Stem cell research and clinical development in tendon repair

Paola Filomeno[1],
Victor Dayan[2],
Cristina Touriño[3]

[1] Hospital de Clínicas. Facultad de Medicina. Universidad de la República
[2] Hospital de Clínicas. Facultad de Medicina. Universidad de la República, Uruguay
[3] Hospital de Clinicas. Facultad de Medicina. UDELAR, Uruguay

Corresponding author:
Cristina Touriño
Hospital de Clínicas. Facultad de Medicina, Universidad de la República
Av. Italia S/N
Montevideo, Uruguay
e-mail: ctourino@hc.edu.uy

Summary

Stem cells are one of the most fascinating areas in regenerative medicine today. They play a crucial role in development and regeneration and are defined as cells that continuously reproduce themselves while maintaining the ability to differentiate into various cell types. Stem cells are found at all developmental stages, from embryonic stem cells (ESCs) which differentiate into all cell types, to adult stem cells (ASCs) which are responsible for tissue regeneration. Studies using animal models have shown promising results following cell therapy for induced injury in musculoskeletal system, including tendon healing, but the results can be variable. Alternative sources for cell therapy in tendon pathology may include ESCs, ASCs (bone marrow, adipose tissue or tendon derived stem cells) or induced pluripotent stem cells (iPSCs). While ethical and safety concerns currently forbid clinical application of ESCs and iPSCs, initial clinical trials with ASCs are promising.

Key words: cell therapy,embryonic stem cell, induced pluripotent stem cells, adult stem cells, mesenchimal stromal cells, tendon repair.

Introduction

Stem cells have several distinct characteristics that distinguish them from other cell types and are one of the most fascinating areas in regenerative medicine today. These cells are unspecialized, self-renewing and can be induced to differentiate into various cell lineages[1], having a crucial role in the development and regeneration of human life. Stem cells are defined as cells that continuously reproduce themselves while maintaining the ability to differentiate into various cell types. They are found at all developmental stages, from embryonic stem cells which differentiate into all cell types found in the human body to adult stem cells which are responsible for tissue regeneration. General opinion postulates that clinical therapies based on stem cells may have the potential to change the treatment of degenerative diseases and severe traumatic injuries in the "near" future.

During embryogenesis, a single fertilized oocyte gives rise to a multicellular organism whose cells and tissues have adopted differentiated characteristics or fates to perform the specified functions of each organ of the body. As embryos develop, cells that have acquired their particular fate proliferate, enabling tissues and organs to grow. Even after an animal is fully grown many tissues and organs maintain a process known as homeostasis, in which as cells die, either by natural death or injury, they are replenished. This remarkable feature has ancient origins, dating back to the most primitive animals, such as sponges and hydrozoans. Mammals seem to have lost at least some of this wonderful plasticity, however, the liver can partially regenerate providing that injury was not too severe, and epidermis and hair can readily repair when wounded or cut. Additionally, epidermis, hair, small intestine, and hematopoietic system are all examples of adult tissues that are naturally in a state of dynamic flux. Even in the absence of injury, these structures continually give rise to new cells, which are able to transiently divide, terminally differentiate and die.

The fabulous ability of an embryo to diversify in all cell types and certain adult tissues to regenerate throughout life is a direct result of stem cells, a nature's gift to multicellular organisms. Stem cells have both the capacity to self-renew, that is, to divide and create additional stem cells, and to differentiate along a specified molecular pathway. Embryonic stem cells are very nearly totipotent, reserving the elite privileges of choosing among most if not all of differentiation pathways. In contrast, stem cells that reside within an adult organ or tissue have more restricted options, often able to select a differentiation program from only a few possible pathways. A new type of cell, which has been recently engineered, is the induced pluripotent stem cell (iPSC). These cells represent the bridge between adult (ASCs) and embryonic stem cells (ESCs), while they are adult cells, genetic modification renders them with embryonic characteristics (Tab. 1).

Table 1 - Attributes, limitations and ethical concerns of different stem cell types for cellular therapy.

	Embryonic Stem Cells		Induced Pluripotent Stem Cells (iPSCs)	Adult Stem Cells
	In Vitro Fertilization (IVF)	Somatic Cell Nuclear Transfer (SCNT)		
Cells Attributes	• can produce all cells types • relatively easy to identify, isolate, maintain and culture • large source of blastocysts from IVF clinics	• can produce all cell types • relatively easy to identify, isolate maintain and culture • cells may be genetically matched to patient	• can produce many cell types • cells may be genetically matched to patient	• demonstrated success in some treatments • cells may be genetically matched to patient
Limitations	• limited number of cell lines available depending of national legislation • immune rejection • risk of creating teratomas	• not extensively demonstrated in human system • very labour-intensive technique • risk of creating teratomas	• low transformation efficiency • risk of mutagenesis insertional when using virus as vectors • risk of senescencia and cancer • risk of creating teratomas	• produce limited number of cell types • not found in all tissues • difficult to identify and isolate • relatively difficult to maintain and culture
Ethical Concerns	• destruction of human blastocysts • donation of blastocysts requires informed consent	• destruction of human blastocysts • donation of oocytes requires informed consent • concern about misapplication form reproductive cloning	• no major ethical concerns	• no major ethical concerns

Embryonic stem cells (ESCs)

Emanating from the pioneering mouse research of Martin Evans[2] in the 1970s and culminating with the recent successful experiments with human tissue[3], cells from the inner cell mass (ICM) of mammalian blastocysts can be maintained in tissue culture under conditions where they can be propagated indefinitely as pluripotent embryonic stem cells. If injected back into a recipient blastocyst, which is then carried to term in a female host, these cells can contribute to virtually all tissues of the chimeric offspring, including the germ cell compartment. To maintain cultured ESCs in their relatively undifferentiated pluripotent state, they must both express the intrinsic transcription factor Oct4 and constitutively receive the extrinsic signal from the cytokine Leukemia Inhibitory Factor (LIF)[4].

Upon LIF withdrawal, cultured ESCs spontaneously aggregate into embryo-like bodies, where they differentiate and spawn many cell lineages, including beating heart muscle cells, blood islands, neurons, pigmented cells, macrophages, epithelia, and fat-producing adipocytes[5]. Similarly, when ESCs are injected into nude mice, they differentiate into multicellular masses, called teratocarcinomas.

Although the programs of gene expression in these structures often bear strong resemblance to the differentiation pathways typical of developing animals, the triggering of these programs is chaotic, yielding a jumbled grab bag of tissue types. These examples graphically illustrate the importance of intercellular interactions and cellular organization in orchestrating development and embryo shape.

Due to their enormous capacity to differentiate into almost every cell type of the body and potentially replenish damaged tissues, ESCs have been used experimentally in diverse animal models. They have been shown to successfully replace neural, cardiac, hepatic, hematopoietic tissue among others[6]. Nonetheless, ethical considerations and safety concerns (risk of teratoma formation and immune rejection upon transplantation) are the main limitations of their use in humans.

First human ESCs were isolated in 1998 by Thomson et al.[3] from *in vitro* fertilization clinics embryos. Few human ESC lines are available and there is a concern over the genetic stability after long-term amplification *in vitro*[7]. Nowadays only 2 trials have been authorized to use ESCs in humans: an US company endorsed trial for neurodegenerative disease which never made it to a peer review journal and recently, the first European trial has been given green light to start recruiting patients with macular degeneration[8]. Both trials have short term follow-up (both started in 2011) and therefore potential side effects such as teratoma formation is not established.

Immunological issues (rejection) associated with the use of ESCs could be overcome by the technique known as somatic cell nuclear transfer (SCNT)[9]. When a nucleus from a differentiated somatic cell, is transplanted into an enucleated oocyte, nuclear reprogramming is initiated, leading to the generation of an entire individual, which is genetically identical clone of the original somatic cell. Generation of pluripotent cells by SCNT has been well documented in mouse and other animal models[7]. This could generate custom-made, patient-specific ESCs which can be induced to differentiate and then transplanted without immune rejection since they have the genetic material of the patient. However, this technique is very labour-intensive. Reprogramming by nuclear transfer technique has not been extensively demonstrated in humans since oocyte provision is not only a rare opportunity, but also an ethical concern of the moment. Another method to generate human pluripotent cells is cell fusion between somatic cells and human ESCs which is leading to the birth of “heterokaryon”. Since the reprogrammed cells contain chromosomal materials from both cell types and exhibit chromosomal tetraploid, it needs to be clarified whether the differentiated progeny from these hybrid cells are functional and their risk for neoplasic cell transformation[7].

Adult stem cells (ASCs)

Adult stem cells were described 50 years ago in the mouse bone marrow[10,11]. Hematopoietic stem cells (HSCs) have been classically defined as bone marrow derived cells that are capable of both self-renewal and multipotential hematopoietic differentiation and give rise to all blood-cell lineages. Later, Friendenstein et al.[12] found another cell population with stem cell-like characteristics in the bone marrow and called them colony forming unit-fibroblast, now known as mesenchymal stromal/stem cells (MSCs). Later on, such cells were found in almost every tissue of the body, and were broadly categorized into adult stem cells.

While diversification of cell types is largely complete at or shortly after birth, many adult tissues undergo self-renewal and accordingly must establish a life-long population of relatively pliable stem cells. Adult stem cells are often relatively slow-cycling cells able to respond to specific environmental signals and either generate new stem cells or select a particular differentiation program. When a stem cell commits to differentiate, it often first enters a transient state of rapid proliferation. Upon exhaustion of its proliferative potential, the cell withdraws from its cycle and executes its terminal differentiation program[13].

Adult stem cells are localized to specific niches, where they use many of the stimulatory and inhibitory signals used by their embryonic counterparts for selecting a specific fate. ASCs have been found in the following tissues: bone marrow, liver, umbilical cord, brain, adipose tissue, gut, among others. Cells found in each of these niches share the same properties: self-renewal and differentiation capacity. In contrast to ESCs, ASCs are more restrained to their differentiation possibilities and are favored towards the tissue in which they reside. Such that muscle stem cells differentiate mainly towards myoblasts and hematopoietic stem cells towards blood cells.

Recent investigations show a regulatory role for blood vessels in these specialized niches. Mesenchymal stromal/stem cells (MSCs) are located surrounding capillaries in a variety of tissues and have the capacity to differentiate into different mesodermal lineages. Angiogenic progenitor cells have also been found in the adventitial layer of large vessels. In the bone marrow, endothelial cells control hematopoietic stem cells release, and in the brain, blood vessels regulate neural stem cells self-renewal and neurogenesis. Similarly, perivascular progenitor cells have also been found in the heart. This intimate connection between stem cells and the vasculature contributes to tissue homeostasis and repair[14].

Knowledge of new stem and progenitor cell populations in the body is accumulating at a rapid pace and a new era of targeting resident stem cell populations for therapeutic purposes is coming into focus.

Hematopoietic Stem Cells (HSCs)

Despite its complexity, blood is probably the best understood developmental system. Easily accessed, blood and bone marrow have been object of study for many years. Direct sampling of the hematopoietic tissues in the bone marrow presents a rather low bar for biopsy acquisition from living donors, especially when compared to other systems such as heart or brain. This relatively easy procedure to obtain cells from primary anatomical location of blood cell genesis and differentiation as well as a reliable transplantation assay and well-described surface markers make HSCs the best understood of all tissue stem cells.

Clinically, HSCs transplantation represents the most widely deployed regenerative therapy, but human HSCs have only been characterized recently. Over the past 10 years, increasing evidence has accumulated that heterogeneity is a feature of HSCs proliferation, self-renewal, and differentiation based on examination of these properties at a clonal level[15]. Recent progress in the hematopoietic field has included identification of HSCs capable of long-term engraftment at the single-cell level, improvements in *ex-vivo* expansion of HSCs, transdifferentiation of somatic cells into hematopoietic progenitors, and the 'correction' of several disease-specific iPSCs using various gene-targeting strategies[16].

The HSCs, commonly used for stem cell transplantation can be obtained from bone marrow, peripheral blood or umbilical cord blood. Adult bone marrow, situated within the bone cavity, comprises three distinct stem cell populations: HSCs, mesenchymal stromal/stem cells (MSCs) and endothelial progenitor/stem cells (EPCs). The homeostasis inside the bone marrow and within the entire body is sustained by an intricate network of growth factors and transcription factors that orchestrate the proliferation and differentiation of these multipotent stem/progenitor cells. A small proportion of cells in peripheral blood are actually pluri/multipotent stem cells. These peripheral blood stem cells (PBSCs) are thought to be heterogeneous and could be exploited for a variety of clinical applications. The exact number of distinct populations is unknown. It is likely that individual PBSC populations detected by different experimental strategies are similar or overlapping but have been assigned different names. Zhang et al.[17] divide PBSCs into seven groups: hematopoietic stem cells (HSCs), CD34- stem cells, CD14+ stem cells, MSCs, very small embryonic-like (VSEL) stem cells, endothelial progenitor cells (EPCs), and other stem cells. Umbilical cord blood (UCB) was initially employed in the treatment of blood malignancies due to its high concentration of hematological precursors and is now a non-controversial and accepted source of HSCs and non-hematopoietic progenitors cells for a variety of emerging cell therapies in clinical trials[18].

Mesenchymal Stem/ Stromal Cells (MSCs)

Mesenchymal stem cells, also called mesenchymal stromal cells, bone marrow stromal stem cells, multipotent adult progenitor cells, mesenchymal adult stem cells or tissue stem cells, exist in almost all tissues and are a key cell source for tissue repair and regeneration. These cells are generally thought to be resident in the perivascular compartment of these tissues[19]. Recent studies have

suggested that resident in almost all tissues are a small number of dormant stem cells that can become activated and specifically migrate to sites of tissue damage, where they then perform repair functions[20]. Isolation of MSCs can be from numerous connective tissues but is most common from bone marrow. MSCs were originally identified by Friedenstein et al.[21] as the primary transplantable component of the bone marrow microenvironment necessary for the maintenance of definitive hematopoiesis.

The term mesenchymal stem cells has been criticized, as there is little data demonstrating self-renewal of definitive single-cell-derived clonal populations from a mesenchymal cell source[22,23]. The differences between MSCs populations derived from different tissues are becoming more apparent, presenting an additional challenge to devising a universal definition[24]. However, accumulating preclinical and clinical evidence indicates that these cells are good candidates for cell therapy. Under pathological conditions, such as tissue injury, MSCs are mobilized towards the site of damage. Tissue damage is usually accompanied by proinflammatory factors, produced by both innate and adaptive immune responses, to which MSCs are known to respond. Indeed, recent studies have shown that there are bidirectional interactions between MSCs and inflammatory cells, which determine the outcome of MSC-mediated tissue repair processes. In addition, MSCs may have potential in suppressing uncontrolled immune responses, providing in situ negative regulation during the inflammatory response. Although the immunomodulatory capacity of MSCs could be use therapeutically, there may also be unwanted effects associated with immunosupression[25].

The International Society for Cellular Therapy has established minimal criteria for defining MSCs[26]. These basal attributes include the abilities to adhere to plastic under normal cell culture conditions, to express a set of cell surface antigens (CD105, CD73, and CD90) while not expressing antigens indicative of other cell lineages, and to differentiate into adipocytes, osteoblasts, and chondroblasts under specific conditions. This has served to allow a basis of comparison between the results of different investigators and has allowed a more focused investigation for clinical trials. MSCs as currently defined are a phenomenon of in vitro culture, suggesting that extrapolating the function of these cells to activity *in vivo* must be done with caution[24].

In culture, most MSCs have a spindle morphology like fibroblasts, and can be maintained for several passages without significant alterations in their major properties. MSCs are multipotent and can differentiate into distinct cell types, such as chondrocytes, osteoblasts, and adipocytes[27]. However, recent findings show that serial passages of MSCs in culture lead to decreased differentiation potential and stem cell characteristics, eventually inducing cellular aging which can limit the success of cell-based therapies. Other studies indicate that *in vitro* aging (passage number in culture) is more important than *in vivo* aging (donor age) when considering the proliferation and differentiation potential of MSC[28]. MSCs derived from adult bone marrow can be cloned and expanded *in vitro* without loss of differentiation potential; these bone marrow-derived MSCs are the most routinely used in studies. However, many properties of these cells remain unknown.

The mechanisms underlying tissue regeneration and immune modulation by therapeutic doses of MSCs also require further elucidation, particularly the extent to which the two processes intersect. The more recent appreciation that MSCs may not mediate tissue regeneration by direct cell replacement is also likely to redirect investigation into more fruitful directions. The effect of MSCs can be through differentiation toward target cell lineage but is more likely to involve trophic modulation by paracrine and autocrine activity; secretion major histocompatability complex (MHC) of angiogenic, chemoattractant, and antiapoptotic factors; and specific anti-inflammatory effects through reduced T-cell activity and MHC suppression[29]. As a result of their extensive proliferative capacity, it is possible to produce relatively large numbers of MSCs for potential clinical applications from bone marrow, umbilical cord, or adipose tissue. Several studies have provided important information about the safety of MSC-based therapy. There are over 100 clinical trials with MSCs registered on clinicaltrials.gov. While a majority of these trials are not completed, the available data, most of which are derived from GvHD treatment, are not conclusive, which suggests the need for larger trials with MSCs. In terms of clinical applications, MSCs are being tested in four main areas: tissue regeneration for cartilage, bone, muscle, tendon and neuronal cells; as cell vehicles for gene therapy; enhancement of hematopoietic stem cell engraftment; and treatment of immune diseases such as graft-versus-host disease, rheumatoid arthritis, experimental autoimmune encephalomyelitis, sepsis, acute pancreatitis and multiple sclerosis[25]. In view of the extraordinarily rapid and extensive use of MSCs clinically, a reappraisal of the approach to the development of clinical protocols based on confirmed laboratory and preclinical observations would be timely and helpful[24].

Tendon-Derived Stem Cells (TDSCs)

The TDSCs have been described in 2007 by Bi et al.[30]. These stem cells present in mature tendon have self-renewal and multilineage differentiation potential. They can differentiate into other cell types, like muscle or fat cells. These cells have been implicated as possible cause of chronic tendinopathy because of the erroneous differentiation into abnormal matrix components causing fatty degeneration and calcification. These cells are still in the preclinical experimentation stage but has great potential for tendon therapy in the future[31,32].

Induced pluripotent stem cells (IPSCs)

The iPSCs are adult somatic cells which acquire embryonic stem cell potential after genetic modification. They are similar in many aspects to natural pluripotent stem cells (like ESCs), such as expression of certain stem cell genes and proteins, chromatic methylation patterns, doubling time, embryoid body formation, teratoma formation, viable chimera formation, potency and differentiability.

The iPSCs were first generated by Shinya Yamanaka's team at Kyoto University, Japan in 2006[33]. Since the ability to reprogram adult cells was demonstrated, they managed to identify four important transcription factors that could induce fibroblasts to become embryonic like-stem cells known as 'Induced pluripotent stem cells'. Yamanaka used retroviruses to transduce mouse fibroblasts with a selection of genes that had been identified as particularly important in ESCs. Eventually, four key pluripotency genes essential for the production of pluripotent stem cells were isolated; Oct-3/4, SOX2, c-Myc, and Klf4 (Yamanaka factors). In 2007, the same investigator managed to accomplish the same achievements using human fibroblasts[34]. The success of iPSCs was confirmed by latter studies from multiple groups, including the combination of different factors like Nanog and Lin28. These cells passed the most stringent examinations for gene expression profile, pluripotency, self-renewal and germ layer differentiation both *in vitro* and *in vivo*, confirming their remarkable similarity to ESCs. The iPSCs share the same advantages and disadvantages of ESCs with the exception of their major ethical concern since they are from adult patients and the fact that cells may be genetically matched to patient. But other potential drawback is the likely presence of inherited or accumulated mutations in the genome from older adult cells that would predispose them to senescence or cancer. There are currently two additional problems with the iPSC technology: the efficiency of human iPSC is substantially low (less than 0.1% of fibroblasts become iPSCs) and the use of virus as a vector can result in the random integration of viral DNA into the host-cell genome[7].

Various groups have tested successfully the use of iPSCs for the regeneration of diverse tissues such as neural, cardiac, hematopoietic, chondrogenic and osteogenic in different models[35-37]. In order to avoid genetic manipulation of adult somatic cells, investigators have tested the possibility of obtaining iPSCs only using the Yamanaka's factors as proteins to stimulate fibroblasts[38]. Furthermore, in order to avoid the main caveat of iPSCs use (which is teratoma formation), various groups have reported direct differentiation of adult somatic cells such as fibroblasts towards neural and cardiac phenotype[39]. Recent studies have shown that an ESC-enriched noncoding RNA (miR-302) induces somatic cell reprogramming to form iPSCs, suggesting its pivotal role in stem cell generation. This miR-302-induced somatic cell reprogramming involves an epigenetic reprogramming mechanism similar to the natural zygotic reprogramming process in the two- to eight-cell-stage embryos and this mechanism can be use to improve iPSC generation. For regenerative medicine, the dual function of miR-302 in both reprogramming and tumor suppression has provided us a convenient means to control the quantity and quality of iPSCs[40].

Tendon regeneration: perspectives and ethical problems using ESCs and IPSs

Cell therapy and stem cell research play an important role in orthopedic regenerative medicine today. However, many elements are required to coordinate the generation of a functional tertiary structure in orthopedic systems, including the choice of most appropriate cell type, a vehicle to support cells, stimulatory and coordinating paracrine factors, malleability to change during tissue regeneration and volume limitation. Current literature provides us with promising results from animal research in the fields of bone, tendon and cartilage repair. While early clinical results using adult stem cells are already published for bone and cartilage repair, the data about tendon repair is limited to animal studies. There are even fewer data using ESCs or iPSCs.

In tendon laceration or strain injury, the local environment is often inundated with fibrovascular and later disorganized fibrous tissue. After a tendon injury, the tendon normally heals through scar tissue formation, which may take up to 1 to 2 years to mature[41]. During this course, the cellularity of the tendon is increased, nevertheless, the infiltrating scar fibroblasts do appear morphologically different from native tenocytes. This lack of regeneration ability of adult tendons has lead to compare the healing properties between adult and fetal tendons[42,43]. Results from a fetal tendon injury model show that no abnormalities occur in the wound, with reconstitution of the collagen architecture[44]. Thus, ESCs may have the potential for tendon regeneration. However, until nowadays, studies have been made only *in vitro* or in animal models (Tab. 2). Fetal-derived embryonic stem cell-like cells have recently been evaluated for tendon and ligament repair. Chen et al.[45] have demonstrated that human ESCs improve both *in vivo* and *in vitro* tendon regeneration after stepwise differentiation from ESCs to tenocytes through a MSCs transition stage. Under *in vitro* mechanical stress, human ESC-derived MSCs (hESC-MSCs) differentiate and form tendon-like tissues. In animal models these cells improved tendon regeneration both structurally and functionally. No teratomas were found at 4 weeks after cell implantation, and even at 8 weeks after ectopic implantation in SCID mice and 8 weeks in rats. Watts et al.[46] studies revealed substantial and clinically relevant improvement in the healing of tendon injury after intra-lesional injection of pluripotent stem cells in a large animal model. Many studies provide evidence for the possibility of using ESCs-derived engineered grafts to replace missing tendon tissue[47-50]. Early-stage fetal tissue has been used to develop an ESC-like cell line that expresses all the markers of ESCs but without the teratogenic potential, providing a better safety profile. Evaluation of these ESCs in a new equine enzymatic/physical defect tendonitis model indicates advantages to the use of ESCs compared with MSCs. There are no published data documenting the outcome in clinical trials in animals, but anecdotal data after ESC injection of flexor tendon injury in several hundred horses used in a variety of athletic pursuits are very supportive of future application in humans[29,51]. The use of human ESCs as a resource for cell therapeutic approaches is an interesting alternative, however, from a legal and ethical point of view, research involving human embryonic cells is highly controversial and many countries are reviewing their legislation. Besides the ethical concerns, the use of embryonic stem cells is problematic, as the use of allogenic pluripotent cells involves an oncogenic potential that currently forbids the application in patients.

Table 2 - Animal models for studying cell-therapy in tendon repair (Modified from Nixon et al.[31] & Ahmad et al.[29])

Model	Tendon	Cell type implanted	Method
Rabbit	Infraspinatus tendon	Fibroblasts	Associated with chitosan- HA hybrid
	Infraspinatus tendon	Periosteal progenitor cells	Associated with PEG-BMP-2 hydrogel
	Achilles tendon	BM-MSCs	Associated with collagen gel or fibrin
	Patellar tendon	BM-MSCs	Associated with collagen gel or fibrin, fresh & cryopreserved cells
	Patellar tendon	Fibroblasts	Associated with alginate-chitosan fibers
	Hamstring tendon	BM-MSCs	Associated with collagen gel
	Supraspinatus tendon	BM-MSCs	Cell impregnated with alginate beads
Rat	Achilles tendon	Syn-MSCs	Associated with collagen gel
	Achilles tendon	BM-MSCs	Associated with fibrin or DMEM as vehicle
	Achilles tendon	Whole BM cells	DMEM as vehicle
	Patellar tendon	BM-MSCs	Associated with fibrin
	Flexor tendon (long DF)	BM-MSCs	Associated with pluronic F-127
	Supraspinatus tendon	BM-MSCs	Associated with fibrin
	Calcaneal tendon	BM-MSCs	Associated with collagen + BMP-12
Chicken	Flexor tendon	Tenocytes	Associated with PGA
Pig	Flexor tendon (SDF)	Dermal fibroblasts	Associated with PGA
Horse	Flexor tendon (SDF)	BM-MSCs	Saline solution as vehicle
	Flexor tendon (SDF)	BM-MNCs	Saline solution as vehicle
	Flexor tendon (SDF)	BDSCs	Erythrocytes lysis & cell incubation with M-CSF for 72 hours
	Flexor tendon (SDF)	Adipose-derived cells	Saline solution as vehicle
	Flexor tendon (SDF)	Fetal-derived ESCs	DMEM as vehicle
ID-rats	Patellar tendon	Human-BMSC	Cells + fibrin

BDSCs, Blood derived stem cells; *BM*, bone marrow; *BM-MSCs*, Bone marrow mesenchimal stromal cells; *BMP,* bone morphogenetic protein; *DMEM,* Dulbecco´s minimal essential medium; *HA*, hyaluronic acid; *ID-rats*, Immunodeficient rats; *long DF*, long digital flexor tendon; *M-CSF*, Macrophage Colony-Stimulating Factor; *MNCs,* Mononucleated Cells; *PGA*, polyglycolic acid; *SFD*, superficial digital flexor tendon.

Given the concurrent social and moral dilemma regarding harvesting of embryonal or fetal tissues as well as the benefits of fetal derived ESCs, the iPSCs may potentially solve some current concerns in stem cell therapy for nonfatal disease such as tendon injury. Thus, the iPSCs could provide the possibility of autologous therapy with easily accessible pluripotent cells. Recently, it has also been developed iPSCs lines from large animals like horses, which comprise a major subset of experimental models of tendon disease and repair[52]. The horse has several well-known models of tendon injury, and iPSCs therapy holds considerable promise as the adult-derived version of fetal-derived ES-like cells. However, this field is still evolving because problems with incomplete reprogramming in some iPSCs lines, including the equine iPSCs. Besides the great potential this technique undoubtedly represents, it also bears some essential safety problems that are currently far from being solved. As ESCs, these cells present a high oncogenic potential, which currently forbids its application in patients.

While ethical and safety concerns currently forbid application of ESCs and iPSCs in patients[53] adult stem cells raise less ethical concerns and have proved to be safer than pluripotent stem cells (Tab. 1). In recent years, the use of cell therapy with ASC in equine veterinary medicine has been intensively studied and their regenerative effect has been described in tendon and ligament injuries[54,55]. ASCs therapy in tendon repair likely has more a role in coordinating regeneration rather than supplementing cell numbers to bridge the void in tendon architecture[29]. Clinical application of cultured bone marrow–derived MSCs in clinical tendonitis in racehorses has resulted in improved return to athletic activity in long-term studies. Application of MSCs in biologic matrices generally improves retention of cells at target sites and may improve tendon repair. One of the consistent findings in the use of bone marrow–derived MSCs and adipose-derived vascular-stromal fraction cells in the equine model has been their anti-inflammatory impact. Simple autologous products such as bone marrow aspirate or platelet-rich plasma (PRP) provide biological agents that seem to enhance repair[29].

In humans the available evidence from clinical trials for the use of stem cells in tendon treatment is limited[31,56]. A clinical trial testing the local application of bone marrow aspirate derived from the proximal humerus at the time of arthroscopic rotator cuff surgery have yielded positive results[57]. Gomes et al.[58] investigated the effect of therapy with bone marrow mononuclear cells extracted from the iliac crest and injected into tendon borders after fixation by transosseous stiches in patients with complete rotator cuff tears. The results suggest that this treatment is safe and has potential to enhance tendon repair. Other studies investigated the use of skin-derived tenocyte-like cells in the treatment of lateral epindondylitis and patellar tendinopathy and the results show a positive effect on tendon healing[31].

Increasing numbers of experimental studies describe improved outcome after use of a combination of stem cells and integrated genes to stimulate stem cell function in the

regenerating tendon. The principal growth factors evaluated include bone morphogenetic protein (BMP) 12, 13, and 14; platelet-derived growth factor subunit B (PDGF-B); basic fibroblast growth factor (bFGF); insulin-like growth factor 1 (IGF-1) and vascular endothelial growth factor (VEGF). Stem cells, either primed by exposure to recombinant growth factors or containing anabolic transgenes, seems to be an appropriate technique for enhanced rotator cuff repair. Stem cells treated with platelet-rich plasma could also become a potential a standard treatment. No results of clinical trials in humans have been published[29].

In summary, more studies that compare stem cells from different compartments, including the recently discovered tendon stem cells, are needed to determine which stem cell population has the greatest ability to enhance tendon repair. In addition, more work will be require to determine the optimal combinations, timing, and cell dosing. Thus, controlled studies are required to have more evidence and define the role of cell therapy strategies in the standard orthopedic care.

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