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REVIEW ARTICLE

Bone marrow derived stem cells in joint and bone diseases: a concise review

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Abstract Stem cells have huge applications in the field of tissue engineering and regenerative medicine. Their use is currently not restricted to the life-threatening diseases but also extended to disorders involving the structural tissues, which may not jeopardize the patients' life, but certainly influence their quality of life. In fact, a particularly popular line of research is represented by the regeneration of bone and cartilage tissues to treat various orthopaedic disorders. Most of these pioneering research lines that aim to create new treatments for diseases that currently have limited therapies are still in the bench of the researchers. However, in recent years, several clinical trials have been started with satisfactory and encouraging results. This article aims to review the concept of stem cells and their characterization in terms of site of residence, differentiation potential and therapeutic prospective. In fact, while only the bone marrow was initially considered as a "reservoir" of this cell population, later, adipose tissue and muscle tissue have provided a considerable amount of cells available for multiple differentiation. In reality, recently, the so-called "stem cell niche" was identified as the perivascular space, recognizing these cells as almost ubiquitous. In the field of bone and joint diseases, their potential to differentiate into multiple cell lines makes their application ideally immediate through three main modalities: (1) cells selected by withdrawal from bone marrow, subsequent culture in the laboratory, and ultimately transplant at the site of injury; (2) bone marrow aspirate, concentrated and directly implanted into the injury site; (3) systemic mobilization of stem cells and other bone marrow precursors by the use of growth factors. The use of this cell population in joint and bone disease will be addressed and discussed, analysing both the clinical outcomes but also the basic research background, which has justified their use for the treatment of bone, cartilage and meniscus tissues.

Keywords Mesenchymal stem cells (MSC) · CD34+ stem cells · Bone marrow concentrate (BMC) · Tissue engineering · Bone · Cartilage · Meniscus · Granulocyte colony-stimulating factor (G-CSF)

> "We have learned to recognize stem cells, not necessarily from what they do in their dependent organism, but rather by what we can make them do."
> (Pamela Gehron Robey; "Stem cells near the century mark". J Clin Invest. 2000)

Introduction

In the orthopaedic field, elements traditionally associated with reparative principles are CD34- mesenchymal stem cells (Fig. 1) [1, 2]. They are also called "mesenchymal stem cells" (MSC) or "marrow stromal cells" or "multipotential stromal cells" and are commonly characterized by positivity for the surface markers CD73, CD90, and CD105, as suggested by the International Society for Cellular Therapy [3], along with other markers such as Stro-1, CD29, CD44, CD106 [4–6] and

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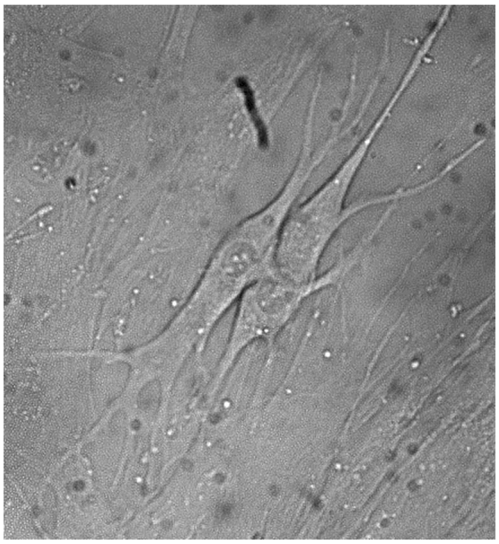

Fig. 1 "Hugging" CD34- mesenchymal cells on a monolayer plastic substrate (original magnification: 40x)

the recently described CD271, that corresponds to the nerve growth factor receptor (NGFR) and seems to be very effective in selecting bone marrow cells with specific inclination toward the osteogenic and the chondrogenic lineages [7, 8].

The potential of MSC to differentiate into multiple cell lines (such as chondrogenic, osteogenic, adipogenic and myogenic lines) makes their application ideally immediate in different pathological conditions, where increased cellularity may lead to an improvement of the healing process (Fig. 2). A milestone in the understanding of this mechanism comes from the work of Mark Pittenger [9], in which these cells were isolated from bone marrow aspirates and subsequently differentiated into the three main lines (osteogenic, adipogenic, chondrogenic), and the work of Arnold Caplan [10]. Caplan created the concept of the "mesengenic process" to elucidate the differentiation of mesenchymal tissues from a single population of precursors, according to a pattern of progressive phenotypic transitions ("stepwise transitions"), that shares many similarities with the differentiation of the hematopoietic line.

Understanding the differentiation potential of MSC was contemporaneous with the recognition of their site of residence. Initially, only the bone marrow was considered as a "reservoir" of this cell population, but later, properly processed adipose and muscle tissues have also provided a considerable amount of cells available for multiple differentiation and, consequently, with "mesenchymal" potential [11, 12]. In reality, however, the view in which to reflect that concept is much broader. Indeed, the microenvironment of mesenchymal stem cells, called the "stem cell niche", corresponds to the perivascular space [6]. This fundamental insight explains how, in vivo, MSC have effectively a systemic localization [13–15] in all places where vessels and, consequently, a perivascular space are present. It is assumed that the "pericytes", observed for some time by conventional histology, actually correspond to mesenchymal cells in their perivascular microenvironment [16]. This position is consistent both with their "systemic" location and with their positive role, in vivo, to post-lesional tissue regeneration processes. From the perivascular space, in

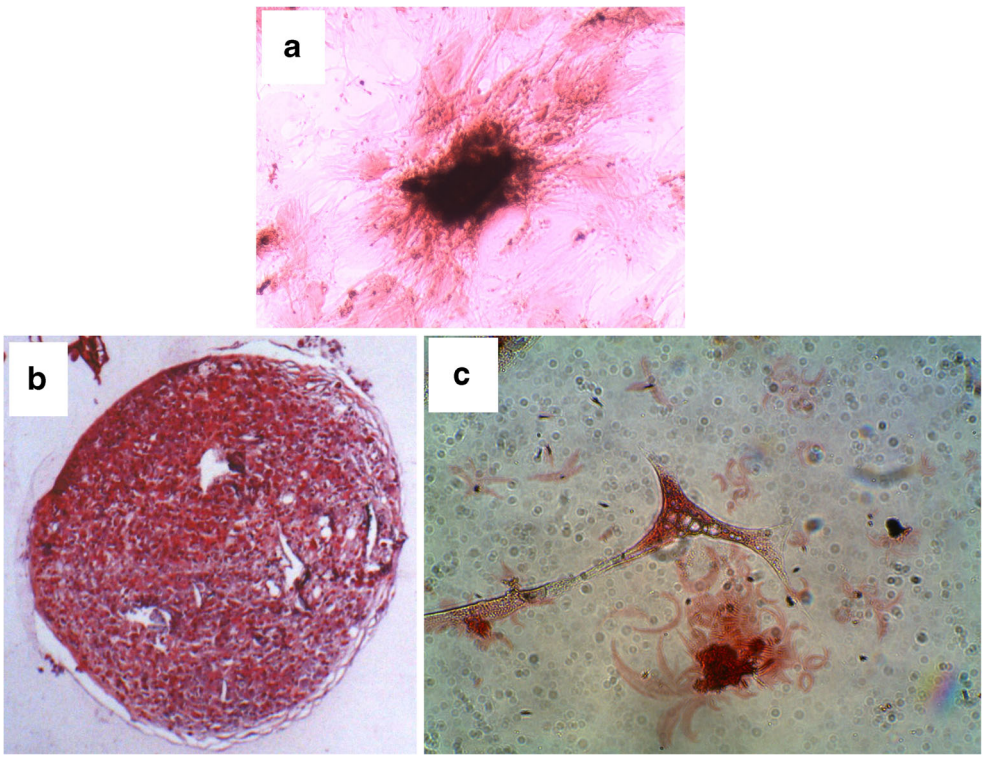

Fig. 2 Osteogenic (**a**), chondrogenic (**b**), and adipogenic (**c**) differentiation of mesenchymal stem cells from bone marrow aspirate. **a** Calcium deposits showed staining with Alizarin Red. **b** Cell pellet with the production of extracellular matrix highlighted with Safranin O staining. **c** Intracellular fat vacuoles highlighted with Oil Red O staining. (**a**,**c**: original magnification 40x; **b**: original magnification 20x)

fact, through mechanisms regulated by the chemokine system, in analogy with the lymphocyte migration behavior, these cells can reach sites of injury and participate in reparative processes. This "participation" has also recently become better understood. Once the MSC have reached the site of injury, they do not only differentiate towards the lines of the damaged tissue, but are also "reservoirs of biological stimuli" (or, more picturesquely, "injury drugstores" [17]), which can stimulate the resident cell population towards cellular repair, as well as immuno-modulating the local immune system, to reduce the fibrous healing process and cellular apoptosis and to stimulate angiogenesis. With this in action, a fundamental role in the communication between MSC and cells of the wound micro-environment is played by microvesicles containing microRNAs which can activate programs in regenerative cell populations surviving the injury site [18, 19].

Tissue regeneration in orthopaedic diseases, nonetheless, may also involve contact between different cell populations for bone and cartilage repair [20, 21]. Recently, a phenomenon of "mutual cooperation" has been observed which includes the sharing of both mesenchymal stem cells (CD34-) and CD34+ stem cells in the realization of a common goal: the vascularization of bioengineered tissues [22]. This concept of "interplay" between different cell populations is very interesting because it introduces the role of new players belonging to seemingly distant cell lines from the mesenchymal area, such as CD34+ cells and the cell populations derived from them. This phenotypic gap is, indeed, only apparent, because many in vitro and in vivo observations have demonstrated the wide "crossover" of the CD34+ and CD34- cell populations. Osteoblast precursors were observed in CD34+ cells derived from bone marrow aspirates [23]; administration of granulocyte colony-stimulating factor (G-CSF) is able to promote osteogenesis and osseointegration to the bone-tendon interface [24]; the in situ application of CD34+ cells has demonstrated the ability to accelerate fracture healing [25] and, even in humans, improvement in healing of tibial nonunion [26] and cartilage lesions after treatment with microperforations [27].

In this experimental evidence one could imagine the foundations of the trophic actions of the bone marrow concentrate aspirate (BMC), which is widely used in orthopaedics, both at the preclinical experimental level, and clinically, as the "readily available" cell source. The BMC represents a cell source of minimal manipulation and, therefore, easily justifiable and directly applicable for clinical use, thus being categorized as "instant cell therapy". However, it is evident that the concentration of both CD34- and CD34+ bone marrow precursors is very low, because the cell "pellets" that are obtained by centrifugation also contain precursor cells of the hematopoietic lineage in various differentiation phases, as well as terminal cells of the white lines, and platelets. It is estimated that, with the most modern systems, a concentration equal to 14.8×10^2 MSC/ml of concentrated bone marrow could be achieved [28].

These theoretical bases, therefore, enable the outline of three main modes for the use of MSC in orthopaedics:

1) Mesenchymal cells selected by withdrawal from bone marrow, subsequent culture in the laboratory, and ultimately transplant at the site of injury (extensive manipulation);
2) Bone marrow aspirate, concentrated and directly implanted into the injury site (minimal manipulation);
3) Systemic mobilization of mesenchymal cells and other bone marrow precursors (CD34+ hematopoietic cells) by the use of "growth factors" such as G-CSF (negligible manipulation).

The application at the site of injury can occur by: (i) direct injection of cell suspension or (ii) by three-dimensional scaffolds infiltrated with candidate cells by direct absorption or through laboratory culture.

Applications in orthopaedics and traumatology

Bone diseases

Apart from the historical usage, with the allogeneic transplant procedure in the systemic disease known as "osteogenesis imperfecta" [29, 30], followed by preclinical studies with local delivery of bone marrow MSC [31], one of the first applications of MSC to increase the bone healing process was studied for the treatment of early-stage idiopathic osteonecrosis. This disease is well suited for measuring the efficacy of cell therapy because it is possible to clinically monitor the improvement of the healing process by means of MRI, in a very reliable manner. The groups of Hernigou and Gangji have investigated the association of lesional "forage" with local injection of concentrated bone marrow aspirate and have obtained, even in the long term, promising results [32–34], although limitations of this technique reside in the early stages of the disease and in the quality of autologous stem cells in patients who underwent prolonged corticoid therapy [35]. The research has, simultaneously, suggested alternatives that, albeit less immediate, could improve wound repair further on. The local application of transgenic MSC for HGF (Hepatocyte Growth Factor) during forage [36], and systemic mobilization of MSC with G-CSF and Stem Cell Factor [37] achieved promising results in small animal models (rabbit).

Even for the treatment of long bone fractures there are currently numerous centres, which are investigating the effect of local injection of BMC during osteosynthesis as improving healing factor by accelerating callus formation [38]. Although the literature has not yet provided clear clinical evidence, basic research supports this therapeutic concept. In mouse models, a considerable improvement of callus formation has been

achieved by systemic injection of autologous MSC through the tail vein [39, 40]. The systemic administration also suggests a wider therapeutic horizon, where the "homing" of autologous mesenchymal stem cells previously expanded in vitro becomes a sufficient means to guarantee the same action at the site of fracture; however, this concept is only currently applicable at a preclinical experimental level.

Another field of application is represented by atrophic pseudarthrosis, where the lack of healing is not so much caused by mechanical failure of the fixation construct but mainly by the lack of cellularity in the lesion site [41]. Local application of BMC was suggested by the Hernigou group, which found that the effectiveness of therapy is dependent on the number of bone marrow precursors conveyed to the site of injury [42]. The delay in the consolidation of the "docking site" during distraction osteogenesis in bone defects of significant size can be considered as a phenomenon similar to atrophic pseudarthrosis. Recent works have suggested the application of BMC associated with demineralized bone matrix with satisfactory results [43], as well as the implant of precultured autologous bone marrow MSC in autologous fibrin clots [44].

In a broader sense, the same bone deficits, including those secondary to traumatic injury, associated with surgical procedures such as opening wedge osteotomy, as well as those derived from the presence of benign growths, are a good model system to test the action of MSC toward bone regeneration. In a clinical study, the BMC was applied, conveyed in a scaffolds of collagen I to promote the healing of bone cysts and enchondromas with restoration of cortical continuity of the site of injury [45]. At the same time, basic research has shown that it is possible, both in vitro [46] and in small (rabbit) [47] and large (goat) animal models [48], to obtain an efficient repopulation of cancellous and cortical allografts by MSC isolated and expanded from bone marrow and, as a consequence, to obtain an improvement in the healing of critical bone lesions; even bone formation around the tendon–bone interface was improved by culturing bone-marrow MSC in scaffolds made by interconnected porous calcium hydroxyapatite ceramics [49]. This therapeutic concept of scaffold "cellularization" by MSC from different sources is broadly proposed in preclinical studies [50–58] and it could also be crucial in humans, to improve the healing of critical bone defects in different settings as revision arthroplasty [59, 60]. A recent clinical study by Marcacci et al. has demonstrated that isolated and expanded MSC have been used in combination with macroporous scaffolds in bioceramics for the treatment of critical bone defects, obtaining promising results [61]. Despite the effectiveness of this approach, it is however of limited use, because it is currently bound to the process of in vitro expansion, necessary to obtain a sufficient number of cells to populate the scaffold. This procedure inevitably leads to a greater manipulation of cells and therefore remains in a strictly experimental area, although recent in vitro and preclinical evidence has shown the great potential of osteogenic differentiation of MCS by means of growth factors from the TGF-beta superfamily [62–64].

More easily applicable at the clinical level is the systemic mobilization of bone marrow precursors by means of subcutaneous administration of G-CSF. This procedure has been associated, in a recent clinical study, with an increase of the processes of osteogenesis and osseointegration at the site of osteotomy, following opening wedge valgus tibial osteotomy [65]. This observation could be the basis for the use of systemic mobilization by G-CSF of bone marrow precursors to promote healing of bone lesions after surgery or secondary to other diseases.

One of the most recent proposals to use the MSC application was in the integration of replacement hip implants. In this area, the only current valid observation was made by the group of Giannini et al. [66]. In large animal models (goat), they observed an increase in newly formed bone around the prosthesis stem after four months of implantation of the prosthesis, where there was simultaneous administration of autologous MSC in the diaphyseal channel. These results, although very interesting, still remain in the preclinical scientific investigation area.

Cartilage pathology

As part of the repair of chondral and osteochondral lesions, tissue engineering has been proposing, for a number of years, the use of MSC as a cell source for repair, along with the use of different growth factors [67, 68], as an example from the superfamily of transforming growth factor beta, the BMPs (bone morphogenetic proteins) [69–72]. Evidence of preclinical animal models have in fact confirmed the effectiveness of this approach, although these studies have found that the mechanism by which MSC promote cartilage regeneration is not only directly, by differentiating into chondrocytes, but also indirectly through "homing" at the site of injury and the recruitment of precursor cells from the joint microenvironment [73, 74]. In fact, in the joint microenvironment, populations of precursors cells have been observed not only in the bone marrow, but also in the upper layers of cartilage (superficial zone) [75, 76] and in the synovial tissue [77]. The healing process may be conducted in a synergistic way by the presence of various cell populations, against which the MSC would act as "directors" in addition to "supporting actors."

In the experimental area, the high chondrogenic potential of MSC transfected with anabolic growth factors such as TGF-β (transforming growth factor beta) [78], FGF-2 (fibroblast growth factor 2) [79], the CDMP-1 (cartilage-derived morphogenetic protein-1) [80] and even BMPs has been also verified. Specifically, a chondrogenic differentiation has been

obtained by transfecting MSC with BMP-2, BMP-4, BMP-7 and BMP-13 [81–89]. Intriguing properties of these BMPs has been recently described regarding cartilage differentiation: BMP-2 and BMP-4 seem to act as inducers of chondrocyte hypertrophy and endochondral ossification, while BMP-13 appears to stimulate chondrogenesis and BMP-7 has been observed to be able to prevent chondrocyte hypertrophy, while maintaining the chondrogenic potential [84, 90, 91]. The group of Madry and Cucchiarini represents one of the main references in Europe in the field of gene transfection for cartilage development [92]. The results obtained in vitro and in vivo in small animal models (rabbit) are convincing in having a faster chondral repair, and with characteristics closer to those of articular hyaline cartilage, although these procedures involve a high manipulation of cells, and are currently only intended for preclinical use.

The first clinical study that demonstrated the efficacy of MSC in the repair of cartilage lesions was performed by the Wakitani group. The first cases were carried out in the early 1990s and later a trial was designed that followed patients for more than 11 years [93]. The cartilage lesions were covered by the periosteum, beneath which was placed a collagen gel containing the population of MSC expanded in culture from bone marrow aspirate. After 42 weeks, repair with metachromatic tissue was obtained, with characteristics similar to that of hyaline cartilage. This study was certainly very courageous and innovative. In fact, he anticipated the concept of "one-stage" cellular repair at a time when the transplantation of autologous chondrocytes in two stages was the more sophisticated perspective to obtain a repair tissue similar to articular cartilage. Despite its limitations, such as the presence of the periosteum and large manipulation necessary to obtain the MSC, the Wakitani study still remains a scientific reference in the history of cartilage repair. Recently, in fact, an experimental study performed by Haleem [94] demonstrated the benefit of the introduction of MSC through a platelet and fibrin gel in femoral chondral lesions, which was then sealed with periosteum, in terms of clinical improvement and evidence of repair tissue similar to cartilage at magnetic resonance imaging.

Currently, however, as with bone lesions, the most widespread clinical use of MSC for the repair of cartilage lesions is related to the use of bone marrow. The reduced manipulation required in obtaining this tissue in large quantities and the ability to apply it in the "one-stage" procedure, makes it an ideal cell source with a low cost. Slynarski et al. proposed the application of fresh bone marrow onto chondral lesions, sealing it with an autologous periosteal membrane; the researchers observed a repair tissue with characteristics similar to cartilage [95]. The orientation of most current clinical research, however, involves the use of BMC to optimize the number of available MSC, as recently reported by de Girolamo et al. [96].

In fact, BMC appears to provide a valid cell source to improve the healing of cartilage defects in preclinical models [97] and both during microfracture technique, in which membranes covering microfractures are soaked with BMC as in the modified AMIC technique described by Gigante et al. [98], and during "one step" cartilage repair recently proposed by Giannini et al., in which BMC carried by collagen or hyaluronic acid-derivative scaffolds is used to fill debrided chondral or osteochondral lesions [99]. The rationale for this "one-step" approach is the ability to convey, through the bone marrow aspirate concentrate, a patrimony of undifferentiated cells containing both CD34- precursors and CD34+ hematopoietic precursors, thus transferring to the chondral defect all the constituent elements of the bone marrow "stem cell niche" in order to maintain the mutual synergy with respect to tissue repair processes. This approach may be even improved by transducing the bone marrow with adenoviral vectors containing cDNA growth factors as transforming growth factor-beta 1, as suggested in a preclinical study by Ivkovic et al. [100].

A more modern perspective, however, presents the use of bone marrow in combination with non-expanded chondrocytes to repair cartilage in a "one-step" method. The key insight is the mutual synergy between chondrocytes and MSC, according to which the chondrocytes would facilitate chondrogenesis in MSC, while the MSC would promote chondrocyte proliferation of the neighboring population. In vitro and in vivo preclinical studies by Hendriks have shown that in co-culture of three-dimensional scaffolds, with 10 % of non-expanded chondrocytes and 90 % of MSC from bone marrow, it is possible to achieve production of glycosaminoglycans (GAGs) equal to that of a culture with 100 % of chondrocytes [101]. Similar results have been achieved by other studies in vitro and in vivo [102, 103]. The advantage of this principle is remarkable, and a recent trial by Bekkers et al. [104] showed promising preclinical results in the goat model. To use the chondrocytes in two-stage cartilage repair procedures, it is in fact necessary to carry out the expensive procedure of isolation and expansion in vitro to obtain sufficient numbers of cells. According to this concept, however, a small number of primary chondrocytes, obtained by lysis of the matrix from a biopsy of cartilage, are combined with cells from bone marrow aspirate concentrate, so that within a single surgical procedure, an efficient cell pool for cartilage repair can be reached. This novel technique, called "Instruct" (CellCotec), combines the insights gained from the experience of transplantation of chondrocytes with the modern concepts of cellular synergy, and has already shown promising results in an initial European trial with ten patients. In the future, it could prove to be a valuable alternative to other "one-step" repair techniques.

Another different way of utilizing the potential of MSC is the recruitment of cells in situ using nanostructured scaffolds. The nanostructuring of a cell growth support enables the observation of unexpected phenomena, as the cells, in contact

with an interface comparable in size to the molecules of the extracellular matrix, exhibit phenotypic and behavioral changes dictated by the interaction with the cell surface nanostructure. This nanostructuring opens up a new "world" where not only cells, but also the scaffold may affect the principles of tissue repair by interacting at a dimensional level, with the order of magnitude of the same cell surface molecules. In this regard, an in vitro study has demonstrated that the MSC in contact with nanofibers of poly-L-lactide (PLLA) coated with nanoparticles of hydroxyapatite are able to spontaneously express genes that are characteristic of the chondrocyte line (such as aggrecan and SOX-9), without being stimulated by chondrogenic growth medium [105]. From "bench" to "bedside", these concepts are only just beginning to be applied, through the use of "biomimetic" scaffolds such as nanostructured Maioregen (Finceramica, Faenza, Italy), equipped with a network of collagen I and hydroxyapatite nanoparticles at increasing concentrations towards the inner layers of the membrane. Studies in animal models have already demonstrated the effectiveness of this support for the conduct of local MSC in the treatment of osteochondral lesions, resulting in satisfactory clinical and histological findings [106]. The Maioregen, however, represents just one of the first proposals for the application of the concepts of nanostructuring for cartilage regeneration using MSC. Other in vitro and in vivo studies in animal models are in fact proposing new scaffolds able to "mimic" the architecture of the extracellular matrix, consisting of poly-L-lactide or polycaprolactone [107, 108], or composites of polycaprolactone-poly-L-lactide (PLLA-b-PCL) [71], poly-DL-lactide-co-glycolide (PLGA) [109], the previously described poly-L-lactide associated with nanoparticles of hydroxyapatite [105] or with the association of extracellular cartilage matrix and PLGA [110].

However, not only the synthetic artificial polymers are proposed for scaffold in cartilage repair by means of stem cells, but also other types of biomaterials and matrices have been studied, such as carbohydrate-based scaffolds (i.e. agarose, alginate, chitosan/chitin, and hyaluronate) and protein-based scaffolds (collagen, fibrin, and gelatin) [89]. For example, hyaluronic acid (HA) has been commonly employed and it has been modified in different ways to obtain a resorbable stable construct. The esterified derivative of HA, named Hyaff-11 sponge, has been widely used in preclinical studies [111] and, more recently, hydrogels made by photocrosslinked hyaluronic acid containing MMP degradable peptide sequences have shown promising in vitro results [112], as well as collagen type II-hyaluronan (HA) composite construct that simulates the extracellular microenvironment of chondrocytes [113]. Collagen alone, also, seems to be very appropriate for cartilage differentiation; in the shape of a collagen I/III membrane (i.e. Chondrogide) [114, 115] or as collagen microspheres [116] or as injectable atelocollagen [117], it has been shown to promote the chondrogenic pathway of the seeded MSC. The chitosan, which derives from crustaceans such shrimps, represents another interesting element for natural resorbsable constructs, both in the shape of microfibers or sponges or as an injectable gel [118, 119]. Finally, the hydrogels (i.e. the gellan gum) may also constitute a promising alternative for the treatment of articular cartilage defects due to their peculiar adhesive properties [120, 121].

A scaffold-free approach has been also hypothesized for cartilage repair, based on the ability of bone marrow MSC to self-assemble in vitro into tissue-engineered cartilage constructs (cell sheets) containing collagen type II and glycosaminoglycans. it has been described in literature by Murdoch et al. in 2007 [122] and it is still a valid alternative to generate chondrogenic constructs [123–125].

Intra-articular injection of MSC through a soluble carrier may also be considered a scaffold free approach for cartilage regeneration. This was suggested in a pilot study from Murphy et al. in 2003 in a caprine model [126] and, later, in several other preclinical models, which include rats [74], donkeys [127], rabbits [128], pigs [73, 129], sheep [130] and monkeys [131]. These studies were performed by means of MSC derived not only from the bone marrow but also from other sources such as synovial tissue, adipose tissue and skeletal muscle, the latter after transduction of the MSC with the genes for a VEGF antagonist and the BMP-4 [132]. Following this approach, clinical pilot studies and case reports have been published in the last six years. Wong et al. [133] showed that intra-articular injections of cultured autologous bone marrow-derived MSC, in association with microfracture and medial opening-wedge high tibial osteotomy three weeks before the cell injection, led to clinical and MRI improvement of degenerative cartilage lesions in clinical pictures of knee medial unicompartimental osteoarthritis. Similar results were obtained with the simple association of arthroscopic bone marrow stimulation and MSC injection for the treatment of symptomatic knee and talar cartilage lesions in the studies of Lee et al. [134] and Kim et al. [135]. Even the simple intra-articular injection of bone marrow MSC showed some clinical and MRI improvement in patients affected by knee osteoarthritis in the studies of Centeno et al. [136], Emadedin et al. [137] and Orozco et al. [138]. To further confirm the encouraging perspective of this concept, a recent review of Peeters et al. has stated that the use of culture-expanded stem cells in human joints "appears to be safe and it is reasonable to continue with the development of articular stem cell therapies" [139]. In this regard, the time needed for a consistent cell adhesion (more than 60 %) was determined as ten minutes in vivo in a preclinical rabbit model [140]. Moreover, to improve cell migration, the use of magnetic fields to "drive" stem cells toward the cartilage defect has been recently proposed by means of magnetically labeled MSC in a preclinical animal model [141, 142]; this fascinating approach is promising in terms of optimizing the "homing" of stem cells at the defect sites and, ultimately, in ameliorating the repair process.

Ultimately, a different approach has been recently introduced by the study of Saw et al. [27]. They obtained cartilage repair through the association of microfracture and delayed (7 days) postoperative intra-articular injections of autologous peripheral blood progenitor cells, collected after a course of G-CSF administration. Along with several recent in vitro and preclinical studies [143–145], this new concept suggests the value of peripheral blood MSC for cartilage repair and the potential of G-CSF both as a trophic factor and as an effective tool for systemic mobilization of precursor cells.

Meniscal pathology

Regarding meniscal lesions, the use of MSC is still limited to preclinical testing.

A scaffold-free approach has been described in the form of intra-articular injection of MSC derived from either the bone marrow or the synovial tissue. In the early experience of Murhpy et al. in 2003 [126], they observed the regeneration of the medial meniscus in caprine knee joints following direct intra-articular injection of autologous bone marrow stem cells and later, in 2006, Agung et al. confirmed the possibility of injecting MSCs for the treatment of intra-articular tissue injuries in a rat model [74]. Later, in 2009, Horie et al. introduced the use of synovium derived MSC [146] and this cell population is still a well-accepted alternative source of stem cells for intra-articular therapy [147]. In their first preclinical model of meniscectomy in mice, three months after the injection, Horie et al. observed the onset of fibrocartilage tissue having histological features showing newly formed fibers with an orientation similar to that of the native meniscus. Horie et al. also obtained comparable positive results even with the use of a xenogenic rat model using human bone marrow MCS [148]. Similar results have been also recently observed in preclinical rabbit [149–151], sheep [130] and porcine models [152] and in a human clinical randomized trial [153]. These observations sustain a promising potential role of MSC injections for improving meniscus regeneration both through the simple injections of the cell solution and, as recently demonstrated, though the administration of cell aggregates [154].

The delivery of MSC through scaffolds represents another interesting experimental approach for the repair of meniscal lesions. In the works of Yamasaki et al., in vitro in 2005 [155] and in a preclinical rat model in 2008 [156], the meniscus itself was considered a potential carrier for rat bone marrow MSC obtaining promising histological and biomechanical results. Nevertheless, many different scaffolds have been proposed during recent years [157] such as type I collagen sponges [158], fibrin glue [159], hyaluronan-collagen or hyaluronan/gelatin composites [160]. In vitro, a meniscal-like tissue was obtained by cultivating MSC in a scaffold consisting of protein derived from silk, in the presence of TGF-β 3 [161] or in scaffolds made of collagen with a cancellous structure [162]. In a recent preclinical study in rabbit, meniscal defects were created with critical dimensions in the avascular area, and were treated using a hyaluronan-collagen based composite scaffold [163]. Better results were observed by means of meniscal-like tissue after treatment with mesenchymal stem cells and scaffolds compared to that of cell-free implants or platelet rich plasma-seeded implants. These results confirm the essential role of MSC in the regeneration of meniscal tissue.

Thus, from this perspective, the importance of three-dimensional nanostructured scaffolds has also been demonstrated in vitro. Indeed the concept of nanostructuration is a relevant conditioning element for the behavior of MSC as the spatial orientation of the nanofibers inside the scaffolds is considered a key factor in determining the phenotype of seeded MSC. In an in vitro study, MSC seeded on nanostructured scaffolds in polycaprolactone consisting of fibers with a precise spatial alignment showed increased proliferation and increased synthesis of extracellular matrix compared to scaffolds made of nanofibers distributed in a random order [164].

Future developments: iPS cells, umbilical cord cells and adipose-derived stem cells

Mesenchymal stem cell therapy is inevitably a perspective still at the experimental level, although it is associated with fascinating results. In this context, basic and preclinical research still has a key role in identifying the mechanisms and the ideal application of these cells in repairing damaged joints, to avoid the risks associated with “enthusiastic” clinical applications considered as being hasty. Despite numerous worldwide trials, a routine clinical application is in fact a distant prospect. Still, there are also three horizons of research that seem promising for the near future.

The first consists in the generation of induced pluripotent stem cells (iPS) by introducing a number of transcription factors into fibroblasts. In mice, this result was obtained by introduction of Octamer-4 and SOX2, proteins involved in the replication of embryonic stem cells: c-Myc, which regulates the expression of approximately 15 % of all genes, and Krüppel-like factor 4, a factor involved in cell differentiation and in the arrest of the cell replication cycle [165]. This method opens up a great number of possibilities that would allow, in theory, the reprogramming of cells normally considered stable, such as fibroblasts, turning them into pluripotent cells capable of undergoing multiple differentiation pathways, and which can participate in the repair of musculoskeletal tissues [166]. Moreover, these cells can be differentiated into a chondroblastic and osteoblast lineage and have shown, in preclinical models, a considerable capacity of improving cartilage repair when implanted at the defect site [167].

The second horizon is represented in the use of umbilical cord cells [168]. Different studies in the

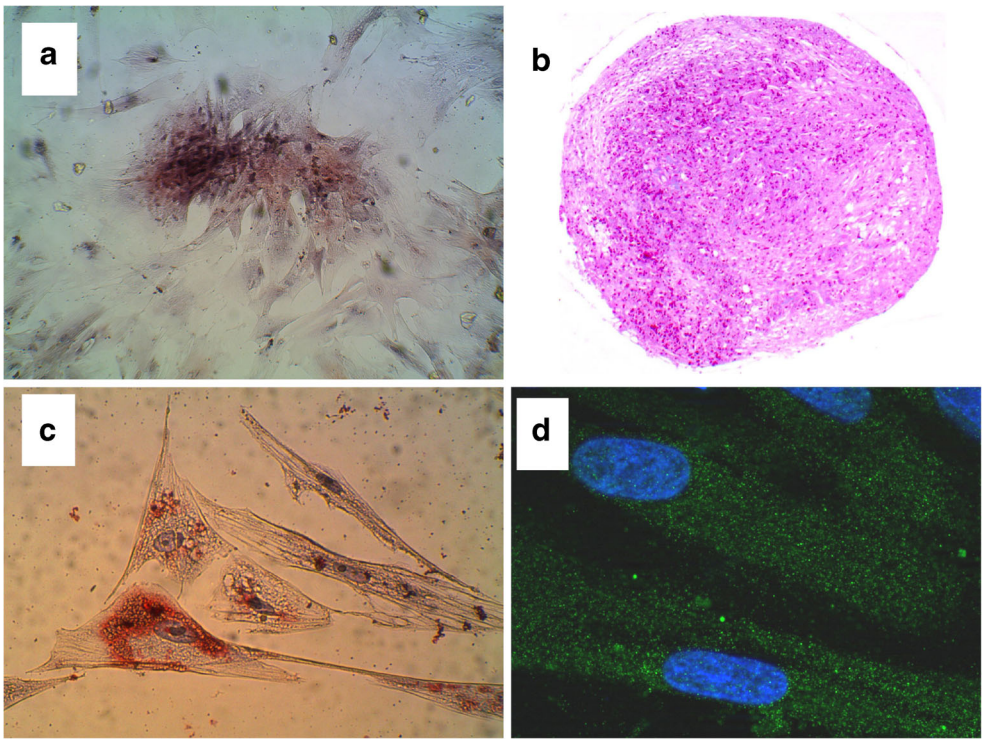

Fig. 3 Osteogenic (**a**), chondrogenic (**b**), adipogenic (**c**), and myogenic (**d**) differentiation of mesenchymal stem cells from umbilical cord (28 days of culture). **a** Calcium deposits highlighted by staining with Alizarin Red. **b** Cellular pellets with the production of extracellular matrix, highlighted by staining with Safranin O. **c** Intracellular fat vacuoles highlighted by Oil Red O staining. d Positive immunofluorescence for myogenin (nuclei stained with DAPI)

literature have shown that cells with mesenchymal potential can be drawn. Cells derived both from the arteries and the veins (perivascular cells), from the Wharton's jelly, from the external membrane of the cord, along with the actual cord blood cells, all showed multiple differentiation potential.

Cells with the greatest osteogenic and chondrogenic potential seem to be those derived from the perivascular space [169] and from cord blood [170, 171], but also those derived from cord stroma have been shown in vitro to have osteogenic and chondrogenic differentiation capacity [172]. However, there are some discrepancies in the differentiation of the MSC from

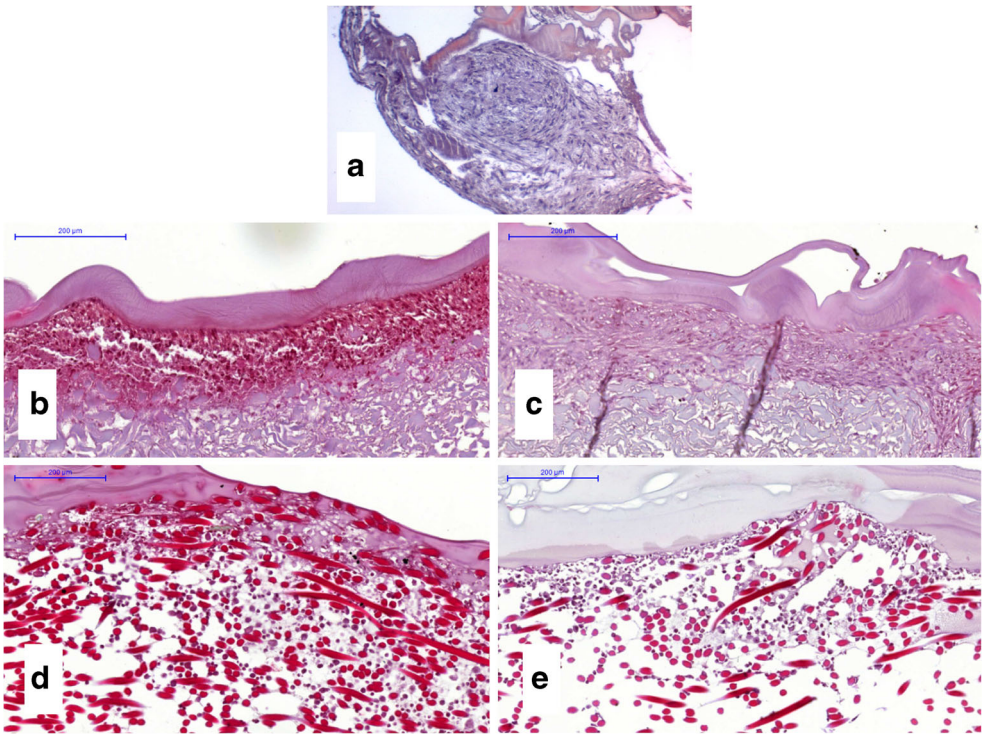

Fig. 4 Osteogenic and chondrogenic differentiation on tridimensional scaffolds. UC-MSC in Orthoss scaffold (30 days of culture), stained with Alizarin Red. **a** UC-MSC in Chondrogide scaffold (28 days of culture), in hypoxic condition (**b**) and in normoxic condition (**c**), stained with Safranin-O. UC-MSC in Hyaff-11 scaffold (28 days of culture), in hypoxic condition (**d**) and in normoxic condition (**e**, stained with Safranin-O

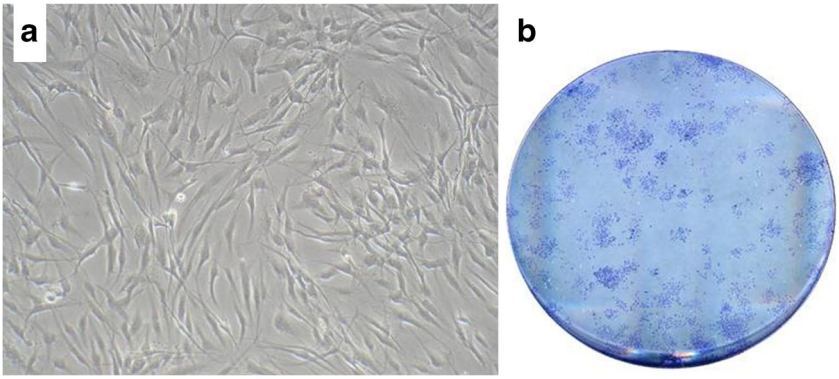

Fig. 5 Monolayer ASCs culture visualized with an optical microscope (**a**) (original magnification 20x) and with macroscopic vision on the culture dish (**b**)

umbilical cord blood compared to their counterparts based in the bone marrow. MSC from cord stroma show slower chondrogenic differentiation in culture and generally an inferior osteogenic potential than MSC from bone marrow. This potential, however, seems to increase when the cord MSC are grown in three-dimensional supports. In addition, the adipogenic differentiation of MSC from the umbilical cord is characterized by the development of small lipid vacuoles, probably in analogy with brown adipose tissue, in contrast to those produced by MSC from bone marrow, which are more similar to those of white adipose tissue [173] (Figs. 3 and 4).

The advantage of using umbilical cord stroma as a source of MSC is potentially significant. In fact, even without selecting a particular population of cells, but utilizing the cord in toto, it is possible to obtain, with simple cell culture methods, a substantial quantity of cells that can be used for musculoskeletal repair processes. In addition, theoretically, the use of the umbilical cord as a source of MSC is ethically acceptable and economical since the material would otherwise be discarded during the process of childbirth. Finally, the immunosuppressive potential of MSC from the umbilical cord [174] make these cells very immuno-privileged with respect to allogeneic use, as already tested in vivo in a combination allogeneic stem cell therapy for neurological lesions of the spinal cord [175]. Thanks to these characteristics, one can imagine the potential use of these cells that would enable in the future the collection in accredited "stem cell factories" of a virtually unlimited population of MSC from different umbilical cords available for homologous use, eliminating costly culture and cell expansion procedures. For all these reasons, cells from umbilical cords, along with induced pluripotent cells, may represent key elements in the near future for the treatment of diseases of the bones and joints.

Finally, a third alternative source for the isolation of mesenchymal stem cells is represented by the subcutaneous adipose tissue isolated by liposuction [176]. Adipose tissue is a complex consisting of mature adipocytes embedded in a extracellular matrix together with the connective tissue surrounding the vessels, named Stromal Vascular Fraction (SVF). The SVF, obtained by lipoaspiration, contains, along with perivascular stem cells, a heterogeneous population of mononucelar cells as preadipocytes, fibroblasts, vascular smooth muscle cells, endothelial cells, resident monocytes and macrophages, and lymphocytes. For about two decades, this undifferentiated SVF has become the object of attention by researchers working on regenerative medicine, because it represents a rich source of stem cells (ASCs, Adipose-derived Stem Cells) [177] to improve cartilage, bone and tendon repair [178–183]. The simple SVF extraction procedure, the mini-invasiveness and reproducibility of the approach, along with the relatively short time for the isolation and the high yield, produces an abundant number of cells with minimal discomfort to the patient. This therefore renders the SVF of adipose tissue a very attractive source in many areas of modern medicine.

In addition, some comparative studies have shown that the ASCs, purified from the other components of the SVF, did not differ morphologically, immuno-phenotypically, clonogenically or in their differentiation capacity from MSC isolated from bone marrow [184] (Fig. 5). ASCs, under appropriate and specific stimuli (Table 1), are in fact able to differentiate in vitro into the osteogenic, adipogenic, chondrogenic and tenocyte lineages [185, 186]. Some factors, such as donor age, sampling technique, location

Table 1 Factors used experimentally to promote the differentiation of ASCs into various cell lines

Type of differentiation	Differentiation factors
Adipogenic	Insulin, IBMX, dexamethasone, indometacin
Chondrogenic	BMP-6, BMP-7, FGF-2, TGF-β1, TGF-β2, TGF-β3, dexamethasone, IGF-1
Osteogenic	1,25(OH)2D3, β- glycerophosphate, ascorbic acid, BMP-2, dexamethasone, valproic acid
Cardiomyogenic	IL-3, IL-6, SCF
Vascular/endothelial	Specific microenvironment?
Neurogenic	Valproic acid, insulin, hydrocortisone, EGF, FGF
Myogenic	Specific microenvironment?

(subcutaneous or visceral adipose tissue) and the different in vitro culture conditions can influence both the rate of proliferation and the differentiation capacity of ASCs. Currently, in humans, the peculiar commitment between the various anatomical sampling sites have not yet been fully described in terms of functionality of the MSC. Nevertheless, a rrecent study of Lopa et al., from the group of de Girolamo and Moretti, have demonstrated the superior chondrogenic potential of ASCs from knee infrapatellar fat pad compared to those from subcutaneous adipose tissue, that seem to display a superior osteogenic commitment [187]. However, since the different anatomical districts possess unique metabolic properties, such as the lipolytic activity and fatty acid composition, it is easy to suppose that the donor site will influence, in the medium to long term, the characteristics of the transplant.

In addition, several preclinical studies show that ASCs are able to differentiate in vivo into the osteogenic lineage, as demonstrated by the production of specific mineralized matrix and the expression of osteoblast specific markers such as osteopontin and alkaline phosphatase. After osteogenic differentiation, ASCs are able to acquire some functional properties typical of osteoblasts, such as responsiveness to stress and mechanical loading by increasing the expression of alkaline phosphatase, collagen I and mechano-sensor genes following exposure to a given stress load. These results show that the ASCs possess the potential to differentiate into mechano-sensitive osteoblast-like cells, and thus may be a valuable tool for skeletal muscle tissue engineering [185, 188]. The chondrogenic potential of ASCs has also been widely demonstrated in vivo in recent works that show the contribution of ASCs in regeneration of chondral and osteochondral defects in animal models [120] and in case series [189, 190].

In conclusion, mesenchymal cells now represent a cell source effective for the treatment of various diseases in orthopaedics and traumatology. Derived from bone marrow, umbilical cord or from adipose tissue, they are the subject of numerous studies for the characterization of their potential clinical use. It is likely that over the next few years they will be used more and more extensively in an effective and safe manner.

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Conflict of interest The authors indicate no potential conflicts of interests.

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