) scaffold
Levy et al [58]	2013	Rat	SST	Autologous BM-MSCs	n/a	Transosseous drilling + cannulated nitinol implant
Oh et al [73]	2014	Rabbit	SSCT	Allogenic ADSCs	10^7/animal	Injection into SSC muscle
Mora et al [74]	2014	Rat	SST	Allogenic ADSCs	2×10^6/animal	Collagen Carrier
Tao et al [83]	2015	Rabbit	SST	Allogenic TDSCs	4×10^6/animal	Fibrin Glue Carrier
Park et al [44]	2015	Rabbit	SSCT	Human UCB-MSCs	0.1mL/animal	Ultrasound-guided injection
Degen et al [61]	2016	Athymic Rat	SST	Human BM-MSCs	10^6/animal	Fibrin Glue Carrier
Zong et al [62]	2016	Athymic Rat	SST	Human BM-MSCs	10^6/animal	Fibrin Glue Carrier

ADSCs: Adipose Derived stem/stromal cells, BMP-13: bone morphogenetic protein-13, IST: Infraspinatus tendon, MSCs: Mesenchymal stem cells, MT1-MMP: membrane type 1 matrix metalloproteinase, N: Number, SCX: Scleraxis, SSCT: subscapularis tendon, SST: Supraspinatus tendon, TDSCs: Tendon Derived stem/stromal cells, UCB: Umbilical Cord Blood.

Table 2

Clinical studies of MSC therapy in rotator cuff repair.

Study	Year	N	Repair Model	Cell Type	N MSCs	Harvested Site	Delivery Method	Time Points
Ellera Gomes et al [67]	2012	14	Complete RC tears	Autologous BM-MSCs	n/a	Dorsal IC	Injection at the repaired tendon insertion site and bony footprint	12 m
Jo et al [63]	2013	124	SST	Autologous BM-MSCs	n/a	Humerus	Multiple channels in the greater tuberosity	24 m
Milano et al [64]	2013	80	Full-thickness RC tears	BMS	n/a	Humerus	Microfractures of the greater tuberosity	28 m
Hernigou et al [68]	2014	90	SST	Autogenous BM-MSC	51,000/12mL	Anterior IC	Injection at the repaired tendon insertion site and bony footprint	6 m and 10 years
Taniguchi et al [65]	2015	111	All-sized + massive RC tears	BMS	n/a	Humerus	Drilling of multiple holes at the footprint	12-14 m
Skoff et al [69]	2015	10	Revision RCr	Autogenous BM-MSC	n/a	Dorsal IC	Graft Incubation and injection of remaining marrow to the RC construct	24 m
Yoon et al [66]	2016	75	Massive RC tears	BMS	n/a	Humerus	Drilling of multiple holes at the footprint + Patch (human dermis) graft	12 m

BMS: bone marrow stimulation, BM-MSCs: Bone marrow mesenchymal stem cells, IC: Iliac Crest, m: Month, MSCs: Mesenchymal stem cells, RC: Rotator cuff, RCr: Rotator cuff repair, SST: Supraspinatus tendon.

Table 3

FDA regulatory categories.

	Category 1	Category 2: Section 361	Category 3: Section 351
Oversight Level	**No HCT/P oversight**	**Minimal**	**Extensive Regulation**
Product Risk Level	Low	Greater risk with regard to safety	Greatest risk
Focus of the Regulations	N/A	Safety (preventing disease transmission)	Safety *and* effectiveness.
Product Example	Vascularized human organs for transplantation, whole blood and blood-derived products, and extracted human products such as collagen and bone marrow [99].	Bone, cartilage, ligament, tendon, and skin [99].	All other products
Establishment and/or Product Description	An establishment that removes HCT/Ps from an individual and implants such HCT/Ps into the same individual during the same surgical procedure. Products must be minimally manipulated, for homologous use and not combined with another article [99].	The product must be: **1** No more than minimally manipulated. For cells or nonstructural tissues, it means that there must be no change in the "relevant biological characteristics of cells or tissues" during processing, storage, etc. **2** Used for a homologous purpose. **3** Combined with no other cells, tissues, or articles except for water, crystalloids, or a sterilizing, preserving, or storage agent, provided that their addition poses no additional concerns regarding clinical safety. **4** These products must also have either **a.** No systemic effect or otherwise depend on the metabolic activity of living cells for their primary function or **b.** A systemic effect or depend on the metabolic activity of living cells for their primary function. and for • Autologous use • Allogeneic use in a first- or second-degree blood relative. or • Reproductive use.	The product is one or more of the following: **1** More than minimally manipulated, which for cells and nonstructural tissue means to present a risk of change in cell morphology, function, expression, or other relevant biological characteristics during processing, storage, etc. **2** Used for a nonhomologous purpose. **3** Combined with other articles that may pose additional concerns regarding clinical safety. **4** Have a systemic effect or otherwise rely on the metabolic activity of living cells for its primary function, and be used in a context other than autologous use, allogeneic use in a first or second degree relative, or

	Category 1	Category 2: Section 361	Category 3: Section 351
Oversight Level	**No HCT/P oversight**	**Minimal**	**Extensive Regulation**
			reproductive use [98].
Main Regulatory Requirements	Physicians must follow current Good Tissue Practices (cGTPs), but otherwise need not register as an establishment with the FDA's CBER or submit a list of the HCT/Ps used.	Physicians must employ current Good Tissue Practices (cGTPs), register their office or clinic as an "establishment" and submit an annually updated list of each HCT/P manufactured to CBER. They need not obtain premarketing approval before using the product or follow current Good Manufacturing Practices (cGMPs) in preparing them [99].	Establishments must register and file a list of their HCT/Ps with CBER each year. Physician or clinic must complete the expensive process of obtaining formal premarket approval from the FDA (this can involve submitting a New Drug Application, an Investigational New Drug Application, Biologics License Application, or, when dealing with a Section 501k medical device, a premarket approval application or premarket notification). Physicians must follow the FDA-prescribed current Good Manufacturing Practices (cGMPs) and prescription drug labeling requirements that govern commercial pharmaceutical manufacturers.