Stem Cell Reviews and Reports (2018) 14:346–369
https://doi.org/10.1007/s12015-018-9800-6

Mesenchymal Stem Cells in the Musculoskeletal System: From Animal Models to Human Tissue Regeneration?

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Published online: 20 March 2018

Abstract
The musculoskeletal system includes tissues that have remarkable regenerative capabilities. Bone and muscle sustain microdamage throughout the lifetime, yet they continue to provide the body with the support that is needed for everyday activities. Our current understanding is that the regenerative capacity of the musculoskeletal system can be attributed to the mesenchymal stem/ stromal cells (MSCs) that reside within its different anatomical compartments. These MSCs can replenish various tissues with progenitor cells to form functional cells, such as osteoblasts, chondrocytes, myocytes, and others. However, with aging and in certain disorders of the musculoskeletal system such as osteoarthritis or osteoporosis, this regenerative capacity of MSCs appears to be lost or diverted for the production of other non-functional cell types, such as adipocytes and fibroblasts. In this review, we shed light on the tissue sources and subpopulations of MSCs in the musculoskeletal system that have been identified in animal models, discuss the mechanisms of their anti-inflammatory action as a prerequisite for their tissue regeneration and their current applications in regenerative medicine. While providing up-to-date evidence of the role of MSCs in different musculoskeletal pathologies, in particular in osteoporosis and osteoarthritis, we share some thoughts on their potential as diagnostic markers in musculoskeletal health and disease.

Keywords Musculoskeletal system · Mesenchymal stem cells · Bone · Muscles · Synovium · Regenerative medicine · Osteoarthritis · Osteoporosis

Mesenchymal Stem Cells Within the Musculoskeletal System: Heterogeneous Obscure Populations Within Several Tissues

The cells that constitute our tissues and organs go through the continuous process of death and replacement. Indeed, the musculoskeletal system, which is of utmost importance for human health and mobility in everyday life activities, is built of tissues that have remarkable regenerative capabilities. Tissues can thus undergo renewal, such as the renewal of the bone of the skeleton every 10 years, and also the growth of new bone after bone fractures [1]. Similarly, skeletal muscles can repair micro-tears that can occur during intense physical activity or injury [2].

These regenerative features are due to the activity of mesenchymal stem/ stromal cells (MSCs), which are multipotent progenitor cells that form the musculoskeletal tissues during embryonic development. What is really intriguing is that MSCs are also retained as rare cell populations in several adult organs and tissues. MSCs were initially described in the bone marrow based on their unique combination of features, which include colony formation, multipotency, and plastic in vitro adherence [3, 4]. Cells with similar in vitro abilities have since been isolated from numerous adult tissues and organs [5]. For example, for the adult knee joint as a vital part of the musculoskeletal system, MSCs-like cells have been identified for the following sites: trabecular bone (epiphysis, metaphysis), bone marrow, periosteum, synovium, infrapatellar fat pad, synovial fluid, and skeletal

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muscle (Fig. 1). At the same time, although the ligaments and tendons have been studied to a lesser extent, they have also been identified as a source of progenitor cells similar to MSCs [17, 39]. Also for cartilage, where despite being an avascular tissue with low healing capacity, a few studies have identified cartilage as a source of mesenchymal progenitor cells [42–44].

Even though the hunt to identify such putative MSC populations in the adult organism has been ongoing for decades, our current knowledge suggests that these MSC-like cells are tissue specific and have distinct phenotypes and differentiation potentials [45, 46]. The hypothesis that currently comes closest to the term MSCs is that they are pericytes, with their CD34-/ CD45-/ CD146 + phenotype. These MSC-like cells thus reside on the walls of the microvasculature, and hence they are ubiquitously present in vascularized tissue [47]. This hypothesis also provides a reasonable explanation why highly vascularized tissues, such as bone marrow, are better sources of these MSCs than poorly vascularized tissues, such as tendons, ligaments and cartilage. However, this hypothesis was challenged recently by Guimarães-Camboa et al. [48], where their tracing of the lineage of the *Tbx18* gene showed that pericytes and vascular smooth muscle cells maintain their identity during aging and in diverse pathological settings, and do not significantly contribute to other cell lineages.

Such studies have now provided a huge amount of knowledge on different MSC-like cell populations and their contributions to cartilage and bone repair in different animal models of musculoskeletal disorders. However, there has been little translation of these data to human, which leaves us with a huge gap between what we know about these cells and how we can take advantage of this knowledge for regenerative medicine. This is partly due to the markers identified in mice being different from those in humans, which still remain to be identified. Also, the results of basic studies have often been brought to clinical trials too early, without sufficient knowledge about how to isolate a specific population of MSC-like cells with the desired regeneration potential. Despite these problems, cell therapies containing MSCs are currently in use in clinical practice, and have shown promising results. However, based on results of basic scientific research, there remains a lot of space for improvement.

In this review, we provide an overview of MSC sources and subpopulations in the musculoskeletal system, we discuss their mechanisms beyond tissue regeneration, their current clinical applications and their potential in the diagnosis and treatment of common disorders of the musculoskeletal system.

Identification of MSCs in the Musculoskeletal System: In Vitro Studies

Since Friedenstein and colleagues [3] established the first MSCs culture, their methodology has been widely used for culture expansion of animal and human MSCs. By virtue of their physical propensity to adhere to plastic and expand in vitro, MSCs are relatively easy to work with, and have been widely identified in numerous laboratory settings. MSCs have been isolated from various tissues of the

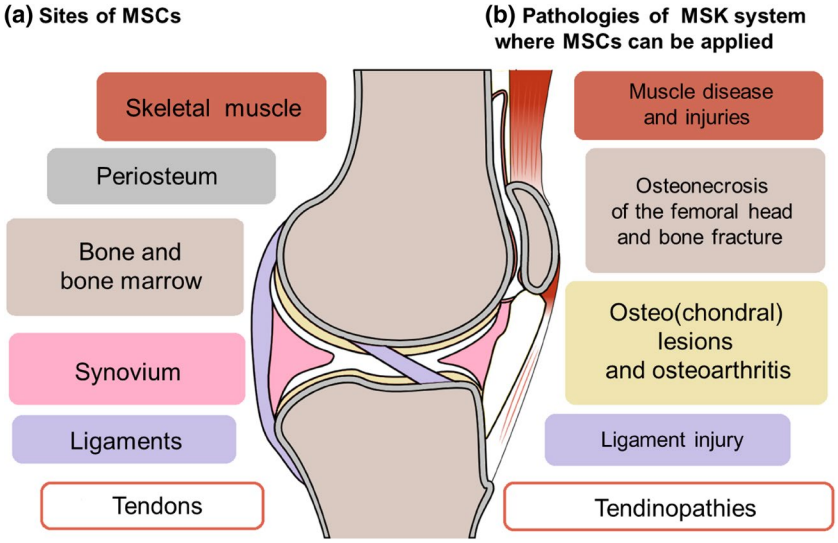

Fig. 1 (**a**) Sites of MSCs that have been identified within the musculoskeletal system. (**b**) Pathologies of the musculoskeletal system where MSCs are already used or have the potential for use in regenerative medicine based on animal studies. MSCs have been identified in skeletal muscle [2], periosteum [6], bone and bone marrow [3, 7–11], synovium [12–15] , ligaments [16] and tendons [17]. MSCs have been tested in muscles disease [18] and injuries [19], osteonecrosis of the femoral head [20–24], bone fracture [25], (osteochondral) lesions and osteoarthritis [26–38], ligament injury [39] and tendinopathies [35, 40, 41].

musculoskeletal system, most often from bone, bone marrow, synovium, infrapatellar fat pads, synovial fluid, and skeletal muscle. In human, these tissues are most commonly acquired from surgically removed biological materials during total or partial arthroplasty for osteoarthritis [7, 12, 49] or anterior cruciate ligament reconstruction surgery following ligament injury [13, 50]. Control tissue, i.e. without musculoskeletal abnormalities is most commonly used from *post-mortem* donors where MSCs from the musculoskeletal system have been shown to be preserved several hours, and even days, following death [12, 51]. Furthermore, there is evidence that MSC-like cells can be isolated from tissue stored in liquid nitrogen for 5 years [52].

There are a number of different methods for isolation of MSCs, with the different procedures mainly depending on the tissue source. Tissues in the musculoskeletal system are most commonly isolated using chemical digestion (e.g., for synovium, skeletal muscle) with additional mechanical dissociation for mineralized tissues, such as trabecular and cortical bone. Due to the high content of hematopoietic cells in human bone marrow, gradient density filtration is applied to isolate MSCs [13]. In mice, there are two approaches to isolate MSCs from bone marrow: either by flushing the bone marrow out of the diaphysis using a syringe; or by crushing the diaphysis and subsequent chemical digestion. Some differences have been shown between these methods, with the crushing approach also including more progenitor cells from the endosteum [53]. Chemical digestion is most often performed with different types of proteases (e.g., collagenase types II, IV, XI, D, dispase), at different concentrations (e.g., commonly 1–3 mg/mL), and for different durations of digestion (e.g., 1 h, to several hours). These systems largely depend on the tissue source, and thus the need to break down the extracellular matrix and release the cells from their niche in these perivascular locations. Synovium and muscle usually require lower collagenase concentrations and shorter digestion times in comparison to bone [13, 14, 54]. When using bone (e.g., trabecular, cortical), some form of mechanical grinding needs to be applied to break down the mineralized part of the bone. Small pieces of bone can be obtained from samples during surgery via the curetting of trabecular bone from the femoral head, or via the sampling of larger bone chips, with subsequent micro-dissection [7, 50, 55]. Either way, such biopsy materials need to be washed extensively under sterile conditions to remove hematopoietic cells. Of note, MSCs isolated from trabecular bone following collagenase digestion appear to be virtually identical in vitro to those isolated from bone marrow [50].

Although plastic adherence is an attribute of MSCs, as a rule, early primary cultures from these tissue sources represent a heterogeneous population, comprised of mature cells of the original tissue (e.g., osteoblasts, fibroblasts, myocytes) and MSCs. As well as the isolation of the cells themselves (e.g., dissection of the specific tissue, method of isolation used), the culture conditions can greatly affect both the expansion of MSC-like cells and their multipotency. The serum used should be either batch tested or from commercially selected lots that are suitable for MSC culture. Hypoxia (i.e., oxygen supply, $< 5\%$ O_2) has been shown to be the preferred condition for culture expansion of MSCs [53, 56]. To obtain a more homogenous population of MSCs at the early passages, methods of negative and positive selection of MSCs have been applied, such as sorting using magnetic beads and fluorescence activated cell sorting. The most common approach used is to select the mesenchymal population as negative lineage for CD45, Ter119, and CD31 in mouse, and CD45, CD34, and CD11b in human, as being depleted of leukocytes, erythrocytes, endothelial cells, and macrophages. Extremely rare populations of cells (i.e., $\leq 0.0025\%$ of single nucleated cells in bone marrow) have been selected and studied in vitro using the appropriate sorting methods [8]. Flow cytometry is also the preferred method for immunophenotyping of these cultured cells.

Due to the heterogeneity of the methods and the cell culture conditions used across numerous experimental settings, minimal criteria for MSC identification have been set by the International Society for Cellular Therapy, according to a list of positive and negative markers that need to be determined for human MSC cultures [57]. Although several novel MSC markers have been identified since the establishment of these criteria, the combination of CD73, CD90 and CD105 remains the gold standard when working with MSC cultures.

Once the plastic-adherent primary culture has been obtained and its MSC phenotype has been established, the next step is to demonstrate their multipotency. MSCs can differentiate along a number of connective tissues, including bone, cartilage, muscle, ligament, tendon, and stroma. In vitro, the possibility to guide these cells along the trilineage differentiation of osteogenesis, chondrogenesis, and adipogenesis needs to be shown, preferably with histochemical staining of the end products; e.g., mineralized tissue, proteoglycans, and adipocytes. Some kind of robust quantification also needs to be carried out, at either the protein (e.g., Alcian blue, Alizarin red S extraction) or gene (e.g., quantitative polymerase chain reaction) level, to avoid false-positive data. The in vitro settings used also include a highly controlled microenvironment that can push these cells down several lineages.

Recently, in vivo differentiation has become much more the norm to demonstrate the multipotency of these cells [45]. In this approach, immunocompromised (i.e., nude) mice are used as carriers to provide the microenvironment for determination of the in vivo differential potential of MSCs populations, which are most commonly transplanted beneath the kidney capsule or subcutaneous tissue of these mice.

Traditionally, following their in vitro isolation, MSCs have been defined based on their three main features, namely plastic adherence, phenotype, and multipotential differentiation [57]. Due to the massive increase in the identification of new MSC markers, these minimal criteria have been exceeded in terms of the fuller identity of MSCs. Much has been done to identify markers of mature lineages, such as osteoblasts (e.g., collagen type I, osteocalcin, alkaline phosphatase) and chondrocytes (e.g., collagen type II, aggrecan), although a specific marker for MSC identification remains to be defined. It appears that no such marker might exist, which might be a reasonable consequence of the multilineage potential of these cells as they need to maintain minimal expression levels of ubiquitously expressed markers to maintain this feature.

Identification of MSCs in the Musculoskeletal System: In Vivo Animal Studies

3.1 Tracing MSCs in Space and Time

There was a massive breakthrough in the identification of MSCs with the implementation of transgenic animal models [58]. The discovery of the genetic elements paved a new way to study MSCs elegantly in time and anatomical space, such as for Cre recombinase that recognizes loxP sites in the transgene construct. Briefly, in these animal models, a cell lineage of interest that expresses a specific marker at some point during embryonic or adult life is labeled with a reporter. Upon gene expression of this specific marker the reporter is switched on, which enables the identification and tracking of all of the cells of a specific population in anatomical space and time.

There are several variations to these models. The Cre model is the simplest one, where switching on the reporter labels all of the daughter cells from that point on (i.e., the progeny of a specific cell lineage), throughout the adult life. Much more sophisticated models such as tamoxifen inducible Cre recombinase (CreER/T) can allow the reporter to be switched on and off to label cells that express a specific marker at a specific time. We can induce Cre recombinase following a specific stimulus for the MSCs, such as injury of the musculoskeletal system. Using mouse lineage tracing models, it is possible to follow the cells of defined developmental structures, such as the joint interzone, and to identify morphogenetic cell populations in adult tissues and organs. Several MSC subpopulations have been identified using this approach, and their roles in embryonic and adult life and in health and disease have been studied (Table 1).

Another sophisticated approach to trace MSCs is double nucleoside labeling. Here, the quiescent and slow-cycling nature of MSCs is taken advantage of. Following a stress stimulus, such as injury, their activity rapidly intensifies, and the slow-cycling cells undergo intense proliferation. Initially, while quiescent, a nucleoside analog such as chlorodeoxyuridine or iodo-deoxyuridine can be administered to these animals via their drinking water for a certain time, which becomes incorporated into the cell DNA. By virtue of their quiescent and slow-cycling nature, the MSCs retain the first nucleoside analog, in contrast to the rest of the cells that divide faster and lose this analog. Following stimulation of the MSCs, such as by cartilage injury, the MSCs exit the quiescent state and become activated. At this point a second nucleoside analog distinct from the first is added, and the MSCs take up the second nucleoside analog and can be detected as double-positive cells. Taking this approach, functional MSCs in mouse synovium have been identified [15].

Animal Models of Musculoskeletal Pathologies

Degenerative disorders such as osteoarthritis and osteoporosis, and injury, most commonly to cartilage, bone and muscle affect the integrity of musculoskeletal system. Millions of people around the globe are impacted in terms of suffering pain and inability of independent motion. Animal models are indispensable when studying the regenerative abilities of MSCs in various disorders of the musculoskeletal system.

Cartilage Damage and Osteoarthritis

Animal models of primary and secondary osteoarthritis are available. Similar to human, primary osteoarthritis in animals is a naturally occurring phenomenon that arises from degenerative changes in joints. Secondary osteoarthritis is normally associated with specific causes and/or risk factors that lead to the joint osteoarthritis, such as trauma and congenital diseases [92]. The animal models of post-traumatic osteoarthritis that are most commonly used are invasive models, where joint injury is induced chemically (e.g., intra-articular injections of collagenase, steroids) or mechanically (e.g., surgical cartilage injury, anterior cruciate ligament transection, partial and total menisectomy, medial meniscal tear). Several small (e.g., mice, rats, rabbits, guinea pigs) and large (e.g., sheep, goats, dogs, horses) animal models are being used. Mice represent the most commonly used animal models of osteoarthritis, as their genetic background can be easily manipulated, while equine articular cartilage is the most comparable to human [92]. The knees are the most widely studied joints.

The MSC regenerative abilities can be studied by tracing the endogenous MSC population (e.g., with lineage tracing in transgenic animal models, or double nucleoside labeling), or by transplantation of freshly isolated or in vitro

Table 1 Overview of MSC subpopulations with demonstrated role in musculoskeletal system

Marker/subset	Phenotype	Relative level (%)	Role in musculoskeletal system	Ref.
Bone and bone marrow				
PαS	PDGFRα/Sca-1(PαS)+	0.067% of CD45/Ter119 − bone marrow cells	Source of hematopoietic niche cells, osteoblasts, adipocytes	[59]
αSMA	CD45/Ter119/CD11b+/−αSMA+	0.02%-0.3% of bone marrow cells	Source of fibrocartilage, skeletal progenitors in fracture-healing model	[6]
Nestin-GFP	CD45/CD31−	4% of CD45- bone marrow cells	Bone marrow niche and hematopoietic stem cell support in skeletal remodeling through differentiation into bone- and cartilage-forming cells	[9]
PDGFRα/ CD51+	CD45/CD31/Ter119− PDGFRα/CD51/CD105+	82%, 79% of Nestin-GFP + bone marrow cells		[60]
Mx-1	CD45/CD31/Ter119− CD105/CD140a+	~44% in bone marrow	Source of new osteoblasts for fracture repair	[61]
Osterix	Nes-GFP/Lepr/PDGFRα/PDGFRβ+	78–89% of P5-iOsx/Tomato + bone marrow cells	Restricted to osteolineage in adult bone marrow callus formation in fracture healing	[62, 63]
Leptin receptor	CD51/PDGFRβ/PDGFRα/ CD105/Prx1/ Cxcl12+ Sca-1+/−Nestin-GFP^{low}	0.3% of bone marrow cells	Main source of adipocytes and osteoblasts in adult bone marrow	[10]
Mouse skeletal stem cells	CD45/Ter-119/Tie2/CD105/Thy/6C3-AlphaV/CD51/CD200+	0.5% of the epiphyseal growth plate cells	Bone, cartilage and stromal cell formation even in nonskeletal tissues	[11]
Gremlin1	CD45/CD31/Ter119− CD105+ Sca-1/PDGFRα−	0.0025% of epiphyseal growth plate cells	Source of osteoblasts, chondrocytes and reticular marrow stromal cells, but not adipocytes	[8]
f-BCSP	CD45/TER119/Tie2/Thy/6C3− AlphaV/CD105/CD49f+	97% of cells in fracture callus	Skeletal growth, regeneration, and repair in bone injury	[64]
Synovium				
Gdf5 lineage	CD45/CD31/CD16/CD11b− PDGFRα/Sca1/gp38/CD200/CD73/CD90+	~60% of nucleated cells in synovial lining	Regeneration of cartilage in injury model, de-novo joint formation	[14]
Skeletal muscle				
Abcg2 skeletal muscle side population cells	CD45/CD31− Sca-1/Pax7/syndecan-3 and 4+	0.25% of all cells in hindlimb muscle	Regeneration of adult skeletal muscle (endogenous, following transplantation after muscle injury)	[65–67]
	CD45/CD31− Sca1/PDGFRα+ Pax7/Myf5/integrin α7/SM/C- 2.6+	0.1% of muscle mononuclear cells	Myogenic in-vitro loss of myogenic potential following muscle injury	[68]
PW1 + interstitial cells (PICs)	CD45/CD31/NG2/PDGFRβ− Sca-1/PDGFRα/CXCR4+	14% of proliferative cells in limb muscles	Adipogenic precursors similar to fibro/adipogenic progenitor cells	[69]
	CD45/CD31/Pax7/PDGFRα− CD34/CD90/CD29+	45% of PW1 + cells in hind limb muscle	Regeneration of skeletal muscle in vivo	[70–72]

Table 1 (continued)

Marker/subset	Phenotype	Relative level (%)	Role in musculoskeletal system	Ref.
Pax7 lineage	CD34/CD29/CD56+ CD105/PDGFRα/c-Kit/CD45−	1% in hind limbs of 1-year-old mouse; 5% of muscle nuclei	Restore dystrophin expression in MDX mice Regeneration of diverse skeletal muscles upon transplantation	[73–75]
Mesoangioblasts	CD45/CD34/CD133− PW1/CD44/CD13/CD49b/ALP+	Not reported	Skeletal muscle regeneration (via systemic delivery) in muscular dystrophy animal models	[76–78]
Pericytes	TNAP-CreERT2 lineage	0.7% of quadriceps muscle fibers	Growth and regeneration of postnatal muscle	[79]
	Nestin-GFP/NG2- DsRed+ PDGFRβ/CD146 + PDGFRα−	3.4% of hindlimb skeletal muscle cells	Skeletal muscle regeneration in vivo	[80]
	ALP/NG2/CD13/CD44+ CD45/CD31/CD34/CD62L/CD71/CD106/ CD117/CD133/CD56−	2%-4% of total skeletal muscle cells	Formation of new fibers when transplanted into dystrophic immunodeficient mice	[81]
Fibro/ adipogenic progenitor cells	PDGFRα/CD90/CD105/CD166+ CD45/CD34/CD31/CD56−	19.7% of regenerating limb muscle cells are CD45/CD31−/PDGFRα+	Major contributor to ectopic fat accumulation and fibrosis in skeletal muscle	[82–84]
	CD15/PDGFRα+ CD56−	87% of in-vitro adherent cells	Major contributor to fat in skeletal muscle support for proliferating myogenic progenitors	[85, 86]
Tendons				
P75 + cells	CD45/CD34/CD54− CD29/CD90/vimentin/Sox10/Snail+	~ 10% of CD45−/p75 + cells in tendon repair tissue	Give rise to scar-forming stromal cells following tendon injury	[87]
Nucleostemin, Oct 3/4 + cells	Nucleostemin+ Oct 3/4+	5%-10% of total cells in rat Achilles tendon	Proliferation and migration to the injury site in tendon healing	[88]
CD146 + cells	CD44/CD90+	0.8% of mononuclear cells from rat patellar tendon	Regeneration of patellar tendon (upon transplantation of CTGF pretreated cells in rat injury model)	[89]
SMA9 lineage	αSMACreERT2	3%-13.5% of cells in tendon midsubstance two and 21 days post- tamoxifen induction	Main contributors to patellar tendon healing	[90]

Abcg2, ATP-binding cassette sub-family G member 2; αSMA, alpha smooth muscle actin; ALP, alkaline phosphatase; Cxcl12, C-X-C motif chemokine ligand 12; CXCR4, C-X-C chemokine receptor type 4; CTFG, connective tissue growth factor; f-BCSP, fracture-induced bone, cartilage, stromal progenitor; GFP, green fluorescent protein; Mx1, myxovirus resistance-1 promoter; Myf5, myogenic factor 5; NG2, neural/glial antigen 2; Oct3/4, octamer-binding transcription factor 3/4; P5-iOsx/Tomato, post-natal day 5 induced Osterix tracing; Pax7, paired box protein 7; PDGFRα/CD140a, platelet derived growth factor receptor α; PDGFRβ, platelet derived growth factor receptor β; Prx1, paired mesoderm homeobox protein 1; PW1/Peg3, paternally-expressed gene 3; p75, low affinity neurotrophin receptor; Sca-1, Stem cells antigen 1; SMA9, αSMACreERT2-Ai9 (tamoxifen-inducible αSMA Ai9 Cre reporter mouse); SM/C- 2.6, monoclonal antibody for mouse satellite cells [91]; Sox-10, (sex determining region Y)-box 10; TNAP, tissue non-specific alkaline phosphatase; Thy, thymus cell antigen 1

manipulated autologous or allogeneic MSCs. The data obtained in this way can provide information relating to what the outcome in human might be. Using animal models, it is also possible to perform randomized controlled studies that provide more evidence of the efficacy of MSC-based cell therapies. Here, the controls are usually a group of animals subjected to sham surgery or the use of the contralateral joint, muscle or limb of the same animal, which is not subjected to the treatment.

Bone Fractures

A variety of different fracture models has been introduced over recent years. Models for normal fracture repair (i.e., primary, secondary), delayed union, nonunion (e.g., atrophy, hypertrophy), segmental defects, and fractures at risk of impaired healing have ranged widely in terms of their accuracy in reflecting the clinical scenario, and of their reproducibility [93]. Open and closed models using different fixation techniques to provide different stiffnesses are now available, in particular with small animal models such as with mice and rats. Here, the outcome of the fracture healing can be influenced by the animal strain, age and sex, while the anesthetic and the postoperative analgesics and antibiotics can also have effects.

Muscle and Tendon Damage

To study muscle regenerative capacity and the efficacy of regenerative treatments for muscle diseases, four different injury models are most commonly used: freeze injury; barium chloride; notexin; and cardiotoxin. The most damaging of these is freeze injury. Although comparison of these models has shown that the muscle regenerates completely, the nature of the injury model should be chosen carefully according to the experimental design and the outcome to be monitored [94].

Animal models for the study of tendon and ligament regeneration are still in their infancy. Again, both small (e.g., rats, rabbits) and large (e.g., dogs, horses, primates) animal models can be used, depending on the type of injury and the tissue to be studied. Rats, for example, appear to be the most suitable model for rotator cuff tendon repair, while rabbits have been most commonly used to study medial collateral ligament tears. Based on the type of experiment and the outcome measures, murine, canine, equine, bovine, ovine, porcine or primate models can also be used. Ligament and tendon injury can be induced in several ways, including by surgical transection, overuse exercise, or chemically (e.g., collagenase, transforming growth factor [TGF]-β1 injections) [95–97].

Subpopulations of MSCs with Roles in the Musculoskeletal System

Taking advantage of the combination of transgenic animal models and animal models of various pathologies of the musculoskeletal system has resulted in the identification of many subpopulations of MSCs, as well as their roles in health and disease. An up-to-date overview of the main MSC subpopulations along with their markers and phenotypes, and their identified roles in the musculoskeletal system, is given in Table 1.

MSCs in Bone and Bone Marrow

Bone marrow is the most studied and well-recognized source of MSCs in adult organisms. MSCs were first discovered in bone marrow of guinea pig, and then later in human, and bone marrow-derived MSCs are those that are most often used in current clinical practice. As well as their role as progenitor cells that can repair damaged tissue, bone marrow MSCs have also been shown to provide meaningful support for hematopoetic stem cells in their niche [98]. Mouse bone marrow MSCs show high concentrations of two positive markers: platelet derived growth factor receptor α (PDGFRα/CD140a) and Sca-1 (PαS subpopulation). Conversely, those that lack Sca-1 and secrete the C-X-C motif chemokine Ligand 12 (CXCL12) represent a key component of the niche for hematopoietic stem cells in adult bone marrow [99]. There has been some controversy over the Nestin–green fluorescent protein (GFP)-positive population of MSCs in bone marrow, which was shown to be an MSC population that was required for maintenance and homing of hematopoietic stem cells [9, 100]. Conversely, another study that used a different Nestin transgenic animal reported that Nestin-GFP + cells were nonMSC populations [101]. Based on these studies, it is of vital importance to validate transgenic animal models, as different transgenes can label different populations, which will produce confounding results. Subsequently, two distinct populations within these Nestin-GFP cell populations were identified [102]. The Nestin-GFP bright cells are rare and positive for the known pericyte marker NG2, while the Nestin-GFP dim cells overlap with the leptin receptor (Lepr)-labeled cells, and they are a subpopulation of bone marrow cells found to be major source of adult bone [10]. Another study indicated that cells defined by promoter/ enhancer activities of genes associated with chondrocytes and their precursors, such as *Sox9, Col2* and *aggrecan*, might be the source of osteoblasts during the rapid phase of bone growth that occurs before Lepr-labeled cells provide precursors for the osteoblast lineage. Combining lineage tracing and various animal models of fractures, it appears that Gremlin1 (i.e., bone morphogenetic protein [BMP] antagonist) and Lepr subpopulations of

MSCs are so far best characterized as MSCs that replenish bone and cartilage in the adult and are involved in regenerative responses of bone and cartilage after injury. Mouse skeletal stem cells have also been defined as mesenchymal lineage negative (CD45/Ter119/Tie2), alpha V/CD200 positive, and CD105/Thy/6C3 negative [11]. Cortical bone has also been shown to be a better source of osteogenic MSCs than bone marrow [103].

Translation of these findings to human is rare, mainly because no specific surface markers have been associated with the lineages identified in transgenic animals. Looking at human MSCs, a subset of so-called pericytes, with the CD34-/CD45-/CD146 + phenotype, would come closest to the term of MSCs [47]. Interestingly, low/ negative expression of PDGFRα identifies candidate primary MSCs in adult human bone marrow [104], in contrast to the PDGFRα-positive population in fetal bone marrow. A subpopulation of bone marrow MSCs with an up-regulated *Lepr* gene was described for human CD271 + cells, which also expressed the low-affinity nerve growth factor receptor. Thus, sorting of these cells for CD271 and for low expression/ absence of CD140a has enabled the enrichment of human adult primary bone marrow MSCs [104]. These provided the highest purity reported for hematopoietically active adult bone marrow MSCs to date, which suggests that CD271 + and CD140(PDGFRα)low/− cells should be the basis used when studying human MSCs. Interestingly, the proportion of CD45-/CD271 + MSCs was higher in subchondral bone marrow lesions compared to non-bone marrow lesions in patients with hip osteoarthritis [105]. Apart from such lineage tracing and surface markers, it has been shown that a minimal set of biophysical markers can be used to identify, isolate, and predict the function of MSCs within mixed cell populations in bone marrow, such as cell diameter, mechanical stiffness, and nuclear membrane fluctuations [106].

Identification of subpopulations of MSCs with specific features is one approach toward their better use in regenerative medicine, which might include those that undergo chondrogenesis rather than osteogenesis in vivo. Another approach is to identify molecules that can guide and stimulate MSCs toward the preferred tissue lineage. In image-based high-throughput screening with primary human bone marrow MSCs, a small molecule known as kartogenin was identified and confirmed to be effective in repairing cartilage in two mouse models of osteoarthritis (i.e., collagenase VII and surgery induced) [107].

MSCs in Skeletal Muscle

For a long time, muscle regeneration was thought to be dependent solely on a heterogeneous population of adult stem cells known as satellite cells. These were first identified in the 1960s using electron microscopy [108], and they were defined as small, mononuclear cells that occupy a niche juxtaposed to multinuclear myofibers in muscle tissue [109]. Since their discovery, however, several distinct types of myogenic progenitor cells that can regenerate skeletal muscle tissue both in vitro and in vivo have been described. One of these populations is the so-called 'side-population' cells. These were initially described in a study of murine hematopoietic stem cells [110], based on their efflux of the fluorescent DNA-binding dye Hoechst 33342 that is taken up by live cells. Later on, these side-population cells were described for other tissues [111], including skeletal muscle. These skeletal muscle side-population cells represent a heterogeneous population with distinct markers and physiological roles [112]. The efflux of the Hoechst 33342 dye from these cells is dependent on a member of the ATP-binding cassette sub-family G member 2 (Abcg2) [113]. A rare subset of these skeletal muscle side-population cells that are characterized by Syndecan-4 and Pax7 constitute a self-renewing muscle stem cell population that can generate both satellite cells and their myonuclear progeny in vivo [65, 114]. Two vessel-associated cell populations with myogenic ability have been described for skeletal muscle. The first of these cell populations are the pericytes in the skeletal muscle interstitium, whereby those of type-1 contribute to fat accumulation and those of type-2 to muscle regeneration [80]. The second of these two vessel-associated cell populations are the so-called mesoangioblasts. These cells can cross the vessel wall when applied systemically, and they contribute to muscle regeneration [76, 115]. Animal models have shown mesoangioblasts to be excellent candidates for cellular therapy of muscle dystrophy [116, 117]; however, human clinical trials have not shown much promise yet [118]. There are two other cell populations in the muscle interstitium. One is the so-called fibro/ adipogenic progenitor cells, which are defined by the expression of PDGFRα. As these cells are highly adipogenic and fibrogenic, they are believed to contribute to skeletal muscle fibrosis and ectopic fat formation [82, 83, 85]. Interestingly, these fibro/ adipogenic progenitor cells are also abundant in young and healthy muscle, although fibrosis and fat formation only appear to occur in diseased and ageing muscle. The second of these last two cell populations is comprised of paternally expressed gene 3 (Peg3/PW1)-positive interstitial cells. These positive interstitial cells show the properties of *bona-fide* tissue-specific stem/ progenitor cells, including clonigenicity, ability to self-renew, and broad differentiation potential. However, clonal positive interstitial cells show a strong tendency toward the skeletal muscle lineage from which they originated [70]. These positive interstitial cells themselves consist of two sub-populations: those that are PDGFRα positive and highly adipogenic; and those that are PDGFRα negative and are myogenic and cannot form fat [69]. These sub-populations account for the two recently

identified populations of fibro/ adipogenic progenitor cells [82, 83, 86]. Pannérec et al. [69] suggested a model of stem-cell hierarchy based on PW1 expression, in which the myogenic potential resides in satellite cells, mesoangioblasts and a subpopulation of positive interstitial cells with adipogenic potential, while cells that do not express PW1 give rise to fibroblasts.

Most of the evidence for regenerative capacity in vivo is attributed to Pax-7–expressing satellite cells [119]. Furthermore, other cell types with regenerative potential depend on the presence of the satellite-cell population [120]. Identification of the pathways that regulate satellite-cell behavior might provide insights toward future applications to modulate satellite-cell fate during muscle regeneration, such as paired like homeodomain 2 transcription factor, Pitx2, that has an important role in muscle morphogenesis [121].

MSCs in Tendons and Ligaments

Tendons connect muscles to bones to enable joint movement. As a result, they are subjected to large mechanical loads, and hence frequently undergo injury [122]. Tendons consist of collagen and elastin embedded in a proteoglycan-rich matrix. The tendon matrix is produced by tenoblasts and tenocytes, which lie parallel with and between the longitudinally arranged collagen fibers. The cellularity of tendon tissue is however low, with approximately 1–4% of total nucleated cells as tendon stem/ progenitor cells [123]. This explains the low turnover and poor self-healing capacity of this tissue [124]. Bi et al. [123] successfully isolated tendon stem/ progenitor cells from both mouse and human tendons, and they showed that these cells possess several of the criteria for stem cells, including clonogenicity, self-renewal, and multipotent differentiation capacity. Unlike MSCs, tendon stem/ progenitor cells express tendon-related markers in vitro, such as scleraxis and tenomodulin, and they can form tendons [125]. Tendon stem/ progenitor cells show greater clonogenicity, cell proliferation, and tenogenic differentiation potential compared to bone marrow MSCs, and so they might represent a better source for musculoskeletal tissue regeneration [16].

Ligaments are the least-studied source of stem cells. However, recent studies that have focused on human anterior cruciate ligament have shown that ligament-derived stem cells have these features of clonogenicity and trilineage multipotency, and that they express common markers found in MSCs, and also in tendon stem/ progenitor cells [126]. Clear variations in characteristics of ligament-derived stem cells isolated from different ligament types have been shown; however, human anterior and posterior cruciate ligaments appear to harbor the same population of ligament-derived stem cells [127]. Further investigations are required to characterize these cells, and to better define their functions and therapeutic potential.

MSCs in Synovium and Synovial Fluid

The synovium is a tiny but complex membrane that encircles synovial joints, and it has been well recognized as a source of MSCs [12]. Synovium can roughly be divided in two anatomical parts: the intima inner layer, with macrophages or fibroblast-like synoviocytes; and the subintima outer layer, with synoviocytes and fibroblasts, which secrete collagen and other extracellular matrix proteins. Synovium is the active site of inflammation in cartilage injury and osteoarthritis, although the hyperplasia that occurs within this tissue can also be attributed to mobilization and activation of MSC-like cells [15]. MSCs identified from synovium have been shown to have superior differential ability in vitro compared to MSCs from other sources [13]. Using a cardiotoxin muscle injury model in nude mice, De Bari et al. [19] showed that human synovial-membrane-derived MSCs can regenerate muscle, in terms of contributions to myofibers and long-term persisting functional satellite cells. Moreover, the Gdf5-lineage cells in adult synovium that are derived from the very first overt joint embryonic structure interzone have been shown to retain a specific joint morphogenetic memory [14]. Superiority of synovium-derived MSCs in osteogenesis and chondrogenesis has been shown in vitro [13, 54].

Synovial fluid, however, has been shown to harbor MSCs from other sources of the synovial joint, as cell populations that show similarities with bone marrow and synovial membrane MSCs have been found [128].

Mechanisms of MSCs in Tissue Regeneration

Anti-inflammatory Actions

Following tissue injury, inflammation occurs as a consequence of danger signals that mobilize different, mainly immune, cells. These first sense and check on nocive factors, and then act to remove them as soon as possible. Acute inflammation is a normal component of a complex multistep wound-healing process. However, under certain circumstances, the acute inflammation does not resolve as expected, but rather continues to persist. This leads to formation of chronic inflammatory foci, where perpetuated collateral lesions of adjacent healthy tissue are generated. As the resolution of inflammation is one of the major prerequisites for successful regeneration of damaged tissue, an understanding of the mechanisms involved in this process and their modulation is of the utmost importance.

It has been known for some time that MSCs act as anti-inflammatory agents that can effectively suppress innate and adaptive immune responses. Furthermore, in recent years, MSCs have been recognized as active participants in tissue damage-repair processes [129, 130]. Consequently, anti-inflammatory immunomodulation of MSCs is now being attempted in numerous clinical studies, and is being exploited in experimental treatments of acute graft-versus-host disease following allogeneic hematopoietic stem cell transplantation, and in Crohn's disease [131, 132]. Although several of their immunomodulatory mechanisms remain to be defined, it is clear that MSCs can suppress inflammation both directly via cell-to-cell interactions and indirectly via soluble paracrine factors that they produce [129, 130, 132]. Interestingly, it appears that MSCs are not spontaneously immunosuppressive. Thus they need to be pre-activated by pro-inflammatory cytokines to acquire this ability, such as interferon-γ, tumor necrosis factor (TNF)-α, and interleukin (IL)-1β [130]. Another interesting feature of MSCs is their very low, or lack of, immunogenicity, which has been attributed to lack of expression of MHC class II molecules and the presence of human leukocyte antigen G on their surface, which is a potent tolerogenic non-classical MHC class I molecule [133, 134]. Through different direct and indirect mechanisms, MSCs target the cells involved in innate immunity: mastocytes, monocytes/ macrophages, immature dendritic cells, natural killer cells, and natural T-killer cells [129, 130, 134, 135]. They disable the process of macrophage polarization through secretion of prostaglandin E2 (PGE2), IL-6, and granulocyte–macrophage colony-stimulating factor, thereby preventing their transition to the pro-inflammatory state (i.e., M1 macrophages). In this way, the M2 macrophages that produce and secrete anti-inflammatory IL-10 can prevail [129, 135]. The PGE2 produced by MSCs inhibits mastocyte degranulation and differentiation and activation of dendritic cells, where in combination with IL-6, it promotes the onset of regulatory/ tolerogenic dendritic cells that produce the strong anti-inflammatory cytokine TGF-β [129, 135]. The numerous soluble factors that are produced by MSCs and affect the functions the of the innate immune system effector cells include TNF-α–stimulated gene protein-6 (TSG-6), TGF-β, and the enzyme indoleamine-2,3-dioxygenase, which catalyses the turnover of tryptophan to kynurenine. TSG-6 activates the CD44 receptor, which basically monitors changes in the extracellular matrix that influence cell growth, survival and differentiation, and consequently inhibits nuclear transcription factor NFκB in macrophages and dendritic cells, and also in helper T-cells (i.e., adaptive immunity) [129]. Both TGF-β and indoleamine-2,3-dioxygenase strongly inhibit cytotoxic functions of natural killer cells [135]. MSCs can also change phenotypic properties and inhibit proliferation and production of pro-inflammatory cytokines by dendritic cells and natural T-killer cells, as well as the cytotoxic functions of the latter. These immunomodulatory effects are a consequence of intercellular interactions and paracrine activities of TGF-β and PGE2 [132]. Additionally, MSCs can induce complement activation and express the CD59 protein and complement factor H, which both protect cells from lysis [130].

Lymphocytes B and T are the effector cells of adaptive immunity, and they are important targets for the immunosuppressive activities of MSCs. As well as inhibition of activation and proliferation of lymphocytes B and chemokine receptor expression on their surfaces, MSCs also prevent maturation of activated B-cells into antibody-producing plasma cells. These effects arise from intercellular interactions (e.g., binding of PD-1 inhibitory receptors on B-cells to their specific PD-1L ligands, expressed by MSCs), and from the activities of MSC-produced soluble factors (e.g., TGF-β, galectin 9) [129, 132, 135]. MSCs inhibit proliferation and effector functions of both CD4+ (helper) and CD8+ (cytotoxic) T-lymphocyte subsets, regardless of antigen-specific restrictions at the level of the major histocombatibility molecules that present antigenic peptides to appropriate clones with T-cell receptors. This is achieved not only via MSC-secreted PGE2, indoleamine-2,3-dioxygenase, hepatocyte growth factor, TGF-β, nitric oxide (mainly in rodents, rabbits, hamsters) and galectin 1, but also by direct cell-to-cell interactions (e.g., binding of PD-1L to PD-1 receptor) [132, 134, 135]. Additionally, MSCs can recruit immunosuppressive natural regulatory T cells and induce the generation of induced regulatory T lymphocytes (e.g., Tr1), which by producing and secreting anti-inflammatory cytokines (e.g., IL-10, TGF-β), strongly inhibits the activation of T-cells [130, 132, 135].

Other Regenerative Mechanisms

For some time the prevailing hypothesis behind the regenerative capabilities of MSCs was that upon their application, MSCs migrate to damaged tissue sites, where they engraft, differentiate, and thereby functionally reconstitute the injured tissue. However, the data from numerous animal and human studies that have been carried out over recent decades have clearly shown that this is not the case. Thus, it has been shown that when applied, MSCs do not engraft in either significant numbers or for sufficient time to satisfy this hypothesis [129]. Additionally, the unresolved enigma regarding their regenerative efficacy in nonmesodermal tissues remains. This was at first ascribed to their trans-germline or cross-germline differentiation, which contradicted the established belief that differentiation of adult stem cells is restricted exclusively to the germ layer of their origin. Later studies showed that most of these data had been misinterpreted due to limitations in the methodologies used, or can be explained by cell fusion, an essential process for

development and tissue repair [136–138]. All of these open questions are stimulating new ideas and investigations into the underlying mechanisms here. Recent aspects have considered several alternative modes of MSC reparative actions, such as enhancement of target tissue cell viability and proliferation, reduction of their apoptosis, and local immunomodulation. These effects can be promoted by MSCs through their paracrine activities (i.e., production, secretion of various growth factors, cytokines, other bioactive molecules), through their cell-to-cell interactions that are mediated by tunneling nanotubes (50-200-nm-diameter filaments) that are involved in the transfer of mitochondria and lysosomal vesicles, and through their release of extracellular vesicles that contain regeneration-stimulating proteins and peptides, as well as mRNA and miRNAs [129].

Interestingly, intravenously administered MSCs have been shown to be preferentially, although transiently, trapped after their first pass through the circulation in the lung microvasculature, regardless of the absence or presence of lung injury [129]. Therefore, after their intravenous application, the circulating and trapped MSCs (i.e., in the microvasculature, capillary network) release their paracrine factors into the blood stream, thereby indirectly providing distal injured or diseased tissues with potentially healing stimuli.

Analysis of the animal and human MSC secretome and proteome have confirmed the presence of a variety of paracrine cell pro-survival trophic factors/ mediators that can stimulate progenitor cell self-renewal and angiogenesis, as well as reduce apoptosis and inflammation. These include secreted frizzled related protein, stromal cell derived factor-1, and vascular endothelial growth factor-A [129]. Another important feature of MSCs is their anti-fibrotic (i.e., anti-scarring) effects. Although these effects can be partly explained by the MSC anti-inflammatory actions, the specific mechanisms remain largely unknown. Usunier et al. [139] suggested that the anti-fibrotic actions of MSCs arise from a combination of their immunomodulatory capabilities, inhibition of TGF-β–induced differentiation of various types of cells into extracellular-matrix–secreting myofibroblasts (e.g., epithelial-to-mesenchymal transition), inhibition of oxidative stress, and matrix remodeling.

It has been shown that MSCs can transfer mitochondria (and/or mtDNA) via tunneling nanotubes to several different types of cells, such as epithelial, endothelial, and cardiac myocytes. This occurs especially when these cells are injured or stressed [129], and this has been shown both in vitro and in vivo [140–142].

It appears that all cells release different extracellular vesicles, exosomes (30–100 nm diameter), microvesicles (50 – 1,000 nm diameter), and apoptotic bodies (1–5 μm diameter). Valadi et al. [143] were the first to demonstrate that mRNA and miRNAs can be exchanged between cells via exosomes. Exosomes from MSCs can reduce apoptosis and necrosis in rodents following ischemic injury to their heart, brain, lungs, liver and kidneys [129]. In addition, these exosomes can reduce inflammation and promote cell proliferation during tissue repair.

Musculoskeletal Regenerative Medicine

MSCs in Musculoskeletal Regenerative Medicine

The pathological tissue degeneration, in particular those of cartilage and bone, that occurs in most musculoskeletal disorders is a result of an imbalance in tissue homeostasis that can be due to inflammation, overuse, or trauma [144]. To restore the structure and function of damaged tissues, regenerative medicine approaches that use the therapeutic actions of MSCs have been tested in numerous clinical trials. These trials have included cells derived from both autologous and allogeneic cell sources [145].

Bone marrow is still the most widespread source for the MSCs used in musculoskeletal regenerative medicine. However, only a small percentage (i.e., 0.01%-0.001%) of the total mononuclear cells used is MSCs [146]. Bone marrow is usually aspirated from the iliac crest, whereby the technique of aspiration can have large effects on the levels of MSCs in the aspirate [147]. Methods for separating cells from bone marrow are based on density gradient centrifugation [147], while new approaches using filter devices have been developed more recently [148, 149]. Adipose tissue has also gained an important role as a source of therapeutic cells for regenerative medicine. In 2001, Zuk et al. [150] showed that adipose tissue contains MSCs (which they termed ASCs – adipose-derived MSCs) that can differentiate into cartilage and bone. Then the first clinical procedure for their use in the treatment of traumatic calvarial defects was reported in 2004 [151]. Subcutaneous adipose depots are ubiquitous, and they are relatively easily accessible using minimally invasive liposuction aspiration. This method is also cheaper and less invasive than bone marrow aspiration [152]. An additional advantage lies in their large numbers of multipotent cells, as it has been reported that the uncultured stromal-vascular fraction can contain up to 3% of the of adipose-derived MSCs; this is 2,500-fold more than the levels of stem cells in bone marrow [153]. To obtain this stromal-vascular fraction the lipoaspirate has to be digested with collagenase [150].

For clinical applications of MSCs for musculoskeletal regeneration, two approaches are generally used, as shown in Fig. 2. The first of these is the nonculture expanded form for the bone marrow aspirate or lipoaspirate, which are also known as the bone marrow concentrate and the stromal vascular fraction, respectively. The second approach involves the use of culture-expanded forms. Typically, nonculture

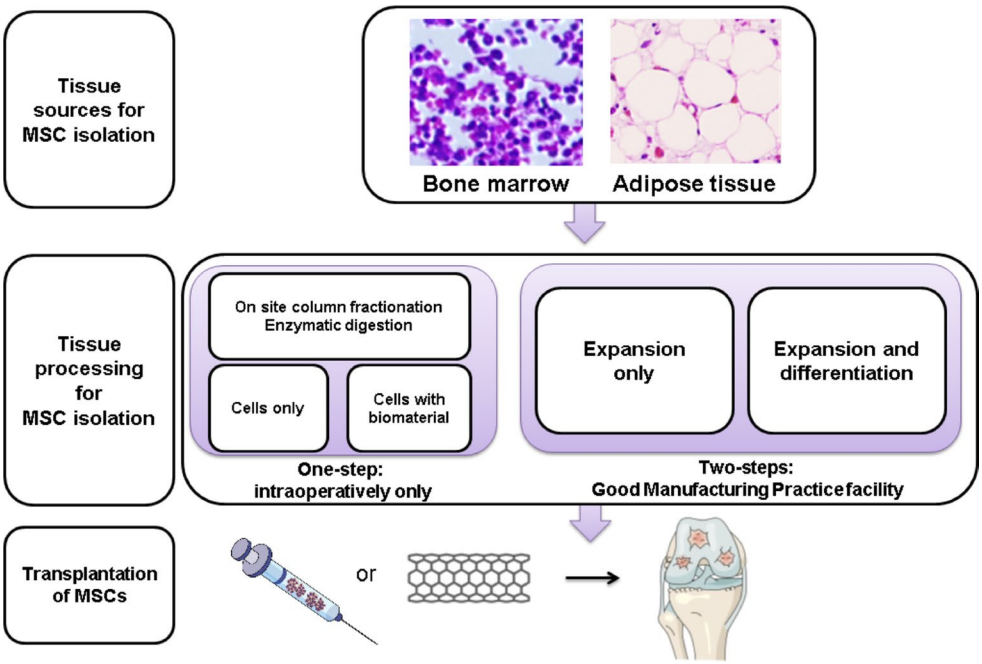

Fig. 2 Current routes of used for MSC isolation, preparation and administration, as used in clinical practice with a view to musculoskeletal regeneration

expanded forms are autologous in nature, and transplantation is performed in one operative procedure, directly in the operating room (i.e., a one-step procedure). On the contrary, in vitro cell manipulation and expansion of MSCs in a Good Manufacturing Practice facility is costly and time consuming [146, 154]. Additionally, culture-expanded MSCs are considered to be a pharmaceutical product, which thus requires regulatory clearance and approval [155]. Depending on the patient indications, both of these non-expanded and expanded forms of MSCs can be delivered into the diseased environment or the lesion by direct intra-articular injection of the cell suspension, or alternatively, they can be seeded into a biocompatible scaffold and implanted using surgical procedures [156].

Clinical Studies Using MSCs for Musculoskeletal Regeneration

All of the above-described features strongly support the application of MSCs in clinical trials of various pathologies where inflammation and degeneration have a role. The numbers of clinical trials that use MSCs for musculoskeletal regeneration have been increasing every year, and an overview of recent studies is given in Table 2. Most of these are case studies that lack a control group. However, a recent review by Reissis et al. [157] demonstrated robust clinical evidence that MSCs have significant potential for the regeneration of hyaline articular cartilage in patients with minimal adverse effects.

In terms of adverse events, a recent study performed in a large group of over 2,000 orthopedic patients treated with MSCs and followed for up to 9 years reported no clinical evidence for increased risk of neoplasm with MSCs of any type [158].

It is still questionable whether the treatment of symptomatic, localized cartilage lesions that are associated with the risk of progressing to generalized osteoarthritis requires concentrated autologous MSCs. The application of a biocompatible scaffold and local microenvironment-derived MSCs, such as those derived from subchondrial bone, appear to provide similar regenerative effects. Recent reviews have also noted the large heterogeneity for these MSC sources, the biomaterials used, and the clinical protocols followed [159, 160]. Both of the approaches with and without cells appear to be safe and to show promising therapeutic outcomes; however, the potential superiority of one over the other here is still something for future clinical studies.

Role of MSCs in Musculoskeletal Disorders: Their Potential in Early Diagnosis

Even though MSC research is mostly focused on the identification of the opportunities for exploiting their regenerative potential, MSCs can also potentially be used in diagnosis of musculoskeletal diseases. Evidence for their use as a diagnostic tool comes from colorectal cancer, where a specific adult intestinal stem cell population that predicts disease relapse has been identified in these patients [161]. With the exception of bone marrow and adipose tissue, MSC subpopulations that reside within the musculoskeletal system are not easily available for diagnostic purposes. There is some

Table 2 Recent clinical research into MSCs according to the musculoskeletal pathologies

Diagnosis	Method	Patients (n)	Age (years)	Cell therapy	Route of transplantation	Follow up	Treatment outcome	Method	Ref.
Osteoarthritis and major joint chondral lesions									
Medial-compartment knee osteoarthritis	Arthroscopy and/or X-ray	56 (randomized)	24–54	Hyaluronic acid with/without cultured bone marrow MSCs	Intra-articular injection 3 weeks after hight tibial osteotomy and microfracture	2 years 1 year	Better activity score Improved cartilage quality	Tegner, Lysholm, Int. Knee Documentation Committee Magnetic Resonance Observation of Cartilage Repair Tissue	[26]
Symptomatic primary knee osteoarthritis; Kellgren-Lawrence grade II to V	X-ray	18	50–75	Autologous ASCs; 3 dose escalation cohorts	Intra-articular injection	6 months	No serius adverse events; improved algofunctional indices	WOMAC, VAS, KOOS, SAS	[27]
Symptomatic knee osteoarthritis; Kellgren-Lawrence grade III, IV	X-ray	12	44–54	Autologous cultured bone marrow MSCs	Intra-articular injection	1 year	Improved cartilage quality Improved algofunctional indices	T2 mapping WOMAC, VAS	[28]
Isolated femoral chondyle chondral lesions	MRI	40 (randomized)	18–50	With/ without ASCs	following arthroscopic microfracture	12 months	Ongoing (started in 2015)	MRI and KOOS	[29]
Kellgren-Lawrence grade III, IV	MRI	12	54–72	Autologous ASCs; 3 dose escalation cohorts	Intra-articular injection	6 months	No treatment-related adverse events; improved algofunctional indices, Improved cartilage quality	WOMAC, VAS, KSS MRI, arthroscopy, histology	[30]
Kellgren-Lawrence grade II, III	X-ray	4	54–65	Autologous cultured bone marrow MSCs	Intra-articular injection	5 years	Improved algofunctional indices	VAS, PGA	[31, 32]
Chronic knee osteoarthritis; Kellgren-Lawrence grade II, IV	X-ray	30 (randomized)	38–73	Allogeneic bone marrow MSCs or hyaluronic acid	Intra-articular injection	1 year	No serious adverse events; improved cartilage quality Improved algofunctional indices	T2 mapping VAS,WOMAC, Lequesne	[33]
Undergoing partial medial meniscectomy	MRI	55 (randomized)	18–60	Hyaluronic acid with/ without allogenic MSCs	7 to 10 days after meniscectomy	2 years	No clinically important safety issues; pain reduction Improved meniscus volume	VAS MRI	[34]

Table 2 (continued)

Diagnosis	Method	Patients (n)	Age (years)	Cell therapy	Route of transplantation	Follow up	Treatment outcome	Method	Ref.
Symptomatic osteoarthritis at glenohumeral joint and/or rotator cuff tears	MRI	102	38–70	Bone marrow concentrate containing PRP and platelet lysate	Intra-articular injection	2 years	Improved algofunctional indices	DASH, NPS	[35]
Knee osteoarthritis	MRI	Case study	46	Autologous cultured bone marrow MSCs	Percutaneous injection	2 years	Improved cartilage and meniscus growth Improved algofunctional indices	MRI VAS, FRI	[36]
Isolated full-thickness chondral lesions of the knee	MRI	14 (randomized)	23–42	Autologous cultured matrix-induced synovial MSCs versus chondrocyte	Implantation via mini-arthrotomy	2 years	Improved algofunctional indices	KOOS, VAS	[37]
Symptomatic knee cartilage defects ICRS grade III, IV	MRI	44(randomized)	18–50	Microfracture with/withouth ASCs		27 months	Improved algofunctional indices	Lysholm, KOOS, VAS	[38]
Osteonecrosis of femoral head and femoral condyles in knees									
ARCO stage II, IIIa	X-ray MRI	9	28–51	Human umbilical cord-derived MSCs	Intra-arterial injection	2 years	Reduced necrotic volume	MRI	[20]
Ficat 0, I, IIA, IIB, or Steinberg A, B, C; sickle cell disease	X-ray	89	18–55	Autologous bone marrow MSCs	Percutaneous injection	5 years	Improved hip function Disease stabilization	HHS X-ray	[21]
Idiopathic SDIC 3A, 3B	X-ray	10	20–48	Autologous cultured bone marrow MSCs with β-tricalcium phosphate and vascularized bone grafts	Surgical transplantation	2 years	Reduced progression of ONFH Increased bone volume Improved clinical score	X-ray CT JOA	[22]
ARCO stage I-III	X-ray MRI	40 (randomized)	18–50	autologous bone marrow mononuclear cells or unprocessed bone marrow	Injection with core decompression	2 years	Improved hip function Reduced lesion size	HHS MRI	[23]

Table 2 (continued)

Diagnosis	Method	Patients (n)	Age (years)	Cell therapy	Route of transplantation	Follow up	Treatment outcome	Method	Ref.
ARCO stage IC, IIC	MRI	100 (randomized)	18–53	Autologous cultured bone marrow MSC	Core decompression	5 years	Delayed or avoided collapse of femoral head; improved hip function Reduced lesion in femoral head	HHS MRI	[24]
Fracture healing									
Fifth metatarsal stress fracture	X-ray	50		With/ without concentrated blood, bone marrow aspirate	Surgery	1 year	Ongoing (started in 2015)	X-ray	[25]
Tendinopathy									
Chronic and intractable lateral epicondylosis	clinical examination	12	42–62	Allogeneic ASCs with fibrin glue	Injection into largest hypoechoic lesion of the CEO tendon under ultrasound guidance	52 weeks	Reduced tendon defects Improved algofunctional indices	Ultrasound VAS, modified Mayo clinic elbow performance index	[40]
Symptomatic glenohumeral joint osteoarthritis and/ or rotator cuff tears	MRI	102	38–70	Bone marrow concentrate contianing PRP and platelet lysate	Intra-articular injection	2 years	improved algofunctional indices	DASH, NPS	[35]
Rotator cuff tendon partial tear	Ultrasound	12		Bone marrow aspirate concentrate with PRP	Injection at tear site under ultrasound guidance	3 months	Reduction in tear size Improved shoulder function	Ultrasound VAS, MMT, ASES	[41]

ACS, Adipose-derived stem/ stromal cells; ARCO, Association Research Circulation Osseous; ASES, American Shoulder Elbow Scores; CT, computed tomography; DASH, disabilities of the arm, shoulder and hand score; FRI, Functional Rating Index; HHS, Harris Hip Score; ICRS, International Cartilage Repair Society; JOA, Japanese Orthopaedic Association; KOOS, Knee injury and Osteoarthritis Outcome Score; KSS, Knee Society Clinical Rating System; MMT, manual muscle test; MRI, magnetic resonance imaging; NPS, numeric pain scale; PGA, Patient Global Assessment; PRP, platelet rich plasma; SAS, Short Arthritis Assessment Scale; SDIC, Specific Disease Investigation Committee; SF, synovial fluid; VAS, Visual Analogue Scale, WOMAC, Western Ontario and McMaster Universities Osteoarthritis Index

evidence that cells with MSC-like features, such as plastic adherence and multipotency, can be isolated from peripheral blood [162, 163]; however, there is no data on how these correlate with those in the musculoskeletal system in health and disease. The following sections provide brief overviews of the roles of MSCs in the pathogenesis of musculoskeletal disorders, with some comments on how these changes might be applied to potential early diagnosis of these conditions.

Osteoporosis

Primary osteoporosis is a progressive musculoskeletal disorder where the complementary actions of two cell players, osteoblasts and osteoclasts, become imbalanced, whereby bone resorption ultimately prevails over bone formation. MSCs have long been suggested to have a role in the pathophysiology of osteoporosis [164, 165]. There is evidence of their reduced numbers or their diverted differentiation toward adipocytes rather than osteoblasts [164, 166]. It is still not entirely clear if it is MSCs themselves or their microenvironment that guides them along the wrong pathway, as there is evidence from both sides.

MSCs isolated from bone marrow of elderly patients have shown lower proliferation and osteogenesis in vitro [167]. Cells termed as circulating osteogenic progenitors can be isolated from peripheral blood according to their phenotype of CD45, CD19 and CD3 negative and osteocalcin positive, and these have been associated with the prevalence of frailty and disability in elderly people [168]. A cocktail of several inflammatory cytokines, adipokines, growth factors and others can create a microenvironment that is not favorable for osteogenesis [169]. A comparison of human MSCs isolated from a control group of patients without osteoporosis and from a group of patients with osteoporosis has shown intrinsic deficiencies in self-renewal and differentiation potential for the osteoporotic stem cells [170]. Patients with osteoporosis who sustain a fracture and undergo total hip replacement have higher rates of prosthesis loosening due to slower and less effective biomaterial osseointegration [171]. MicroRNAs identified in sera of patients with osteoporosis have been shown to either stimulate (i.e., miR-382-3p) or inhibit (i.e., miR-550a-5p) osteogenesis, while both of these miRNAs stimulate adipogenesis [172].

Osteoarthritis

As endogenous MSCs contribute to the maintenance of healthy joints by acting as reservoirs to repair cells or as immunomodulatory sentinels to reduce inflammation, their role in promoting changes that can lead to osteoarthritis appears inevitable. The onset of degenerative changes in joints is associated with aberrant activity or depletion of these cell reservoirs, which can lead to loss of the chondrogenic potential and preponderance of a fibrogenic phenotype [173]. Murphy et al. [49] reported that MSCs isolated from the bone marrow of the iliac crest of donors with osteoarthritis appear to differ in vitro from those in the normal population in several respects: they have less proliferative capacity, and they are less active in chondrogenic and adipogenic differentiation. Synovial membranes from patients with osteoarthritis contain more cells positive for CD44, CD90, and CD105 than those from joints with undamaged cartilage, although no differences in chondrogenic potential was indicated [174]. In a comparison of subchondral bone without and with bone marrow lesions in patients with hip osteoarthritis, numeric and topographic changes in the native MSCs were shown [79]. As osteoarthritis is a whole joint disease that involves several joint tissues [175], a more comprehensive approach needs to be taken to identify which MSCs contribute to the pathogenesis of this complex disease, and how this contribution is mediated. However, it cannot be excluded that the MSCs abnormalities observed in subjects with osteoarthritis are the consequence rather than the cause of osteoarthritis.

Sarcopenia

Sarcopenia is a multifactorial syndrome that affects more than 40% of people over 70 years of age [176]. Clinical criteria for the diagnosis of sarcopenia are not clearly defined and have differed greatly between studies. Sarcopenia is characterized by a loss of muscle mass and a decrease in muscle strength that is caused by atrophy of type II muscle fibers, heterogeneity in muscle fiber size, accumulation of intramuscular connective tissue and fat, and decreased oxidative capacity. It is also accompanied by a significant decline in satellite-cell function and numbers [177]. Although it remains unclear whether satellite-cell loss contributes to the age-associated loss of muscle mass, they do contribute to age-dependent muscle fibrosis and compromised recovery capacity of sarcopenic muscles in response to injury [178, 179]. It has also been shown that muscle damage affects the potential lineages of skeletal muscle side-population cells, by promoting their differentiation along fibro-adipogenic lineages while inhibiting myogenesis [67]. PDGFRα-positive cells have been shown to be conspicuous in muscles of patients with both genetic and nongenetic muscle diseases, and their pathological relevance to human muscle diseases has been suggested [125]. Studies on sarcopenia treatment have been mainly focused on the promotion of muscle fiber hypertrophy and the activation of endogenous satellite cells using pharmacological and nonpharmacological interventions [180, 181].

Duchenne Muscular Dystrophy

Duchenne muscular dystrophy is an X-linked recessive severe progressive and degenerative muscle disease that affects approximately 1 in 3,500 new-born boys [182]. It is caused by mutations in the dystrophin gene, which result in synthesis of an abnormal dystrophin protein, and the consequent muscle degeneration [183, 184]. Dystrophin provides the required stability for the connection between the cytoskeleton and the plasma membrane [184]. It also has an essential role in satellite-cell polarity [185]. In the absence of dystrophin, satellite-cell polarity is impaired, which in turn leads to dysregulation of mitotic spindle orientation and affects cell-proliferation kinetics. Consequently, this leads to a deficit in cell division, lack of asymmetric division, and reduced generation of myogenic progenitor cells.

Therefore, in addition to muscle fragility, Duchenne muscular dystrophy is a muscle stem-cell disease. Therapies based on the use of stem cells are one of the most promising methods for treating such muscular dystrophies. Although the results of Duchenne muscular dystrophy therapies using satellite cells are encouraging, these approaches have limitations, the greatest of which is the death of most of the injected satellite cells within the first 72 h. More studies are therefore being focused on the use of different cell populations that show myogenic potential, such as pericytes, mesangioblasts and positive interstitial cells [18, 186].

Future Directions Towards Better Human Tissue Regeneration

While there are many positive indications from basic studies and a growing body of evidence coming from clinical trials in favour of MSCs in human tissue regeneration, there is still a lot to be done in future endeavours of regenerative medicine. Despite a myriad of research being done to identify and characterize MSCs isolated from different human tissues, we still do not know exactly how they behave in vivo. Are impaired MSCs responsible for degenerative changes observed in musculoskeletal disorders or does a given disorder itself affect the local microenvironment which then induces changes in MSC phenotype? Does aging affect MSC numbers, their proliferation and differentiation potential? Studies so far have produced conflicting evidence that can be attributed at least to differences in anatomical locations of MSCs sources, isolation protocols used, musculoskeletal pathologies and cell culture conditions.

Another major obstacle is a lack of knowledge on their adverse effects in humans. The results of clinical studies have clearly shown their short and long-term safety not only in orthopaedic conditions [158], but numerous extra musculoskeletal disorders such as Chron's and other inflammatory bowel diseases, multiple sclerosis, idiopathic pulmonary fibrosis, etc. (reviewed elsewhere). However safety, in particular of culture expanded MSCs [187], remains the main concern. We still need to bear in mind that MSCs are multipotent stem cells responding to growth factors and cytokines in their microenvironment. Their specific combinations could, at least in theory, cause MSCs maldifferentiation and possible oncogenic transformation.

In light of all the above described obstacles, much is still left to be done. To start with, we need to identify human MSCs in their physiologic state and learn more about how they are influenced by chronic inflammation, oxidative stress or other conditions accompanying musculoskeletal disorders. We then need to seek further for better alternative sources of MSCs intended for more targeted musculoskeletal tissue regeneration, e.g. autologous or allogeneic tissues other than bone marrow, adipose tissue or cord blood. Moreover, we need to standardize cell therapies by developing new optimized procedures for their isolation, characterization, cultivation and therapeutic applications. Next, more well-designed clinical trials are needed to prove the efficacy and confirm long-term safety of such cell therapies. Last, but not least, development of prognostic tests that would predict the regenerative efficacy of autologous, i.e. patient-derived cells before their clinical application should be strongly encouraged.

MSCs are not the only population of stem cells which can be used human musculoskeletal regeneration. Pluripotent stem cells, in particular induced pluripotent stem cells (iPSC) created by reprogramming autologous somatic cells, which are not a subject of ethical issues, are becoming a promising source in musculoskeletal regenerative medicine [188]. However, it is not yet clear whether the act of reprogramming itself or factors used for reaching this goal can cause genomic instabilities of iPSCs, making them potentially tumorigenic. There is also some evidence that both autologous and allogeneic iPSCs can trigger immune responses [189]. Since extensive preclinical experiments are required to prove their efficacy and safety, at least for now, the use of classical MSCs seems to have an advantage in musculoskeletal regenerative medicine.

Conclusions

Mesenchymal stem/stromal cells are rare, and due to their heterogeneity, they represent largely unknown progenitor cells. MSCs can be found in several adult tissues, where they have been preserved from embryonic development throughout adult life. With the advances in sophisticated animal models to trace MSCs, rare subpopulations that show different regenerative potentials have been identified in several structures of the musculoskeletal system. In other words,

there is substantial evidence from basic studies that every individual harbors their own unique tool to repair damaged tissues.

The question here is now how well we can translate this knowledge to human, to identify these putative cells within human musculoskeletal system, and to exploit them for the purpose of regenerative medicine. Accumulating data from clinical trials and various case studies show promising efficacies and safeties for cell therapies based on MSCs. However, much remains to be learnt about these unique cells within adult human musculoskeletal system. Only by obtaining better understanding of their nature and their behaviour in health and disease can we then seek to investigate new options for their stimulation to regenerate various tissues, including cartilage, bone and muscle, with the ultimate aim being to improve the quality of life of affected individuals.

Acknowledgements The authors acknowledge Chris Berrie for scientific English editing of the manuscript. J. Zupan was funded by UK Arthritis Research as a Postdoctoral Research Fellow at the University of Aberdeen (2014–2016) and by P3-0298 research program of Slovenian Research Agency (2009–2014 and since 2016) as Researcher at the University of Ljubljana. Figures were created using the Mind the Graph platform (http://www.mindthegraph.com).

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

References

1. Walmsley, G. G., Ransom, R. C., Zielins, E. R., et al. (2015). Stem cells in bone regeneration. *Stem Cell Reviews and Reports*. https://doi.org/10.1007/s12015-016-9665-5.
2. Tedesco, F. S., Dellavalle, A., Diaz-Manera, J., Messina, G., & Cossu, G. (2010). Repairing skeletal muscle: regenerative potential of skeletal muscle stem cells. *Journal of Clinical Investigation*. https://doi.org/10.1172/JCI40373.
3. Friedenstein, A. J., Chailakhjan, R. K., & Lalykina, K. S. (1970). The development of a fibroblast colonies in monolayer cultures of guinea pig bone marrow and spleen cells. *Cell Proliferation*. https://doi.org/10.1111/j.1365-2184.1970.tb00347.x.
4. Pittenger, M., Mackay, A., Beck, S., et al. (1999). Multilineage potential of adult human mesenchymal stem cells. *Science*. https://doi.org/10.1126/science.284.5411.143.
5. Hass, R., Kasper, C., Böhm, S., & Jacobs, R. (2011). Different populations and sources of human mesenchymal stem cells (MSCs): a comparison of adult and neonatal tissue-derived MSC. *Cell Communication and Signaling: CCS*. https://doi.org/10.1186/1478-811X-9-12.
6. Grcevic, D., Pejda, S., Matthews, B. G., et al. (2012). In vivo fate mapping identifies mesenchymal progenitor cells. *Stem Cells*. https://doi.org/10.1002/stem.780.
7. Tuli, R., Seghatoleslami, M. R., Tuli, S., et al. (2003). A simple, high-yield method for obtaining multipotential mesenchymal progenitor cells from trabecular bone. *Molecular Biotechnology*. https://doi.org/10.1385/MB:23:1:37.
8. Worthley, D. L., Churchill, M., Compton, J. T., et al. (2015). Gremlin 1 identifies a skeletal stem cell with bone, cartilage, and reticular stromal potential. *Cell*. https://doi.org/10.1016/j.cell.2014.11.042.
9. Méndez-Ferrer, S., Michurina, T. V., Ferraro, F., et al. (2010). Mesenchymal and haematopoietic stem cells form a unique bone marrow niche. *Nature*. https://doi.org/10.1038/nature09262.
10. Zhou, B. O., Yue, R., Murphy, M. M., Peyer, J. G., & Morrison, S. J. (2014). Leptin-receptor-expressing mesenchymal stromal cells represent the main source of bone formed by adult bone marrow. *Cell Stem Cell*. https://doi.org/10.1016/j.stem.2014.06.008.
11. Chan, C. K. F., Seo, E. Y., Chen, J. Y., et al. (2015). Identification and specification of the mouse skeletal stem cell. *Cell*. https://doi.org/10.1016/j.cell.2014.12.002.
12. De Bari, C., Dell'Accio, F., Tylzanowski, P., Luyten, F. P. (2001) Multipotent mesenchymal stem cells from adult human synovial membrane. *Arthritis and Rheumatism*, https://doi.org/10.1002/1529-0131(200108)44:8<1928::AID-ART331>3.0.CO;2-P
13. Sakaguchi, Y., Sekiya, I., Yagishita, K., & Muneta, T. (2005). Comparison of human stem cells derived from various mesenchymal tissues: superiority of synovium as a cell source. *Arthritis and Rheumatism*. https://doi.org/10.1002/art.21212.
14. Roelofs, A. J., Zupan, J., Riemen, A. H. K., et al. (2017). Joint morphogenetic cells in the adult synovium. *Nature Communications*. https://doi.org/10.1038/ncomms15040.
15. Kurth, T. B., Dell'Accio, F., Crouch, V., Augello, A., Sharpe, P. T., & De Bari, C. (2011). Functional mesenchymal stem cell niches in adult mouse knee joint synovium in vivo. *Arthritis and Rheumatism*. https://doi.org/10.1002/art.30234.
16. Tan, Q., Lui, P. P., Rui, Y. F., & Wong, Y. M. (2012). Comparison of potentials of stem cells isolated from tendon and bone marrow for musculoskeletal tissue engineering. *Tissue Engineering Part A*. https://doi.org/10.1089/ten.TEA.2011.0362.
17. Lui, P. P. (2015). Markers for the identification of tendon-derived stem cells in vitro and tendon stem cells in situ – update and future development. *Stem Cell Research & Therapy*. https://doi.org/10.1186/s13287-015-0097-y.
18. Sienkiewicz, D., Kulak, W., Okurowska-Zawada, B., Paszko-Patej, G., Kawnik, K (2015). Duchenne muscular dystrophy: current cell therapies. *Therapeutic Advances in Neurological Disorders*. https://doi.org/10.1177/1756285615586123.
19. De Bari, C., Dell'Accio, F., Vandenabeele, F., Vermeesch, J. R., Raymackers, J. M., & Luyten, F. P. (2003). Skeletal muscle repair by adult human mesenchymal stem cells from synovial membrane. *The Journal of Cell Biology*. https://doi.org/10.1083/jcb.200212064.
20. Chen, C., Qu, Z., Yin, X., et al. (2016). Efficacy of umbilical cord-derived mesenchymal stem cell-based therapy for osteonecrosis of the femoral head: a three-year follow-up study. *Molecular Medicine Reports*. https://doi.org/10.3892/mmr.2016.5745.
21. Daltro, G. C., Fortuna, V., de Souza, E. S., et al. (2012). Efficacy of autologous stem cell-based therapy for osteonecrosis of the femoral head in sickle cell disease: a five-year follow-up study. *Stem Cell Research & Therapy*. https://doi.org/10.1186/s13287-015-0105-2.
22. Aoyama, T., Goto, K., Kakinoki, R., et al. (2014). An exploratory clinical trial for idiopathic osteonecrosis of femoral head by cultured autologous multipotent mesenchymal stromal cells augmented with vascularized bone grafts. *Tissue Engineering Part B*. https://doi.org/10.1089/ten.teb.2014.0090.
23. Rastogi, S., Sankineani, S. R., Nag, H. L., et al. (2013). Intralesional autologous mesenchymal stem cells in management of osteonecrosis of femur: a preliminary study. *Musculoskeletal Surgery*. https://doi.org/10.1007/s12306-013-0273-0.

24. Zhao, D., Cui, D., Wang, B., et al. (2012). Treatment of early stage osteonecrosis of the femoral head with autologous implantation of bone marrow-derived and cultured mesenchymal stem cells. *Bone*. https://doi.org/10.1016/j.bone.2011.11.002.
25. Weel, H., Mallee, W. H., van Dijk, C. N., et al. (2015). The effect of concentrated bone marrow aspirate in operative treatment of fifth metatarsal stress fractures; a double-blind randomized controlled trial. *BMC Musculoskeletal Disorders*. https://doi.org/10.1186/s12891-015-0649-4.
26. Wong, K. L., Lee, K. B., Tai, B. C., Law, P., Lee, E. H., & Hui, J. H. (2013). Injectable cultured bone marrow-derived mesenchymal stem cells in varus knees with cartilage defects undergoing high tibial osteotomy: a prospective, randomized controlled clinical trial with two years' follow-up. *Arthroscopy*. https://doi.org/10.1016/j.arthro.2013.09.074.
27. Pers, Y.-M., Rackwitz, L., Ferreira, R., et al. (2016). Adipose mesenchymal stromal cell-based therapy for severe osteoarthritis of the knee: a phase I dose-escalation trial. *Stem Cells Translational Medicine*. https://doi.org/10.5966/sctm.2015-0245.
28. Orozco, L., Munar, A., Soler, R., et al. (2013). Treatment of knee osteoarthritis with autologous mesenchymal stem cells. *Transplantation*. https://doi.org/10.1097/TP.0b013e318291a2da.
29. Freitag, J., Ford, J., Bates, D., et al. (2015). Adipose derived mesenchymal stem cell therapy in the treatment of isolated knee chondral lesions: design of a randomised controlled pilot study comparing arthroscopic microfracture versus arthroscopic microfracture combined with postoperative mesenchymal. *British Medical Journal Open*. https://doi.org/10.1136/bmjopen-2015-009332.
30. Jo, C., Lee, Y., & Shin, W. (2014). Intra-articular injection of mesenchymal stem cells for the treatment of osteoarthritis of the knee: a proof-of-concept clinical trial. *Stem Cells*. https://doi.org/10.1002/stem.1634.
31. Davatchi, F., Sadeghi Abdollahi, B., Mohyeddin, M., Nikbin, B. (2016). Mesenchymal stem cell therapy for knee osteoarthritis: five years follow-up of three patients. *International Journal Rheumatic Diseases*. https://doi.org/10.1111/1756-185X.12670.
32. Davatchi, F., Abdollahi, B. S., Mohyeddin, M., Shahram, F., & Nikbin, B. (2011). Mesenchymal stem cell therapy for knee osteoarthritis. Preliminary report of four patients. *International Journal Rheumatic Diseases*. https://doi.org/10.1111/j.1756-185X.2011.01599.x.
33. Vega, A., Martín-Ferrero, M. A., Del Canto, F., et al. (2015). Treatment of knee osteoarthritis with allogeneic bone marrow mesenchymal stem cells. *Transplantation*. https://doi.org/10.1097/TP.0000000000000678.
34. Vangsness, C. T., Farr, J., Boyd, J., Dellaero, D. T., Mills, C. R., & LeRoux-Williams, M. (2014). Adult human mesenchymal stem cells delivered via intra-articular injection to the knee following partial medial menisectomy. *The Journal of Bone and Joint Surgery*. https://doi.org/10.2106/JBJS.M.00058.
35. Centeno, C. J., Al-Sayegh, H., Bashir, J., Goodyear, S. H., & Freeman, M. D. (2015). A prospective multi-Site registry study of a specific protocol of autologous bone marrow concentrate for the treatment of shoulder rotator cuff tears and osteoarthritis. *Journal of Pain Research*. https://doi.org/10.2147/JPR.S80872.
36. Centeno, C. J., Busse, D., Kisiday, J., et al. (2008). Increased knee cartilage volume in degenerative joint disease using percutaneously implanted, autologous mesenchymal stem cells. *Pain Physician, 11*(3), 343–353.
37. Akgun, I., Unlu, M. C., Erdal, O. A., et al. (2015). Matrix-induced autologous mesenchymal stem cell implantation versus matrix-induced autologous chondrocyte implantation in the treatment of chondral defects of the knee: a 2-year randomized study. *Archives of Orthopaedic and Trauma Surgery*. https://doi.org/10.1007/s00402-014-2136-z.
38. Koh, Y.-G., Kwon, O.-R., Kim, Y.-S., Choi, Y.-J., & Tak, D.-H. (2016). Adipose-derived mesenchymal stem cells with microfracture versus microfracture alone: two-year follow-up of a prospective randomized trial. *Arthroscopy*. https://doi.org/10.1016/j.arthro.2015.09.010.
39. Chamberlain, C. S., Saether, E. E., Aktas, E., & Vanderby, R. (2017). Mesenchymal stem cell therapy on tendon/ ligament healing. *Journal of Cytokine Biology, 2*(1), 112.
40. Lee, S. Y., Kim, W., Lim, C., & Chung, S. G. (2015). Treatment of lateral epicondylosis by using allogenic adipose-derived mesenchymal stem cells: a pilot study. *Stem Cells*. https://doi.org/10.1002/stem.2110.
41. Kim, S. J., Song, D. H., Park, J. W., Park, S., & Kim, S. J. (2017). Effect of bone marrow aspirate concentrate-platelet-rich plasma on tendon-derived stem cells and rotator cuff tendon tear. *Cell Transplantation*. https://doi.org/10.3727/096368917X694705.
42. Alsalameh, S., Amin, R., Gemba, T., & Lotz, M. (2004). Identification of mesenchymal progenitor cells in normal and osteoarthritic human articular cartilage. *Arthritis and Rheumatism*. https://doi.org/10.1002/art.20269.
43. Pretzel, D., Linss, S., Rochler, S., et al. (2011). Relative percentage and zonal distribution of mesenchymal progenitor cells in human osteoarthritic and normal cartilage. *Arthritis Research and Therapy*. https://doi.org/10.1186/ar3320.
44. Williams, R., Khan, I. M., Richardson, K., et al. (2010). Identification and clonal characterisation of a progenitor cell subpopulation in normal human articular cartilage. *PLoS One*. https://doi.org/10.1371/journal.pone.0013246.
45. Sacchetti, B., Funari, A., Remoli, C., et al. (2016). No identical "mesenchymal stem cells" at different times and sites: human committed progenitors of distinct origin and differentiation potential are incorporated as adventitial cells in microvessels. *Stem Cell Reports*. https://doi.org/10.1016/j.stemcr.2016.05.011.
46. Al-Nbaheen, M., Vishnubalaji, R., Ali, D., et al. (2013). Human stromal (mesenchymal) stem cells from bone marrow, adipose tissue and skin exhibit differences in molecular phenotype and differentiation potential. *Stem Cell Reviews and Reports*. https://doi.org/10.1007/s12015-012-9365-8.
47. Crisan, M., Yap, S., Casteilla, L., Chen, C. W., et al. (2008). A perivascular origin for mesenchymal stem cells in multiple human organs. *Cell Stem Cell*. https://doi.org/10.1016/j.stem.2008.07.003.
48. Guimarães-Camboa, N., Cattaneo, P., Sun, Y., et al. (2017). Pericytes of multiple organs do not behave as mesenchymal stem cells in vivo. *Cell Stem Cell*. https://doi.org/10.1016/j.stem.2016.12.006.
49. Murphy, J. M., Dixon, K., Beck, S., Fabian, D., Feldman, A., & Barry, F. (2002). Reduced chondrogenic and adipogenic activity of mesenchymal stem cells from patients with advanced osteoarthritis. *Arthritis and Rheumatism*. https://doi.org/10.1002/art.10118.
50. Sakaguchi, Y., Sekiya, I., Yagishita, K., Ichinose, S., Shinomiya, K., & Muneta, T. (2009). Suspended cells from trabecular bone by collagenase digestion become virtually identical to mesenchymal stem cells obtained from marrow. *Stem Cells*. https://doi.org/10.1182/blood-2003-12-4452.
51. Latil, M., Rocheteau, P., Châtre, L., et al. (2012). Skeletal muscle stem cells adopt a dormant cell state post mortem and retain regenerative capacity. *Nature Communications*. https://doi.org/10.1038/ncomms1890.
52. Valente, S., Alviano, F., Ciavarella, C., et al. (2014). Human cadaver multipotent stromal/stem cells isolated from arteries stored in liquid nitrogen for 5 years. *Stem Cell Research & Therapy*. https://doi.org/10.1186/scrt397.
53. Baustian, C., Hanley, S., & Ceredig, R. (2012). Isolation, selection and culture methods to enhance clonogenicity of mouse

bone marrow derived mesenchymal stromal cell precursors. *Stem Cell Research & Therapy*. https://doi.org/10.1186/s13287-015-0139-5.
54. Futami, I., Ishijima, M., Kaneko, H., et al. (2012). Isolation and characterization of multipotential mesenchymal cells from the mouse synovium. *PLoS One*. https://doi.org/10.1371/journal.pone.0045517.
55. Zhu, H., Guo, Z.-K., Jiang, X.-X., et al. (2010). A protocol for isolation and culture of mesenchymal stem cells from mouse compact bone. *Nature Protocols*. https://doi.org/10.1038/nprot.2009.238.
56. Tsai, C.-C., Yew, T.-L., Yang, D.-C., Huang, W.-H., & Hung, S.-C. (2012) Benefits of hypoxic culture on bone marrow multipotent stromal cells. *American Journal of Blood Research*.
57. Dominici, M., Le Blanc, K., Mueller, I., et al. (2006) Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. *Cytotherapy*. https://doi.org/10.1080/14653240600855905.
58. Fuchs, E., & Horsley, V. (2011). Ferreting out stem cells from their niches. *Nature Cell Biology*. https://doi.org/10.1038/ncb0511-513.
59. Morikawa, S., Mabuchi, Y., Kubota, Y., et al. (2009). Prospective identification, isolation, and systemic transplantation of multipotent mesenchymal stem cells in murine bone marrow. *The Journal of Experimental Medicine*. https://doi.org/10.1084/jem.20091046.
60. Pinho, S., Lacombe, J., Hanoun, M., et al. (2013). PDGFRa and CD51 mark human Nestin + sphere-forming mesenchymal stem cells capable of hematopoietic progenitor cell expansion. *The Journal of Experimental Medicine*. https://doi.org/10.1084/jem.20122252.
61. Park, D., Spencer, J. A., Koh, B. I., et al. (2012). Cell stem cell endogenous bone marrow MSCs are dynamic, fate-restricted participants in bone maintenance and regeneration. *Stem Cell*. https://doi.org/10.1016/j.stem.2012.02.003.
62. Mizoguchi, T., Pinho, S., Ahmed, J., et al. (2014). Osterix marks distinct waves of primitive and definitive stromal progenitors during bone marrow development. *Developmental Cell*. https://doi.org/10.1016/j.devcel.2014.03.013.
63. Liu, Y., Strecker, S., Wang, L., et al. (2013). Osterix-Cre labeled progenitor cells contribute to the formation and maintenance of the bone marrow stroma. *PLoS One*. https://doi.org/10.1371/journal.pone.0071318.
64. Marecic, O., Tevlin, R., McArdle, A., et al. (2015). Identification and characterization of an injury-induced skeletal progenitor. *Proceedings of the National Academy of Sciences*. https://doi.org/10.1073/pnas.1513066112.
65. Tanaka, K. K., Hall, J. K., Troy, A. A., Cornelison, D. D. W., Majka, S. M., & Olwin, B. B. (2009). Syndecan-4-expressing muscle progenitor cells in the SP engraft as satellite cells during muscle regeneration. *Cell Stem Cell*. https://doi.org/10.1016/j.stem.2009.01.016.
66. Doyle, M. J., Zhou, S., Tanaka, K. K., et al. (2011). Abcg2 labels multiple cell types in skeletal muscle and participates in muscle regeneration. *The Journal of Cell Biology*. https://doi.org/10.1083/jcb.201103159.
67. Meeson, A. P., Hawke, T. J., Graham, S., et al. (2004). Cellular and molecular regulation of skeletal muscle side population cells. *Stem Cells*. https://doi.org/10.1634/stemcells.2004-0077.
68. Penton, C. M., Thomas-Ahner, J. M., Johnson, E. K., McAllister, C., & Montanaro, F. (2013). Muscle side population cells from dystrophic or injured muscle adopt a fibro-adipogenic fate. *PLoS One*. https://doi.org/10.1371/journal.pone.0054553.
69. Pannérec, A., Formicola, L., Besson, V., Marazzi, G., & Sassoon, D. A. (2013). Defining skeletal muscle resident progenitors and their cell fate potentials. *Development*. https://doi.org/10.1242/dev.089326.
70. Cottle, B. J., Lewis, F. C., Shone, V., & Ellison-Hughes, G. M. (2017). Skeletal muscle-derived interstitial progenitor cells (PICs) display stem cell properties, being clonogenic, self-renewing, and multi-potent in vitro and in vivo. *Stem Cell Research & Therapy*. https://doi.org/10.1186/s13287-017-0612-4.
71. Mitchell, K. J., Pannerec, A., Cadot, B., et al. (2010). Identification and characterization of a non-satellite cell muscle resident progenitor during postnatal development. *Nature Cell Biol*. https://doi.org/10.1038/ncb2025.
72. Lewis, F. C., Henning, B. J., Marazzi, G., Sassoon, D., Ellison, G. M., & Nadal-Ginard, B. (2014). Porcine skeletal-muscle-derived multipotent PW1 pos /Pax7 neg interstitial cells: isolation, characterization, and long-term culture. *Stem Cells Transational Medicine*. https://doi.org/10.5966/sctm.2013-0174.
73. Bosnakovski, D., Xu, Z., Li, W., et al. (2008). Prospective isolation of skeletal muscle stem cells with a Pax7 reporter. *Stem Cells*. https://doi.org/10.1634/stemcells.2007-1017.
74. Seale, P., Sabourin, L. A., Girgis-Gabardo, A., Mansouri, A., Gruss, P., & Rudnicki, M. A. (2000) Pax7 is required for the specification of myogenic satellite cells skeletal muscle. *Cell, 102*(6), 777–786.
75. Xu, X., Wilschut, K. J., Kouklis, G., et al. (2015). Human satellite cell transplantation and regeneration from diverse skeletal muscles. *Stem Cell Reports*. https://doi.org/10.1016/j.stemcr.2015.07.016.
76. Morosetti, R., Mirabella, M., Gliubizzi, C., et al. (2007). Isolation and characterization of mesoangioblasts from facioscapulohumeral muscular dystrophy muscle biopsies. *Stem Cells*. https://doi.org/10.1634/stemcells.2007-0465.
77. Bonfanti, C., Rossi, G., Tedesco, F. S., et al. (2015). ARTICLE PW1/Peg3 expression regulates key properties that determine mesoangioblast stem cell competence. *Nature Communications*. https://doi.org/10.1038/ncomms7364.
78. Morosetti, R., Mirabella, M., Gliubizzi, C., et al. (2006). MyoD expression restores defective myogenic differentiation of human mesoangioblasts from inclusion-body myositis muscle. *Proceeding of the National Academy of Sciences*. https://doi.org/10.1073/pnas.0603386103.
79. Dellavalle, A., Maroli, G., Covarello, D., et al. (2011). Pericytes resident in postnatal skeletal muscle differentiate into muscle fibres and generate satellite cells. *Nature Communications*. https://doi.org/10.1038/ncomms1508.
80. Birbrair, A., Zhang, T., Wang, Z. M., et al. (2013). Role of pericytes in skeletal muscle regeneration and fat accumulation. *Stem Cells and Development*. https://doi.org/10.1089/scd.2012.0647.
81. Dellavalle, A., Sampaolesi, M., Tonlorenzi, R., et al. (2007) Pericytes of human skeletal muscle are myogenic precursors distinct from satellite cells. *Nature Cell Biology*, https://doi.org/10.1038/ncb1542.
82. Uezumi, A., Ito, T., Morikawa, D., et al. (2011). Fibrosis and adipogenesis originate from a common mesenchymal progenitor in skeletal muscle. *Journal of Cell Science*. https://doi.org/10.1242/jcs.086629.
83. Uezumi, A., Fukada, S., Yamamoto, N., Takeda, S., & Tsuchida, K. (2010). Mesenchymal progenitors distinct from satellite cells contribute to ectopic fat cell formation in skeletal muscle. *Nature Cell Biology*. https://doi.org/10.1038/ncb2014.
84. Uezumi, A., Fukada, S., Yamamoto, N., et al. (2014). Identification and characterization of PDGFRa + mesenchymal progenitors in human skeletal muscle. *Cell Death & Disease*. https://doi.org/10.1038/cddis.2014.161.
85. Arrighi, N., Moratal, C., Clément, N., et al. (2015). Characterization of adipocytes derived from fibro/adipogenic progenitors

resident in human skeletal muscle. *Cell Death & Disease*. https://doi.org/10.1038/cddis.2015.79.
86. Joe, A. W., Yi, L., Natarajan, A., et al. (2010). Muscle injury activates resident fibro/adipogenic progenitors that facilitate myogenesis. *Nature Cell Biology*. https://doi.org/10.1038/ncb2015.
87. Xu, W., Sun, Y., Zhang, J., et al. (2015). Perivascular-derived stem cells with neural crest characteristics are involved in tendon repair. *Stem Cells and Development*. https://doi.org/10.1089/scd.2014.0036.
88. Runesson, E., Ackermann, P., Karlsson, J., & Eriksson, B. I. (2015). A randomised controlled trial of percutaneous fixation with kirschner wires versus volar locking-plate fixation in the treatment of adult patients with a dorsally displaced fracture of the distal radius. *BMC Musculoskeletal Disorders*. https://doi.org/10.1186/s12891-015-0658-3.
89. Donahue, T., Gregersen, C., Hull, M., et al. (2015). Harnessing endogenous stem/progenitor cells for tendon regeneration. *Journal of Biomechanical Engineering*. https://doi.org/10.1172/JCI81589.
90. Dyment, N. A., Hagiwara, Y., Matthews, B. G., Li, Y., Kalajzic, I., & Rowe, D. W. (2014). Lineage tracing of resident tendon progenitor cells during growth and natural healing. *PLoS One*. https://doi.org/10.1371/journal.pone.0096113.
91. Fukada, S., Higuchi, S., Segawa, M., et al. (2004). Purification and cell-surface marker characterization of quiescent satellite cells from murine skeletal muscle by a novel monoclonal antibody. *Experimental Cell Research*. https://doi.org/10.1016/j.yexcr.2004.02.018.
92. Kuyinu, E. L., Narayanan, G., Nair, L. S., & Laurencin, C. T. (2016). Animal models of osteoarthritis: classification, update, and measurement of outcomes. *Journal of Orthopaedic Surgery and Research*. https://doi.org/10.1186/s13018-016-0346-5.
93. Mills, L. A., & Simpson, A. H. (2012). In vivo models of bone repair. *Bone Joint and Journal*. https://doi.org/10.1302/0301-620X.94B7.27370.
94. Hardy, D., Besnard, A., Latil, M., et al. (2016). Comparative study of injury models for studying muscle regeneration in mice. *PLoS One*. https://doi.org/10.1371/journal.pone.0147198.
95. Carpenter, J. E., & Hankenson, K. D. (2004). Animal models of tendon and ligament injuries for tissue engineering applications. *Biomaterials*. https://doi.org/10.1016/S0142-9612(03)00507-6.
96. Docheva, D., Müller, S. A., Majewski, M., & Evans, C. H. (2015). Biologics for tendon repair. *Advanced Drug Delivery Reviews*. https://doi.org/10.1016/j.addr.2014.11.015.
97. Hast, M. W., Zuskov, A., & Soslowsky, L. J. (2014). The role of animal models in tendon research. *Bone and Joint Research*. https://doi.org/10.1302/2046-3758.36.2000281.
98. Kfoury, Y., & Scadden, D. T. (2015). Cell stem cell mesenchymal cell contributions to the stem cell niche. *Stem Cells*. https://doi.org/10.1016/j.stem.2015.02.019.
99. Omatsu, Y., Seike, M., Sugiyama, T., Kume, T., & Nagasawa, T. (2014). Foxc1 is a critical regulator of haematopoietic stem/progenitor cell niche formation. *Nature*. https://doi.org/10.1038/nature13071.
100. Isern, J., Martín-Antonio, B., Ghazanfari, R., et al. (2013). Self-renewing human bone marrow mesenspheres promote hematopoietic stem cell expansion. *Cell Reports*. https://doi.org/10.1016/j.celrep.2013.03.041.
101. Ding, L., Saunders, T. L., Enikolopov, G., & Morrison, S. J. (2012). Endothelial and perivascular cells maintain haematopoietic stem cells. *Nature*. https://doi.org/10.1038/nature10783.
102. Kunisaki, Y., Bruns, I., Scheiermann, C., et al. (2013). Arteriolar niches maintain haematopoietic stem cell quiescence. *Nature*. https://doi.org/10.1038/nature12612.
103. Fernandez-Moure, J. S., Corradetti, B., Chan, P., et al. (2015). Enhanced osteogenic potential of mesenchymal stem cells from cortical bone: a comparative analysis. *Stem Cell Research & Therapy*. https://doi.org/10.1186/s13287-015-0193-z.
104. Li, H., Ghazanfari, R., Zacharaki, D., et al. (2014). Low/ negative expression of PDGFR-α identifies the candidate primary mesenchymal stromal cells in adult human bone marrow. *Stem Cell Reports*. https://doi.org/10.1016/j.stemcr.2014.09.018.
105. Campbell, T. M., Churchman, S. M., Gomez, A., et al. (2016). Mesenchymal stem cell alterations in bone marrow lesions in patients with hip osteoarthritis. *Arthritis & Rheumatology*. https://doi.org/10.1002/art.39622.
106. Lee, W. C., Shi, H., Poon, Z., et al. (2014). Multivariate biophysical markers predictive of mesenchymal stromal cell multipotency. *Proceedings of the National Academy of Science*. https://doi.org/10.1073/pnas.1402306111.
107. Johnson, K., Zhu, S., Tremblay, M. S., et al. (2013). A stem-cell–based approach to cartilage repair. *Science*. https://doi.org/10.1126/science.1229223.
108. Mauro, A. (1961). Satellite cell of skeletal muscle fibers. *The Journal of Biophysical and Biochemical Cytology*. https://doi.org/10.1083/jcb.9.2.493.
109. Muir, A. R., Kanji, A. H., & Allbrook, D. (1965). The structure of the satellite cells in skeletal muscle. *Journal of Anatomy, 99*(Pt 3), 435–444.
110. Goodell, M. A., Brose, K., Paradis, G., Conner, A. S., & Mulligan, R. C. (1996). Isolation and functional properties of murine hematopoietic stem cells that are replicating in vivo. *The Journal of Experimental Medicine*. https://doi.org/10.1084/jem.183.4.1797.
111. Montanaro, F., Liadaki, K., Schienda, J., Flint, A., Gussoni, E., & Kunkel, L. M. (2004). Demystifying SP cell purification: Viability, yield, and phenotype are defined by isolation parameters. *Experimental Cell Research*. https://doi.org/10.1016/j.yexcr.2004.04.010.
112. Uezumi, A., Ojima, K., Fukada, S., et al. (2006). Functional heterogeneity of side population cells in skeletal muscle. *Biochemical and Biophysical Research Communications*. https://doi.org/10.1016/j.bbrc.2006.01.037.
113. Zhou, S., Schuetz, J. D., Bunting, K. D., et al. (2001). The ABC transporter Bcrp1/ABCG2 is expressed in a wide variety of stem cells and is a molecular determinant of the side-population phenotype. *Nature Medicine*. https://doi.org/10.1038/nm0901-1028.
114. Asakura, A., Seale, P., Girgis-Gabardo, A., & Rudnicki, M. A. (2002). Myogenic specification of side population cells in skeletal muscle. *The Journal of Cell Biology*. https://doi.org/10.1083/jcb.200202092.
115. Sampaolesi, M., Torrente, Y., Innocenzi, A., et al. (2003). Cell therapy of α-sarcoglycan-null dystrophic mice through intra-arterial delivery of mesoangioblasts. *Science*. https://doi.org/10.1126/science.1082254.
116. Galvez, B. G., Sampaolesi, M., Brunelli, S., et al. (2006). Complete repair of dystrophic skeletal muscle by mesoangioblasts with enhanced migration ability. *The Journal of Cell Biology*. https://doi.org/10.1083/jcb.200512085.
117. Sampaolesi, M., Blot, S., D'Antona, G., et al. (2006). Mesoangioblast stem cells ameliorate muscle function in dystrophic dogs. *Nature*. https://doi.org/10.1038/nature05282.
118. Cossu, G., Previtali, S. C., Napolitano, S., et al. (2015). Intra-arterial transplantation of HLA-matched donor mesoangioblasts in Duchenne muscular dystrophy. *EMBO Molecular Medicine*. https://doi.org/10.15252/emmm.201505636.
119. Relaix, F., & Zammit, P. S. (2012). Satellite cells are essential for skeletal muscle regeneration: the cell on the edge returns centre stage. *Development*. https://doi.org/10.1242/dev.069088.

120. Sambasivan, R., Yao, R., Kissenpfennig, A., Van Wittenberghe, L., et al. (2011). Pax7-expressing satellite cells are indispensable for adult skeletal muscle regeneration. *Development*. https://doi.org/10.1242/dev.067587.
121. Hernandez-Torres, F., Rodríguez-Outeiriño, L., Franco, D., & Aranega, A. E. (2017). Pitx2 in embryonic and adult myogenesis. *Frontiers in Cell and Development Biology*. https://doi.org/10.3389/fcell.2017.00046.
122. Zhang, J., & Wang, J. (2013). Human tendon stem cells better maintain their stemness in hypoxic culture conditions. *PLoS One*. https://doi.org/10.1371/journal.pone.0061424.
123. Bi, Y., Ehirchiou, D., Kilts, T. M., et al. (2007). Identification of tendon stem/progenitor cells and the role of the extracellular matrix in their niche. *Nature Medicine*. https://doi.org/10.1038/nm1630.
124. Po, P., & Lui, Y. (2015). Stem cell technology for tendon regeneration: current status, challenges, and future research directions. *Stem Cells Cloning: Advances and Applications*. https://doi.org/10.2147/SCCAA.S60832.
125. Zhang, X., Lin, Y. C., Rui, Y. F., et al. (2016). Therapeutic roles of tendon stem/ progenitor cells in tendinopathy. *Stem Cells International*. https://doi.org/10.1155/2016/4076578.
126. Lee, K. J., Clegg, P. D., Comerford, E. J., & Canty-Laird, E. G. (2017). Ligament-derived stem cells: identification, characterisation, and therapeutic application. *Stem Cells International*. https://doi.org/10.1155/2017/1919845.
127. Cheng, M. T., Yang, H. W., Chen, T. H., & Lee, O. K. (2009). Isolation and characterization of multipotent stem cells from human cruciate ligaments. *Cell Proliferation*. https://doi.org/10.1111/j.1365-2184.2009.00611.x.
128. de Sousa, E., Casado, P., Neto, V., Duarte, M. E., & Aguiar, D. (2014). Synovial fluid and synovial membrane mesenchymal stem cells: latest discoveries and therapeutic perspectives. *Stem Cell Research & Therapy*. https://doi.org/10.1186/scrt501.
129. Spees, J. L., Lee, R. H., & Gregory, C. A. (2016). Mechanisms of mesenchymal stem/stromal cell function. *Stem Cell Research & Therapy*. https://doi.org/10.1186/s13287-016-0363-7.
130. Zachar, L., Bačenková, D., & Rosocha, J. (2016). Activation, homing, and role of the mesenchymal stem cells in the inflammatory environment. *Journal of Inflammation Research*. https://doi.org/10.2147/JIR.S121994.
131. Schepers, K., & Fibbe, W. E. (2016). Unraveling mechanisms of mesenchymal stromal cell-mediated immunomodulation through patient monitoring and product characterization. *Annals of the New York Academy of Sciences*. https://doi.org/10.1111/nyas.12984.
132. Zhao, Q., Ren, H., & Han, Z. (2016). Mesenchymal stem cells: Immunomodulatory capability and clinical potential in immune diseases. *Journal of Cellular Immunotherapy*. https://doi.org/10.1016/j.jocit.2014.12.001.
133. Regulski, M. J. (2017). Mesenchymal stem cells: "guardians of inflammation." *Wounds, 29*, 20–27.
134. Nishizawa, K., & Seki, R. (2016). Mechanisms of immunosuppression by mesenchymal stromal cells: a review with a focus on molecules. *Biomedical Research and Clinical Practice*. https://doi.org/10.15761/BRCP.1000116.
135. Glenn, J. D. (2014). Mesenchymal stem cells: emerging mechanisms of immunomodulation and therapy. *World Journal of Stem Cells*. https://doi.org/10.4252/wjsc.v6.i5.526.
136. Gruh, I., & Martin, U. (2009). Transdifferentiation of stem cells: a critical view. *Engineering of Stem Cells*. https://doi.org/10.1007/10_2008_49.
137. Prockop, D. J., & Oh, J. Y. (2012). Medical therapies with adult stem/progenitor cells (MSCs): a backward journey from dramatic results in vivo to the cellular and molecular explanations. *Journal of Cellular Biochemistry*. https://doi.org/10.1002/jcb.24046.
138. Sottile, F., Aulicino, F., Theka, I., & Cosma, M. P. (2016). Mesenchymal stem cells generate distinct functional hybrids in vitro via cell fusion or entosis. *Scientific Reports*. https://doi.org/10.1038/srep36863.
139. Usunier, B., Benderitter, M., Tamarat, R., & Chapel, A. (2014). Management of fibrosis: the mesenchymal stromal cells breakthrough. *Stem Cells International*. https://doi.org/10.1155/2014/340257.
140. Islam, M. N., Das, S. R., Emin, M. T., et al. (2012). Mitochondrial transfer from bone marrow-derived stromal cells to pulmonary alveoli protects against acute lung injury. *Nature Medicine*. https://doi.org/10.1038/nm.2736.
141. Chinnery, H. R., Pearlman, E., & McMenamin, P. G. (2008). Cutting edge: Membrane nanotubes in vivo: a feature of MHC class II + cells in the mouse cornea. *Journal of Immunology, 180*(9), 5779–5783.
142. Liu, K., Ji, K., Guo, L., et al. (2014). Mesenchymal stem cells rescue injured endothelial cells in an in vitro ischemia–reperfusion model via tunneling nanotube like structure-mediated mitochondrial transfer. *Microvascular Research*. https://doi.org/10.1016/j.mvr.2014.01.008.
143. Valadi, H., Ekström, K., Bossios, A., Sjöstrand, M., Lee, J. J., & Lötvall, J. O. (2007). Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. *Nature Cell Biology*. https://doi.org/10.1038/ncb1596.
144. Viganò, M., Sansone, V., d'Agostino, M. C., Romeo, P., Perucca Orfei, C., & de Girolamo, L. (2016). Mesenchymal stem cells as therapeutic target of biophysical stimulation for the treatment of musculoskeletal disorders. *Journal of Orthopaedic Surgery and Research*. https://doi.org/10.1186/s13018-016-0496-5.
145. Coelho, M. B., Cabral, J. M. S., & Karp, J. M. (2012). Intraoperative stem cell therapy. *Annual Review of Biomedical Engineering*. https://doi.org/10.1146/annurev-bioeng-071811-150041.
146. Veronesi, F., Giavaresi, G., Tschon, M., Borsari, V., Nicoli Aldini, N., & Fini, M. (2013). Clinical use of bone marrow, bone marrow concentrate, and expanded bone marrow mesenchymal stem cells in cartilage disease. *Stem Cells and Development*. https://doi.org/10.1089/scd.2012.0373.
147. Hernigou, P., Homma, Y., Flouzat Lachaniette, C.H., et al (2013). Benefits of small volume and small syringe for bone marrow aspirations of mesenchymal stem cells. *International Orthopaedics*. https://doi.org/10.1007/s00264-013-2017-z.
148. Otsuru, S., Hofmann, T. J., Olson, T. S., Dominici, M., & Horwitz, E. M. (2013). Improved isolation and expansion of bone marrow mesenchymal stromal cells using a novel marrow filter device. *Cytotherapy*. https://doi.org/10.1016/j.jcyt.2012.10.012.
149. Ito, K., Aoyama, T., Fukiage, K., et al. (2010). A novel method to isolate mesenchymal stem cells from bone marrow in a closed system using a device made by nonwoven fabric. *Tissue Engineering Part C Methods*. https://doi.org/10.1089/ten.TEC.2008.0693.
150. Zuk, P. A., Zhu, M., Mizuno, H., et al. (2001). Multilineage cells from human adipose tissue: implications for cell-based therapies. *Tissue Engineering*. https://doi.org/10.1089/107632701300062859.
151. Lendeckel, S., Jödicke, A., Christophis, P., et al. (2004). Autologous stem cells (adipose) and fibrin glue used to treat widespread traumatic calvarial defects: case report. *Journal of Cranio-Maxillofacial Surgery*. https://doi.org/10.1016/j.jcms.2004.06.002.
152. Baer, P. C., & Geiger, H. (2012). Adipose-derived mesenchymal stromal/stem cells: tissue localization, characterization, and heterogeneity. *Stem Cells International*. https://doi.org/10.1155/2012/812693.
153. Fraser, J. K., Zhu, M., Wulur, I., & Alfonso, Z. (2008). Adipose-derived stem cells. *Methods in Molecular Biology*. https://doi.org/10.1007/978-1-60327-169-1_4.

154. Pak, J., Lee, J. H., Park, K. S., Park, M., Kang, L. W., & Lee, S. H. (2017). Current use of autologous adipose tissue-derived stromal vascular fraction cells for orthopedic applications. *Journal of Biomedical Sciences*. https://doi.org/10.1186/s12929-017-0318-z.
155. Ancans, J. (2012). Cell therapy medicinal product regulatory framework in Europe and its application for MSC-based therapy development. *Frontiers in Immunology*. https://doi.org/10.3389/fimmu.2012.00253.
156. Kristjánsson, B., & Honsawek, S. (2014). Current perspectives in mesenchymal stem cell therapies for osteoarthritis. *Stem Cells International*. https://doi.org/10.1155/2014/194318.
157. Reissis, D., Tang, Q. O., Cooper, N. C., et al. (2016). Current clinical evidence for the use of mesenchymal stem cells in articular cartilage repair. *Expert Opinion on Biological Therapy*. https://doi.org/10.1517/14712598.2016.1145651.
158. Centeno, C. J., Al-Sayegh, H., Freeman, M. D., Smith, J., Murrell, W. D., & Bubnov, R. (2016). A multi-center analysis of adverse events among two thousand, three hundred and seventy two adult patients undergoing adult autologous stem cell therapy for orthopaedic conditions. *International Orthopaedics*. https://doi.org/10.1007/s00264-016-3162-y.
159. Deng, Z., Jin, J., Zhao, J., & Xu, H. (2016). Cartilage defect treatments: With or without cells? Mesenchymal stem cells or chondrocytes? Traditional or matrix-assisted? A systematic review and meta-analyses. *Stem Cells International*. https://doi.org/10.1155/2016/9201492.
160. Kon, E., Roffi, A., Filardo, G., Tesei, G., & Marcacci, M. (2015). Scaffold-based cartilage treatments: with or without cells? A systematic review of preclinical and clinical evidence. *Arthroscopy*. https://doi.org/10.1016/j.arthro.2014.11.017.
161. Merlos-Suárez, A., Barriga, F. M., Jung, P., et al. (2011). The intestinal stem cell signature identifies colorectal cancer stem cells and predicts disease relapse. *Cell Stem Cell*. https://doi.org/10.1016/j.stem.2011.02.020.
162. Cao, C., Dong, Y., & Dong, Y. (2005). [Study on culture and in vitro osteogenesis of blood-derived human mesenchymal stem cells]. *Zhongguo Xiu Fu Chong Jian Wai Ke Za Zhi, 19*, 642–647.
163. Kassis, I., Zangi, L., Rivkin, R., et al. (2006). Isolation of mesenchymal stem cells from G-CSF-mobilized human peripheral blood using fibrin microbeads. *Bone Marrow Transplant*. https://doi.org/10.1038/sj.bmt.1705358.
164. Pino, A. M., Rosen, C. J., & Rodríguez, J. P. (2012). In Osteoporosis, differentiation of mesenchymal stem cells (MSCs) improves bone marrow adipogenesis. *Biological Research*. https://doi.org/10.4067/S0716-97602012000300009.
165. Rodríguez, J. P., Garat, S., Gajardo, H., Pino, A. M., & Seitz, G. (1999). Abnormal osteogenesis in osteoporotic patients is reflected by altered mesenchymal stem cells dynamics. *Journal of Cellular Biochemistry*. https://doi.org/10.1002/(SICI)1097-4644(19991201)75:3<414::AID-JCB7>3.0.CO;2-C
166. Rosen, C. J., & Bouxsein, M. L. (2006). Mechanisms of disease: is osteoporosis the obesity of bone? *Nature Clinical Practice Rheumatology*. https://doi.org/10.1038/ncprheum0070.
167. D'Ippolito, G., Schiller, P. C., Ricordi, C., Roos, B. A., & Howard, G. A. (1999). Age-related osteogenic potential of mesenchymal stromal stem cells from human vertebral bone marrow. *Journal of Bone and Mineral Research*. https://doi.org/10.1359/jbmr.1999.14.7.1115.
168. Gunawardene, P., Bermeo, S., Vidal, C., et al. (2016). Association between circulating osteogenic progenitor cells and disability and frailty in older persons: the Nepean osteoporosis and frailty study. *The Journals of Gerontology Series A, Biological Sciences and Medical Sciences*. https://doi.org/10.1093/gerona/glv190.
169. Yu, B., & Wang, C. Y. (2016). Osteoporosis: the result of an "aged" bone microenvironment. *Trends Mol Med*. https://doi.org/10.1016/j.molmed.2016.06.002.
170. Benisch, P., Schilling, T., Klein-Hitpass, L., et al. (2012). The transcriptional profile of mesenchymal stem cell populations in primary osteoporosis is distinct and shows overexpression of osteogenic inhibitors. *PLoS One*. https://doi.org/10.1371/journal.pone.0045142.
171. Marco, F., Milena, F., Gianluca, G., & Vittoria, O. (2005). Peri-implant osteogenesis in health and osteoporosis. *Micron*. https://doi.org/10.1016/j.micron.2005.07.008.
172. Heilmeier, U., Hackl, M., Skalicky, S., et al. (2016). Serum miRNA signatures are indicative of skeletal fractures in postmenopausal women with and without type 2 diabetes and influence of osteogenic and adipogenic differentiation of adipose-tissue-derived mesenchymal stem cells in vitro. *Journal of Bone and Mineral Research*. https://doi.org/10.1002/jbmr.2897.
173. Barry, F., & Murphy, M. (2013). Mesenchymal stem cells in joint disease and repair. *Nature Reviews Rheumatology*. https://doi.org/10.1038/nrrheum.2013.109.
174. Hermida-Gómez, T., Fuentes-Boquete, I., Gimeno-Longas, M. J., et al. (2011). Quantification of cells expressing mesenchymal stem cell markers in healthy and osteoarthritic synovial membranes. *The Journal of Rheumatology*. https://doi.org/10.3899/jrheum.100614.
175. Findlay, D. M., & Kuliwaba, J. S. (2016). Bone–cartilage crosstalk: a conversation for understanding osteoarthritis. *Bone Research*. https://doi.org/10.1038/boneres.2016.28.
176. Kalinkovich, A., & Livshits, G. (2015). Sarcopenia–The search for emerging biomarkers. *Ageing Research Reviews*. https://doi.org/10.1016/j.arr.2015.05.001.
177. Sousa-Victor, P., & Muñoz-Cánoves, P. (2016). Regenerative decline of stem cells in sarcopenia. *Molecular Aspects of Medicine*. https://doi.org/10.1016/j.mam.2016.02.002.
178. Sousa-Victor, P., Gutarra, S., García-Prat, L., et al. (2014). Geriatric muscle stem cells switch reversible quiescence into senescence. *Nature*. https://doi.org/10.1038/nature13013.
179. Fry, C. S., Lee, J. D., Mula, J., et al. (2015). Inducible depletion of satellite cells in adult, sedentary mice impairs muscle regenerative capacity without affecting sarcopenia. *Nature Medicine*. https://doi.org/10.1038/nm.3710.
180. Dennison, E. M., Sayer, A. A., & Cooper, C. (2017). Epidemiology of sarcopenia and insight into possible therapeutic targets. *Nature Reviews Rheumatology*. https://doi.org/10.1038/nrrheum.2017.60.
181. Snijders, T., Verdijk, L. B., & van Loon, L. J. C. (2009). The impact of sarcopenia and exercise training on skeletal muscle satellite cells. *Ageing Research Reviews*. https://doi.org/10.1016/j.arr.2009.05.003.
182. Emery, A. E. H. (1991). Population frequencies of inherited neuromuscular diseases-A world survey. *Neuromuscular Disorders*. https://doi.org/10.1016/0960-8966(91)90039-U.
183. Song, Y., Yao, S., Liu, Y., et al. (2017). Expression levels of TGF-β1 and CTGF are associated with the severity of Duchenne muscular dystrophy. *Experimental and Therapeutic Medicine*. https://doi.org/10.3892/etm.2017.4105.
184. Almeida, C. F., Martins, P. C., & Vainzof, M. (2016). Comparative transcriptome analysis of muscular dystrophy models Large^{myd}, Dmd^{mdx}/Large^{myd} and Dmd^{mdx}: what makes them different? *European Journal of Human Genetics*. https://doi.org/10.1038/ejhg.2016.16.
185. Dumont, N. A., Xin Wang, Y., & von Maltzahn, J. (2015). Dystrophin expression in muscle stem cells regulates their polarity and asymmetric division. *Nature Medicine*. https://doi.org/10.1038/nm.3990.

186. Marg, A., Escobar, H., Gloy, S., et al. (2014). Human satellite cells have regenerative capacity and are genetically manipulable. *Journal of Clinical Investigation*. https://doi.org/10.1172/JCI63992.
187. Wang, Y., Han, Z., Song, Y., & Han, Z. C. (2012). Safety of mesenchymal stem cells for clinical application. *Stem Cells International*. https://doi.org/10.1155/2012/652034.
188. Lu, X., & Zhao, T. (2013). Clinical Therapy using iPSCs: Hopes and Challenges. *Genomics, Proteomics & Bioinformatics*. https://doi.org/10.1016/j.gpb.2013.09.002.
189. Liu, X., Li, W., Fu, X., & Xu, Y. (2017). The immunogenicity and immune tolerance of pluripotent stem cell derivatives. *Frontiers in Immunology*. https://doi.org/10.3389/fimmu.2017.00645.