

Review

# Mesenchymal stem cells for bone, cartilage, tendon and skeletal muscle repair

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## Abstract

Literature data concerning the biology and differentiation potential of mesenchymal stem cells (MSCs) have become huge in less than 10 years, although some of these data still remain contradictory. MSCs seem to be a very promising tool for cell therapy because of their peculiar characteristics, which mimic partially those of embryonic stem cells, but with some advantages in terms of availability, expandability, transplantability, and ethical implications. We discuss here the potential use of MSCs in degenerative or inflammatory diseases involving bone, cartilage, tendon and muscle tissues, on the basis of the experimental evidence.

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**Keywords:** Mesenchymal stem cells; Regenerative medicine; Tissue repair

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## Introduction

MSCs have been initially identified in bone marrow as non-hematopoietic stem cells that may differentiate into tissues of mesodermal origin, such as adipocytes, osteoblasts, chondrocytes, tenocytes, skeletal myocytes and visceral stromal cells [1–5]. Indeed, MSCs can differentiate also into tissues of ectodermal (such as neurons) [6] and endodermal origin, such as hepatocytes [7], thus resembling embryonic stem cells. This

multilineage stem cell potential permits to distinguish normal marrow stromal precursors from MSCs that are normally rare in adult human bone marrow ( $1/10^5$ ) [1–5]. Cell suspensions derived from bone marrow or other tissues are the normal sources of MSCs. Cells may be expanded with complete medium in culture plates or flasks, where they adhere, start proliferating and form fibroblastic-like cell clusters (fibroblast colony forming units, CFU-F), whose number depends on MSC clonogenic potential of the sample [1–5,8]. Before cells become confluent, they are split and expanded in larger flasks, thus becoming a more and more homogenous adherent cell population that may proliferate without differentiating up to 40 generations [1–5,8]. For clinical use, MSCs must be expanded in dedicated

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rooms with laminar flux (Stem Cell Factory), according to the same rules of good manufacturing practice (GMP) used for drug production, which requires very stringent conditions of sterility, specific reagents without heterologous proteins and different kinds of quality controls, including microbiological, immunological and functional tests [9].

Specific differentiation media can easily reveal MSCs nature of expanded, adherent stromal-like cells, which do not express specific markers, but a complex pattern of molecules, including CD105 (SH2), CD73 (SH3 and SH4), CD106 (VCAM-1), CD54 (ICAM-1), CD44, CD90, CD29, STRO-1 [1–5,8,10], as well as immune molecules such as HLA class I and II (the latter only upon the effect of interferon-gamma, IFN- $\gamma$ ) and CD119 (IFN- $\gamma$  receptor) [10]. Hemopoietic markers, such as CD45 and CD34, are normally not expressed [1–5,8,10].

Source of MSCs is not only bone marrow, but also other adult tissues such as fat [11], hair follicles and scalp subcutaneous tissue [12], periodontal ligament [13], thymus and spleen (personal data), as well as pre-natal tissues, such as placenta [14], umbilical cord blood [15], fetal bone marrow, blood, lung, liver and spleen [16]. Circulating MSCs can be detected in peripheral blood [17], similarly to what happens for hemopoietic stem cells. It is likely that a mesenchymal reservoir, involved in tissue homeostasis, may be supported by circulating bone marrow MSCs, as shown by injecting MSCs intravenously: these cells spread into all tissues, but preferentially survive and proliferate in the presence of regenerating tissues and tumors, where they become vasculo-stromal fibroblasts. For this reason, engineered MSCs may be used as specific carriers of anti-cancer drugs [18] as the systemic infusion of MSCs is feasible and safe in the short term [18,19].

An additional, but not less important property of MSCs is their immunomodulatory effect towards a large number of immune effector cells, including CD4<sup>+</sup> and CD8<sup>+</sup> T cells [10,20–25], NK cells [10,26,27], B cells [28], monocytes and dendritic cells [29–31]. This effect, although the underlying mechanisms are only partially clear, is operational in vivo as MSC infusion can significantly prolong the survival of MHC-mismatched skin grafts in baboons [32], lower the incidence [33] and cure the refractoriness to treatment [34] of graft-versus-host disease after allogeneic hemopoietic stem cell transplantation in humans and improve experimental autoimmune encephalomyelitis in mice [35]. It is likely that the immunomodulatory effect of MSCs may be broad and long-lasting inside the tissue microenvironment because it seems to depend, at least in humans, mainly on soluble factors rather than cell–cell contact [10,20,22–24]; among them, transforming growth factor (TGF)- $\beta$ 1, hepatocyte growth factor (HGF) [20,26], prostaglandin (PG)E<sub>2</sub> [26,31], indoleamine 2,3-dioxygenase (IDO) [10,23] and interferon-gamma (IFN- $\gamma$ ) [10] have been shown to play an important role in MSC-mediated inhibition of immune effector cells. This evidence suggests that local or systemic therapeutic benefit of MSC infusion may be achieved also with low numbers of MSCs.

Considering together regenerative potential and immunoregulatory effect of MSCs, it is easy to imagine what powerful tool for cell therapy these cells may become in degenerative and inflammatory diseases [36–39]. Such an approach has been

employed, using animal models, to repair and regenerate various tissues [40,41]. However, the reconstruction of any tissue requires not only repairing cells (i.e. MSCs), but also adequate scaffolds where the implanted MSCs can proliferate and interact with specific growth factors and cytokines. Thus, regenerative medicine has become a discipline that joins cell biology, tissue engineering and surgery to renew tissues with vital cells, biomatrices and signaling molecules [42,43]. Interaction between cells and scaffolds, cell adhesion on the matrix surface, cell proliferation, maturation and differentiation and extracellular matrix production are all important factors for the success of cell therapy procedures.

## Mesenchymal stem cells and regenerative medicine

### Bone repair

On the basis of in vitro observation that MSCs can differentiate into osteocytes and chondrocytes, many attempts have been made to use expanded MSCs for in vivo tissue repair [44–46]. Bone-marrow-derived MSCs have been seeded on extracellular matrices such as hydroxyapatite and then implanted in vivo into NOD/SCID mice, subsequently observing bone formation [47]. In various animal models, MSCs have been used to repair segmental bone defects of critical size [48,49]. Similarly, hydroxyapatite matrices loaded with MSCs have been applied into a canine segmental bone defect [50,51]. Normal marrow-derived stromal cells have been infused into irradiated mice with osteogenesis imperfecta, a genetic disorder of mesenchymal tissues, determining functional bone and cartilage formation from the transplanted cells [4]. Similarly, bone marrow cells infused in children with osteogenesis imperfecta not only engrafted without any side effect, but also increased 3 months later the mean number of osteoblasts, the formation of new lamellar bone and the total body mineral content. In addition, they eventually lowered the frequency of fractures and enhanced the body growth rate [3]. Other studies in animals showed that the best route of MSC administration to induce local repair or regeneration of bone, cartilage or tendon is the in situ injection or implant [52,53].

Particularly promising for orthopedic applications, especially for bone formation, is the use of natural or synthetic biomaterials as carriers for MSCs delivery [54]. Recent advances in the field of biomaterials have determined a transition from the use of non-porous, biologically inert materials (i.e. ceramics or titanium) to porous, resorbable and osteoconductive biomaterials (i.e. hydroxyapatite and tricalcium phosphate) [55,56]. Cell–matrix composites, made of hydroxyapatite/tricalcium phosphate ceramics loaded with autologous MSCs expanded in vitro, have been successfully used in vivo, leading to resolution of critical segmental bone defects that had been not healed by resident cells or the addition of the osteoconductive device alone [57]. The comparison of the biodegradable polymers poly-L-lactide (PLA) and poly-L-lactide-co-glycolide (PLGA), as far as adherence, proliferation and differentiation of trabecular-bone-derived osteoprogenitor cells are concerned, showed that PLGA is the best substrate for this purpose [58]. A number of clinical studies have shown the efficacy of this approach in humans. Porous ceramic

scaffolds loaded with in vitro expanded autologous bone-marrow-derived MSCs were successfully implanted in 3 patients with large bone defects [59]. Similarly, periosteal-derived stem cells in conjunction with hydroxyapatite scaffold were used to reconstruct an avulsed phalanx [60]. More recently, an extended mandible discontinuity was successfully repaired through a heterotopic bone induction with biomaterials, patient's bone marrow and growth factors [61]. Consequently, an efficient approach to repair bone defects seems to be the local implantation of porous cell-matrix composites loaded with autologous bone marrow MSCs, previously collected from the patient and expanded in vitro under stringent culture conditions [9].

### Cartilage repair

The clinical benefit derived from the local injection of chondrocyte suspension to cure human joint cartilage defects (autologous chondrocyte transplantation, ACT) was first described in 1994 [62]. This evidence has led to the development of MSC-based strategies of tissue engineering to induce in situ differentiation of mesenchymal progenitors into cartilage [63,64]. MSCs have been used in vivo to repair full-thickness, joint cartilage defects in animal models using various carrier matrices [65–70]. Allogeneic, goat MSCs derived from bone marrow were injected, in combination with a hyaluronan carrier, into goat knees after medial meniscectomy and resection of the anterior crucial ligament [67]. Most goats treated with MSCs and hyaluronan had evidence of regeneration of the meniscus in comparison with controls treated with the carrier alone. In addition, those animals showed a significant decrease of bone resorption, subchondral bone remodeling, osteophyte formation and cartilage destruction, as well as a better preservation of the joint lining. Tissue biopsy showed no signs of inflammation, suggesting that any immune rejection had not occurred, probably because of the down-modulation of immune effector functions by MSCs [67]. In rabbits, full repair of full-thickness defects of joint cartilage was observed after transplantation of autologous MSCs dispersed in a type I collagen gel [65]. Similarly, in the same animal model, encouraging results have been obtained by injecting calcium phosphate and hyaluronan sponge, previously loaded with autologous bone-marrow-derived MSCs, in knees with osteochondral defects [71]. The use of synthetic polymers, particularly PLA and PLGA, is also promising and applicable for the clinical reconstruction of joint cartilage defects [43,72]. In addition, the induction of chondrogenic differentiation of bone marrow MSCs by tridimensional matrices and scaffolds has been studied in vivo in the presence of cytokines and recombinant human bone morphogenetic protein-2 (rhBMP-2). This approach that combines regenerating cells, bioactive matrices, and osteoinductive growth factors seems to be mostly effective for the treatment of joint cartilage defects [73–77]. Recently, autologous bone-marrow-derived MSCs have been applied to patients with osteoarthritis [68]. MSCs were injected in twelve patients inside the knee, on the medial femoral condyle, at the time of high tibial osteotomy, and were covered with autologous periosteum. Twelve control patients underwent the same procedure without receiving MSCs. Patients

treated with MSCs had better arthroscopic and histological grading scores, although the clinical improvement was not significantly different between the two groups [68]. However, this study shows the feasibility, safety and potential efficacy of MSC therapy for cartilage repair, which probably requires both cartilage regeneration and MSC immunoregulatory effect that may play a role in reducing arthritis signs and symptoms due to inflammatory reaction.

Finally, MSCs have been successfully used for intervertebral disc regeneration in a rat model [78]. Local injection of fluorescently labeled MSCs was carried out using 15% hyaluronan gel as a carrier. After an initial decrease at 7 and 14 days after injection, fluorescent MSCs inside the disc returned to the initial number of injected cells at 28 days, with 100% cell viability, thus suggesting that MSCs are vital and proliferate within the rat intervertebral disc [78].

### Tendon and skeletal muscle repair

Induction of MSC differentiation into connective tissues other than bone and cartilage, such as tendons and ligaments, has been investigated for a potential clinical application [43]. Several animal studies and human clinical trials have been carried out to evaluate the efficiency of MSC local injection in tendon repair [79,80]. Different autologous MSC concentrations (1, 4 and  $8 \times 10^6$  cells/ml) in a type I collagen gel significantly improved tendon repair, but not in a dose-dependent manner and with the evidence of ectopic bone formation in almost 30% of the cases. In this study, the maximum force and maximum stress for the cell-gel-suture repairs were 20% of normal [81]. Similarly, MSCs-collagen composites (MSC density:  $4 \times 10^6$  cells/ml) were implanted into long gap defects in the rabbit Achilles tendon: biochemical and histological analysis revealed an improvement of biomechanical properties, tissue architecture and functionality of the tendon after injury. At 12 weeks post-surgery, the modulus and maximum stress for the repair tissue were 34% and 37%, respectively, of normal values [82]. A further enhancement of tendon and ligament tissue regeneration derives from the use of exogenous growth/differentiation factors (GDF), for example, GDF-5, GDF-6 and GDF-7, which have been implicated in tendon formation [83]. In addition, PLGA scaffold have been used instead of collagen gel with suture, leading to better results at 4 and 12 weeks than scaffold alone [84,85]. Other authors showed that decreasing cell-to-collagen ratio by 20 times (from 0.8 to 0.04 M cells/mg collagen) improved cell viability in culture, eliminates ectopic bone in the repair site and improves repair biomechanics and histological appearance at 12 weeks post-surgery [86]. In addition, the introduction of stem cells into a gel-sponge composite, as well as the mechanical stimulation using a strain signal that matched estimated peak in vivo strains, significantly improved repair biomechanics and cellular organization of rabbit patellar tendon repairs at 12 weeks post-surgery [87].

MSCs can differentiate into skeletal, smooth and cardiac muscle cells [88,89]. Most studies are focused on cardiomyocyte differentiation potential of MSCs for cardiac regeneration after infarction [90–96]. After the evidence that human or murine MSCs implanted into murine myocardium differentiate

into cardiomyocytes and induce angiogenesis [91,92], some groups used autologous MSCs to treat myocardial infarction in animal models, showing engraftment, differentiation and improved cardiac function and myocardial perfusion [94], thus suggesting that this approach could be useful to regenerate cardiomyocytes and reduce the complications of cardiac disease in humans [90–93]. However, some data were achieved with the use of bone-marrow-derived mononuclear cells rather than purified MSCs. The first randomized clinical trial of intracoronary infusion of autologous bone-marrow-derived progenitor cells was carried out in a group of 30 patients with myocardial infarction: 6 months later, significant increase of left ventricular ejection fraction (LVEF) and reduced end-systolic volume without adverse events was observed in treated patients as compared to control group [97]. Similar results were achieved in the first randomized clinical trial with autologous MSCs administered intracoronary, which was carried out in 69 patients with acute myocardial infarction: 3 months later, left ventricular perfusion and LVEF improved significantly [98], thus suggesting that myocardial regeneration potential of bone marrow mononuclear cells is probably due to the presence of MSCs. Recently, a clear correlation between in vivo transplantation of MSCs and repair of scarred myocardium after myocardial infarction has been shown in rats [99].

Congenital skeletal muscle defects, such as muscular dystrophy and other myopathies, may theoretically benefit from MSC transplantation to restore muscle structure and function. Adult human synovial membrane-derived mesenchymal stem cells have shown in vivo myogenic potential in the mdx mouse model of Duchenne muscular dystrophy, contributing to myofibers and to long-term persisting functional satellite cells. When administered into mouse muscles, MSCs restored sarcolemmal expression of dystrophin, reduced central nucleation and rescued the expression of mouse mechano growth factor, thus providing proof for their potential clinical use in human Duchenne muscular dystrophy [100]. Bone-marrow-derived human mesenchymal stem cells, genetically modified with the full-length Dys-coding sequence to engage in myogenesis, could participate in myotube formation when cultured together with differentiating human myoblasts through cell fusion. In addition, MSCs transduced with a tropism-modified high-capacity hybrid viral vector encoding full-length Dys could complement the genetic defect of dystrophic myotubes [101]. These and other data are consistent with the feasibility and potential usefulness of MSC infusion in human myopathies, but clear evidence of de novo muscle regeneration by MSCs and clinical improvement is still lacking.

### Conclusions and future perspectives

The knowledge of the biology and the potential clinical use of MSCs have dramatically improved in the last few years. On the basis of in vitro evidence of MSC multilineage differentiation, most experiments with tissue engineering have been carried out with small animals or small size defects. However, human defects are normally larger and more complicated, thus requiring larger repair tissues and structural and mechanical properties similar to human normal tissues. For this reason, bioreactors have been developed in the last few years [102].

Within the bioreactor, cells are continuously loaded into the scaffold and nutrients dynamically provided through the cell–scaffold composite. Moreover, bioreactors can also expose forming tissue (i.e. cartilage) to specific physical stimuli that may improve tissue growth and maturation [54]. In the next future, the ex vivo formation of complex tridimensional hybrid tissues (i.e. joint cartilage with subchondral bone and integrated vascular access for implantation) would revolutionize the treatment of damaged skeletal tissue.

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