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REVIEW ARTICLE

# Chemokines in mesenchymal stem cell therapy for bone repair: a novel concept of recruiting mesenchymal stem cells and the possible cell sources

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**Abstract** Skeletal injury is one of the most prevalent clinical problems that jeopardize the activities of daily life, especially in our aging society. Mesenchymal stem cells (MSCs) play pivotal roles in regenerating bones after bone injury. MSCs come from the surrounding tissues and/or circulation. Cell sources may be the bone marrow, periosteum, vessel walls, muscle, circulation, and elsewhere, and the migration of MSCs is necessary for bone healing. The mechanism(s) of recruitment and crucial molecules for cell migration are still unclear, but chemokines and their receptors seem to play critical roles. The induction of MSC recruitment from surrounding tissues or from the circulation can be a helpful modality to induce or to support cell-based therapy for bone regeneration.

**Keywords** Mesenchymal stem cell · Migration · Bone repair · Fracture · Chemokine

## Introduction

Skeletal injuries remain among the most prevalent clinical problems that jeopardize the activities of daily life, especially in an aging society. This problem is accentuated in patients with rheumatoid arthritis, who tend to have lower bone mineral density and a lesser ability to form new bones because of a combination of their disease-induced generalized osteopenia [1] and the consequences of steroid-induced osteoporosis [2]. Cell-based therapy has become one of the most promising modalities to treat difficult aspects of bone repair, such as nonunion and massive bone defects, in such patients. Mesenchymal stem cells (MSCs) are without doubt almost the only candidate, albeit a very attractive one, for cell-based bone regeneration.

The concept of stem cells likely originated at the end of the nineteenth century as a theoretical postulate to account for the ability of certain tissues or cells of an organism to self-renew. The identification of stem cells as discrete cellular entities possibly benefitted from the development of methods for isolation of stem cells in vitro in parallel with bioassays to examine their potency. The term MSCs was first coined by Caplan in 1991 [3], and the concept can be traced to classical experiments demonstrating that the transplantation of bone marrow to heterotopic sites results in de novo bone formation. Tavassoli and Crosby obtained clear evidence for the inherent osteogenic potential of bone marrow [4], but their study failed to distinguish the progenitor cell of nonhematopoietic, differentiated bone cells because the experiments were conducted with entire fragments of bone marrow. However, Friedenstein and colleagues in a series of studies in the 1960s and 1970s demonstrated that the osteogenic potential of bone marrow was associated with a minor subpopulation of bone marrow cells (reviewed by Freidenstein [5]). These cells were distinguishable from the majority of bone marrow cells (hematopoietic cells) by their rapid adherence to tissue culture plates and by the fibroblast-like shape of their progeny in culture, suggesting that their origin was clearly different from that of hematopoietic bone marrow cells. The second major breakthrough was provided by Friedenstein and colleagues [6], who showed that seeding bone marrow cell suspensions at clonal density results in the establishment of discrete colonies initiated by single cells. In vivo transplantation of those cells led to the

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recognition that multiple skeletal tissues could be experimentally generated in vivo by the progeny of a single bone marrow stromal cell. However, despite several important publications, the concept of a nonhematopoietic stem cell in bone marrow did not gain the worldwide popularity befitting its potential until various pioneering works were published describing appropriate methods for the isolation and differentiation of these cells, such as that reported by Pittenger and colleagues [7]. In conjunction with the isolation of human embryonic stem (ES) cells, the term mesenchymal stem cell gained wide popularity. Because of massive, energetic studies all over the world, MSCs became identified as one kind of postnatal human stem cell with a differentiation potential that could be as broad as that of ES cells. This assumption evoked feverish attention and generated much confusion, and it remains highly controversial. It remains to be clearly established what MSCs actually are, how the stem cell concept should be applied, what their possible clinical applications could be, and what nomenclature would be most appropriate [8].

Despite these profound scientific questions, the therapeutic use of stem cells has attracted much attention in medicine. The use of ES cells is still debated in public because of ethical concerns, but the application of ES cells in human therapy is also controversial because of immunological incompatibilities and concerns about uncontrolled development of malignancies from administered cells. Inducible pluripotent stem cells have recently been discovered by Takahashi and Yamanaka [9]. This discovery induced more enthusiasm in this field than ES cells because induced pluripotent stem cells raise few ethical concerns, although there is still debate on their use because of the recently reported possibilities of teratoma development [10]. In contrast, adult stem cells (without any gene modification with viruses or other vectors) are free of such ethical concerns and can be used in the autologous setting, thereby avoiding rejection. Furthermore, allogeneic stem cells have already been used extensively in human bone marrow transplantation for the treatment of otherwise deadly diseases [11]. Recognition of these advantages of adult stem cells has translated into a large number of clinical trials with bone marrow-derived or other tissue-derived cells for organ repair and regeneration. Together, the promising results with stem cell therapy have led to the development of a new discipline in medicine, regenerative medicine [12].

Initial attempts using MSCs for bone regeneration were made as adjuncts to support bone healing. Historically, orthopedic surgeons routinely used freshly isolated bone marrow to provide rapid and extensive repair of nonunions, large bone defects, or spinal fusions—not because of any convincing scientific evidence but because they knew the method worked well. During the past decade, accumulating evidence has shown that the therapeutic use of MSCs is one of the most promising advancing technologies for bone regeneration in this era of 'biologic treatment'. Although a large number of experimental and clinical studies have attempted to regenerate bones with MSCs, the results have several notable shortcomings, such as vulnerability to infection, uncertainty regarding the differentiation capability of MSCs in specific in vivo situations, the high cost of ex vivo cell handling, concern regarding the limited number of cells that can actually contribute to bone formation, and possibly even malignant transformation of the cells during ex vivo cell expansion [13–15]. These factors are especially relevant in a highly demanding situation, such as a large bone defect. Therefore, a new strategy is required to support or supplement current methods for promoting bone regeneration using MSCs.

In this review, I attempt to describe an intriguing mechanism by which MSCs can be recruited from the surrounding tissues or from the circulation by migratory factors in vivo. From that starting point, a promising modality could be developed for cell-based therapy in regenerative medicine. Although I recognize the definition of 'stem cell' will be controversial, and some readers may think 'progenitor cell' would be appropriate, I use the term 'stem cell' for this review. In addition, I adopt the term 'migration' rather than 'trafficking', because I feel it is more appropriate for describing the mobilization of a group of MSCs rather than a single cell.

## Bone healing mechanisms and MSCs

Bone healing is thought to rely upon two types of healing mechanisms. Endochondral ossification is the major type of healing for most bone injuries. In this process, undifferentiated MSCs undergo proliferation, chondrogenic differentiation, hypertrophic change, and calcification, eventually being replaced by bone produced by osteoclasts and osteoblasts. This type of healing mechanism resembles the mechanism of development of the growth plate in long bones, and most researchers think this series of events 'recapitulates' growth plate development, although some controversy certainly exists. The other type of healing is intramembranous ossification, in which MSCs or undifferentiated bone-forming cells directly differentiate into osteoblasts and efficiently form new bones. This process requires certain stable environmental and mechanical conditions. One of the most enigmatic yet fascinating questions would be where the stem (or progenitor) cells come from and how the cells are recruited to the injured sites. I first attempt to summarize the possible sources of MSCs and then discuss the migration of those cells to the injured sites, followed by a discussion of possible chemotactic factors.

## Possible sources of MSCs for bone repair

### The bone marrow

The bone marrow is 'historically' still the most attractive source of stem cells. As mentioned above, Tavassoli et al. [2] and Friedenstein et al. [3] reported the existence of such cells in the bone marrow as early as 1968. Moreover, clinical applications of bone marrow aspirates have been adopted for many years for the treatment of nonunion in orthopedic surgery. The most favorable features of bone marrow in terms of clinical application are its accessibility with little morbidity and the abundance of the cells. However, how many or what percentage of cells from the bone marrow actually contribute to bone healing remains unknown. Additionally, the proportion of mesenchymal cells in bone marrow is different for each bone, and which bone should be used for bone marrow harvest in each clinical situation remains to be investigated. Also, some strongly argue that human bone marrow MSCs represent a phenotypically homogeneous cell population that shares an identical phenotype with marrow adventitial reticular cells, which are stromal cells similar in nature to pericytes [16]. This question should be clarified in the near future. The studies of bone marrow stem cells have been summarized in other reviews [11, 12, 16].

### The periosteum

Based on an abundance of clinical experience, the periosteum is unanimously considered by orthopedic surgeons to be one of the most crucial components for successful bone healing. If the periosteum is destroyed by the injury itself or the operation, the healing process will be much delayed, causing many subsequent problems. Thus, every effort should be made to preserve the periosteum of the injured bone. A number of studies have also shown that when the periosteum is autografted at heterotopic sites, either as free grafts or in diffusion chambers, mineralized and cartilaginous tissue can be detected [17]. However, the nature and kinetics of the cells involved have not been well studied. One of the major reasons for this is that the number of cells is limited, and the extraction of the cells or of the periosteum itself is relatively difficult, especially in small animals such as rodents.

The periosteum consists of two layers: the outer fibrous layer and the inner cambium layer. The latter is considered to be the main source of stem cells, and it plays a key role in bone healing. The former lacks such cells but provides not only attachment to tendons and ligaments but also a crucial blood supply to the cambium layer; it also plays a key role in the association between the cambium layer and surrounding muscles. Although it is relatively difficult to show the biological contribution of the periosteum to bone healing, the existence of 'stem cells' and the nature of those cells have been reported. It has been reported that culture-expanded cells obtained by enzymatic digestion or outgrowth of periosteal explants retain their osteochondrogenic potential [18, 19]. Gruber et al. showed that under osteogenic conditions, periosteum-derived cell populations express messenger RNA for the bone markers osteocalcin, osteopontin, and collagen type I and for the chondrogenic markers collagen type II and aggrecan under chondrogenic conditions. They also showed that growth factors enhance osteogenic differentiation of these cells [20]. These studies provide evidence of the potential of periosteal cells at the cellular and molecular levels, but only in vitro. Scientific investigations in vivo have recently become available because of the development of cell tracking and imaging techniques. Zhang et al. [21] reported that using a bone graft model, they could attribute 70% of osteogenesis to the cellular proliferation and differentiation of donor progenitor cells on the surface of the live bone graft, indicating the importance of the periosteum in bone healing in vivo. Colnot [22] recently reported that in bone graft and fracture models, the periosteum and bone marrow/endosteum both give rise to osteoblasts, whereas the periosteum is the major source of chondrocytes in bone healing. However, how much and by what method the periosteal cells contribute to actual bone healing in humans remains to be demonstrated, and the molecular and physiological mechanisms underlying their contributions are unclear.

### The vessel wall

Brighton and Hunt [23] first reported a possible contribution to fracture healing of cells derived from vessels. Few reports had investigated the roles that mesenchymal pluripotent cells from vessel walls (pericytes) play in bone healing [24–28] until Farrington-Rock et al. [29] demonstrated the chondrogenic and adipogenic potential of microvascular pericytes. Their study showed that when cultured under defined conditions, pericytes could be induced to express chondrogenic and adipogenic markers. They also showed the presence of chondrocytes and adipocytes and the formation of cartilage, fibrocartilage, and mineralized cartilage when pericytes were loaded into diffusion chambers and implanted in vivo.

Vessels are available virtually everywhere in the body except for certain 'avascular' tissues such as cartilage, so they are easily envisaged as a possible source of stem cells. Some hypothesize that pericytes play a role in normal bone growth and development. For example, it is well established that bone formation is suppressed when angiogenesis is inhibited [31]. Pericytes are an essential part of the angiogenic process, so these cells may directly contribute

to skeletogenesis. Moreover, pericytes may serve as a reservoir of primitive precursor cells. Indeed, there are many phenotypic similarities between pericytes and stem cells isolated from adult tissues [27, 29]. However, the isolation of distinct pericytes separate from other possible stem cells is required to prove this hypothesis in vivo, and it seems that some ambiguity and confusion exist in these experiments. It is also possible that pericytes and other types of stem cells, such as 'circulating' stem cells (described below), are unidentifiable in certain in vivo experiments. In addition, pericytes may not be a good candidate for cell therapy because of their limited availability or difficulty in harvesting, with the exception of those in the bone marrow [16]. Still, the potential of pericytes in bone healing mechanisms is fascinating, and scientific investigations to elucidate their mechanism of action and possible therapeutic use are urgently needed.

### Muscle

Muscle has been one of the most plausible sources of cells for bone repair because of its proximity to bones and its ample blood supply. However, the lack of scientific evidence on its contribution to bone repair has led to some doubt about its candidacy. Lee et al. [31] provided some of the first scientific evidence by showing that muscle-derived cells that had been highly purified using their preplate technique expressed stem cell markers and had the capacity to differentiate along both myogenic and osteogenic lineages in vitro and in vivo. The group led by J. Huard has extensively reported the capability and possible therapeutic characteristics of muscle-derived cells [32], but its work still seems to lack definite physiological evidence of the role of these cells in normal bone repair. However, the muscle is one of the most abundant tissues in the human body, making it a good candidate for cell harvest for cell-based therapy. The development of practical therapeutic methods is required.

### In the circulation

Complete reconstitution of the hematopoietic system after the systemic infusion of hematopoietic stem cells (HSCs) has shown that HSCs are capable of homing to the bone marrow in normal physiology. It remains to be determined, however, whether any physiological events in extravascular mesodermal tissues require naturally circulating progenitor cells. Kuznetsov et al. published a breakthrough report in which they isolated adherent and clonogenic cells from whole blood of adult animals of four different species, including humans. The study demonstrated that some polyclonal strains and several single colony-derived strains form bone upon in vivo transplantation [33]. Several studies have shown similar results for circulating stem cells [34–39], but others counter with negative results [16, 40, 41]. Even Kuznetsov et al. [42] reported the extreme rarity of these cells in humans, suggesting the likelihood that the abundance of such cells is species dependent.

While the role of circulating MSCs in bone healing remains controversial, a recent study formally demonstrated the participation of circulating osteogenic connective tissue progenitor cells in a parabiotic mouse model of fracture healing [43]. A second interesting study demonstrated that circulating bone marrow-derived osteoblast progenitor cells were recruited to the bone-forming site in a model of bone morphogenic protein-2-induced ectopic bone formation [44], while yet another recent study demonstrated the migration of intravenously transplanted MSCs to the bone repair site in a live bone graft model [45]. While the existence of circulating MSCs remains controversial, it is safe to say that recent investigations show their existence and that they have roles in bone healing, at least in rodents. Furthermore, the possibility has arisen that MSCs in circulation could be a transient phenomenon following skeletal trauma (an unavoidable consequence of skeletal injuries), and one probable scenario is that the circulation of bone marrow MSCs helps the injured bone to heal [16].

### Others

Recent vigorous attempts to find any other sources of stem cells have led to the demonstration of several types of intriguing MSC-like cells, one of which is adipose tissue-derived stem cells. Pioneering work by Zuk et al. [46] showed that a putative stem cell population could be isolated from adipose tissue and differentiated toward the osteogenic, adipogenic, myogenic, chondrogenic, and even neurogenic lineages. Another recent finding is that human and mouse tendons harbor a unique cell population that has universal stem cell characteristics, such as clonogenicity, multipotency, and self-renewal capacity [47]. Together with other types of stem cell-like cells, such as skin-derived stem cells [48], these have a certain potential for therapeutic applications, but it seems inconceivable that they would make a physiological contribution to bone healing. For the clinical application of these cells in the treatment of bone diseases, their precise profiles and practical efficacy for bone regeneration should be demonstrated.

The characteristics of the possible sources of MSCs are summarized in Table 1.

## Migration of MSCs

It has been demonstrated that both local MSCs derived from the injured tissue and circulating MSCs collaborate in the

**Table 1** Characteristics of different sources of mesenchymal stem cells for bone repair

| |
|---|
| The bone marrow |
| Favorable features—therapeutic accessibility with less morbidity, abundance of cells |
| Questions—percentage of available stem cells, differences of various bones, differences or similarities to pericytes |
| The periosteum |
| Favorable features—well-known importance from practical experiences |
| Questions—percentage of available stem cells, molecular mechanisms of the contributions, harvest difficulty |
| The vessel wall (pericytes) |
| Favorable features—physiological accessibility |
| Questions—differences or similarities to other stem cells, limited availability, harvest difficulty |
| Muscle |
| Favorable features—abundance for cell sources |
| Questions—lack of physiological evidence for normal repair, harvest difficulty, limited capability for differentiation |
| In circulation |
| Favorable features—physiological accessibility, therapeutic accessibility |
| Questions—lack of physiological evidence for normal repair, rarity of available stem cells |
| Other possible sources |
| Adipose tissue-derived stem cells, tendon-derived stem cells, skin-derived stem cells |

healing of damaged organs during the course of organ regeneration. A series of reports have supported the hypothesis that a particular set of molecules upregulated during bone injury is released around the injured site and/or into circulation, stimulating MSCs to downregulate the adhesion molecules that hold them in their niche. Subsequently, resident or circulating MSCs 'sense' a tissue injury, migrate to the sites of damage from surrounding tissues or from the circulation, and undergo tissue-specific differentiation [49–51]. However, the mechanisms responsible for MSC migration to the site of bone injury have not yet been shown. Cytokines and chemokines probably play critical roles in these processes, and many of these factors are chemoattractants.

## Possible factors inducing the migration of MSCs

What factors or mechanisms contribute to the migration of MSCs? Chemokines (and their receptors) are the molecules with which leukocytes are targeted to areas of inflammation, infection, or injury [52]. MSCs have been shown to express a variety of chemokine receptors [53, 54], and chemokine-mediated MSC migration has been demonstrated in vitro and in vivo. The importance of adhesion molecules, such as VLA-1, VCAM-1, ICAM-1, VAL-4, $\beta$1-integrin, and P-selectin, and matrix-degrading enzymes, such as MMP-2, MT1-MMP, and TIMP-2, is undeniable [55]. The roles of these potent chemoattractants in MSC migration processes are described below.

One of the most likely and well-investigated factors is stromal cell-derived factor 1 (SDF-1)/pre-B cell growth-stimulating factor/CXCL-12. SDF-1 plays many important roles through its activation of the G protein-coupled receptor CXCR4, and the interaction of SDF-1/CXCR4 and HSCs has been extensively reported. In the bone marrow, endothelial cells and stromal cells express SDF-1, which not only acts as a chemoattractant for HSCs to a bone marrow niche but also supports their survival and proliferation [56, 57]. During the past decade, data have been accumulating that support an emerging hypothesis in which SDF-1/CXCR4 also plays a pivotal role in the biologic and physiological functions of MSCs [58, 59]. SDF-1 is upregulated at sites of injury and serves as a potent chemoattractant to recruit circulating or residing CXCR4-expressing MSCs, which are necessary for tissue-specific organ repair or the regeneration of many organs, such as the liver [60], heart [61], and skin [62] (Fig. 1). Moreover, the local delivery of SDF-1 into injured tissue promotes the recruitment of circulating mesenchymal stromal and progenitor cells to lesions in the heart [61] and brain [63], and the implantation of MSCs expressing CXCR4 improves the performance of infarcted myocardium [64]. However, the involvement of the SDF-1/CXCR4 axis of MSCs in bone repair has not been fully elucidated. Otsuru et al. [44] recently showed (using an ectopic bone formation model induced by implantation of a bone morphogenic protein-2-containing collagen pellet in mouse muscle tissues) that circulating bone marrow-derived osteoblast progenitor cells migrated to the region of bone formation through chemoattraction by SDF-1 expressed on vascular endothelial cells and on the de novo osteoblasts of the region. Granero-Moltó et al. [65] very recently published an interesting article in which they showed that implanted MSCs migrated to a fracture site in an exclusively CXCR4-dependent manner. Another recent study also showed that

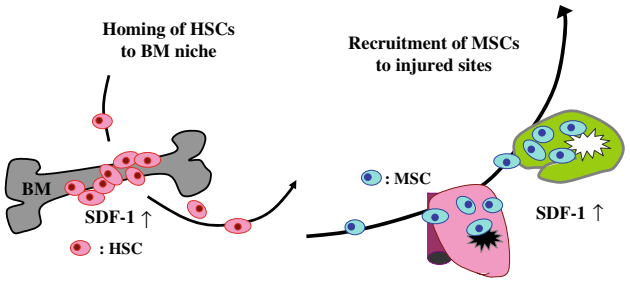


**Fig. 1** Stromal cell-derived factor 1 (*SDF-1*) recruits hematopoietic stem cells (*HSCs*) and mesenchymal stem cells (*MSCs*) to injured sites for the induction of organ repair. SDF-1 is upregulated at sites of injury and serves as a potent chemoattractant to recruit circulating or residing CXCR4-expressing MSCs, which are necessary for tissue-specific organ repair or the regeneration of the many organs such as the liver, heart, and skin. *BM* Bone marrow

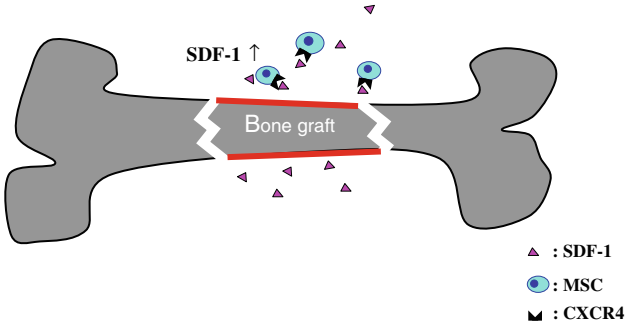


**Fig. 2** SDF-1/CXCR4 can recruit MSCs to induce fracture repair in skeletal repair. After bone injury, SDF-1 is expressed on the periosteum of the bone graft and recruits CXCR4-expressing MSCs to bone repair sites in the acute phase of bone repair

SDF-1 was induced in the periosteum in bone injury and promoted endochondral bone repair by recruiting MSCs to the site of injury [45]. In mouse models of structural femoral live and dead bone grafts, bone formation was decreased in SDF-$1^{+/-}$ and CXCR$4^{+/-}$ mice. This was rescued by grafted bones from CXCR$4^{+/-}$ mice transplanted into the SDF-$1^{+/-}$ femur, but not vice versa. These results demonstrate that following a bone injury, SDF-1 is expressed on the periosteum of the bone graft and recruits CXCR4-expressing MSCs to bone repair sites in the acute phase of bone repair (Fig. 2). Therapeutic studies are promised in the near future.

Another possible factor is monocyte chemotactic protein-1 (MCP-1) (also known as CCL2). In a recent study by Belema-Bedada et al. [66], systemically induced green fluorescent protein (GFP)-labeled MSCs that expressed the MCP-1 receptor CCR2 on their surface were infused into transgenic mice, with MCP-1 specifically expressed in the myocardium. GFP-positive cells were found in the myocardium at high frequencies, compared with none found in the hearts of the control mice. To eliminate the possibility of indirect effects of MCP-1 on MSCs via its receptor CCR2, MSCs were transfected with a vector expressing a truncated version of FROUNT (DN-FOUNT). FROUNT binds to CCR2, enabling CCR2-mediated chemotaxis toward MCP-1, while DN-FOUNT competes with endogenous FROUNT for CCR2 binding and acts as a dominant negative effector of CCR2-mediated chemotaxis. DN-FROUNT-transfected MSCs lacked the ability to home to the hearts of the MCP-1 transgenic mice. Therefore, the results showed that direct interaction of CCR2 with MCP-1 is crucial to the engraftment of MSCs, at least in ischemic heart tissue.

Other possible chemokines include MIP-1$\alpha$ (CCL3) and MCP-2 (CCL8) [67], fractalkine (CX3CL1) [68], and others [53, 54]. There are possibly also nonchemokine molecules that may act as chemoattractants for stem cells in vitro and in vivo, but the whole set of chemoattractants for MSCs is largely unknown. The candidate chemokines of MSC migration induction are summarized in Table 2.

This field remains insufficiently investigated, and it is assumed that many more factors can contribute to the migration of MSCs to the site of bone injury. The induction of MSC migration may be less efficient and less applicable than cell transplantation for stimulating bone healing. However, the method has certain clinical advantages, such as fewer ethical issues and a decreased possibility of infection, and it could be used to augment cell transplantation to enhance cell targeting. More studies must be conducted to elucidate the mechanisms so that practical therapeutic modalities can be developed in the near future.

## Conclusion

Skeletal injuries remain among the most prevalent clinical problems, especially in an aging society. The structural bone loss that occurs in compound fractures and periprosthetic osteolysis also exemplifies a serious clinical problem requiring massive bone reconstruction. In order to overcome the limitations of current treatments, it is of great importance to develop novel biologic strategies. However, these first require the elucidation of the molecular signals responsible for successful bone repair. MSCs are, without doubt, the most attractive candidate for cell-based bone regeneration, but current results have several notable shortcomings, such as vulnerability to infection, the uncertainty of the capability of MSCs for differentiation in specific in vivo situations, the high cost of ex vivo cell handling, the limited number of cells actually obtainable, and even possible malignant transformation of the cells during ex vivo cell expansion. The induction of MSC migration could be a promising approach to overcome these issues. The phenomenon of MSC migration to bone healing sites has recently gained wider acceptance. Rigorous studies have

**Table 2** Possible chemokines of MSC migration induction in bone repair

| Chemokines | Receptor(s) | Reference(s) |
|---|---|---|
| CXCL12 (SDF-1) | CXCR4, CXCR7 | [58–65] |
| CCL2 (MCP-1/MCAF) | CCR2, CCR11 | [66] |
| CCL3 (MIP-1$\alpha$) | CCR1, CCR5 | [67] |
| CCL8 (MCP-2) | CCR2, CCR3, CCR11 | [67] |
| CX3CL1 (fractalkine) | CX3CR1 | [68] |
| Other candidates [53, 54] | | |
| CXC chemokines—CXCL9 (MIG), CXCL13 (BLC/BCA-1), CXCL16 (SR-PSOX) | | |
| CC chemokines—CCL5 (RANTES), CCL17 (TARC), CCL19 (ELC/MIP-3$\beta$), CCL20 (LARC/MIP-3$\alpha$/exodus), CCL25 (TECK) | | |

been conducted to elucidate the mechanism(s) and crucial molecules, but meticulous and practical studies should be performed to make this method clinically applicable. Cell sources may be the bone marrow, periosteum, vessel walls, muscle, circulation, and elsewhere, but differences among these sources still cause controversy among experts and require more elaborate investigations. The induction of MSC migration by appropriate molecules could emerge as an efficient method to treat difficult bone regeneration issues. Molecules that efficiently induce the migration of MSCs should be sought, and practical aspects of the use of those molecules, such as injection, coating, transduction, and even the blocking of antagonized factors, should be investigated. The recruitment of MSCs from surrounding tissues or from circulation would be a helpful modality to induce or support bone regeneration.

**Conflict of interest** None.

## References


1. Goldring S, Gravallese E. Mechanisms of bone loss in inflammatory arthritis: diagnosis and therapeutic implications. Arthritis Res. 2000;2:33–7.
2. Paget S. Steroids cause osteoporosis. Ann Rheum Dis. 2002; 61:1–3.
3. Caplan AI. Mesenchymal stem cells. J Orthop Res. 1991; 9:641–50.
4. Tavassoli M, Crosby WH. Transplantation of marrow to extramedullary sites. Science. 1968;161:54–6.
5. Freidenstein AJ. Osteogenic stem cells in bone marrow. In: Heerschem JNM, Kanis JA, editors. Bone and mineral research. Amsterdam: Elsevier; 1990. p. 243–72.
6. Friedenstein AJ, Chailakhjan RK, Lalykina KS. The development of fibroblast colonies in monolayer cultures of guinea-pig bone marrow and spleen cells. Cell Tissue Kinet. 1970;3:393–403.
7. Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, Moorman MA, Simonetti DW, Craig S, Marshak DR. Multilineage potential of adult human mesenchymal stem cells. Science. 1999;284:143–7.
8. Bianco P, Robey PG, Simmons PJ. Mesenchymal stem cells: revisiting history, concepts, and assays. Cell Stem Cell. 2008; 2:313–9.
9. Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell. 2006;126:663–76.
10. Miura K, Okada Y, Aoi T, Okada A, Takahashi K, Okita K, Nakagawa M, Koyanagi M, Tanabe K, Ohnuki M, Ogawa D, Ikeda E, Okano H, Yamanaka S. Variation in the safety of induced pluripotent stem cell lines. Nat Biotechnol. 2009; 27:743–5.
11. Thomas ED. Bone marrow transplantation: a review. Semin Hematol. 1999;36[4 Suppl 7]:95–103.
12. Tögel F, Westenfelder C. Adult bone marrow-derived stem cells for organ regeneration and repair. Dev Dyn. 2007;236:3321–31.
13. Castano-Izquierdo H, Alvarez-Barreto J, van den Dolder J, Jansen J, Mikos A, Sikavitsas V. Pre-culture period of mesenchymal stem cells in osteogenic media influences their in vivo bone forming potential. J Biomed Mater Res A. 2007;82:129–38.
14. Hernigou P, Poignard A, Beaujean F, Rouard H. Percutaneous autologous bone-marrow grafting for nonunions. Influence of the number and concentration of progenitor cells. J Bone Joint Surg Am. 2005;87:1430–7.
15. Kucia M, Ratajczak M. Stem cells as a two edged sword–from regeneration to tumor formation. J Physiol Pharmacol. 2006; 57[Suppl 7]:5–16.
16. Jones E, McGonagle D. Human bone marrow mesenchymal stem cells in vivo. Rheumatology (Oxford). 2008;47:126–31.
17. Nishimura T, Simmons DJ, Mainous EG. The origin of bone formed by heterotopic periosteal autografts. J Oral Maxillofac Surg. 1997;55:1265–8.
18. Nakahara H, Bruder SP, Haynesworth SE, Holecek JJ, Baber MA, Goldberg VM, Caplan AI. Bone and cartilage formation in diffusion chambers by subcultured cells derived from the periosteum. Bone. 1990;11:181–8.
19. Nakahara H, Goldberg VM, Caplan AI. Culture-expanded human periosteal-derived cells exhibit osteochondral potential in vivo. J Orthop Res. 1991;9:465–76.
20. Gruber R, Mayer C, Bobacz K, Krauth MT, Graninger W, Luyten FP, Erlacher L. Effects of cartilage-derived morphogenetic proteins and osteogenic protein-1 on osteochondrogenic differentiation of periosteum-derived cells. Endocrinology. 2001; 142:2087–94.
21. Zhang X, Xie C, Lin AS, Ito H, Awad H, Lieberman JR, Rubery PT, Schwarz EM, O'Keefe RJ, Guldberg RE. Periosteal progenitor cell fate in segmental cortical bone graft transplantations: implications for functional tissue engineering. J Bone Miner Res. 2005;20:2124–37.
22. Colnot C. Skeletal cell fate decisions within periosteum and bone marrow during bone regeneration. J Bone Miner Res. 2009; 24:274–82.
23. Brighton CT, Hunt RM. Early histological and ultrastructural changes in medullary fracture callus. J Bone Joint Surg Am. 1991;73:832–47.
24. Schor AM, Allen TD, Canfield AE, et al. Pericytes derived from the retinal microvasculature undergo calcification in vitro. J Cell Sci. 1990;97:449–61.
25. Brighton CT, Lorich DG, Kupcha R, et al. The pericyte as a possible osteoblast progenitor cell. Clin Orthop. 1992; 275:287–99.
26. Diaz-Flores L, Gutierrez R, Lopez-Alonso A, et al. Pericytes as a supplementary source of osteoblasts in periosteal osteogenesis. Clin Orthop. 1992;275:280–6.
27. Doherty MJ, Ashton BA, Walsh S, et al. Vascular pericytes express osteogenic potential in vitro and in vivo. J Bone Miner Res. 1998;13:828–83.

28. Tintut Y, Alfonso Z, Saini T, et al. Multilineage potential of cells from the artery wall. Circulation. 2003;108:2505–10.
29. Farrington-Rock C, Crofts NJ, Doherty MJ, Ashton BA, Griffin-Jones C, Canfield AE. Chondrogenic and adipogenic potential of microvascular pericytes. Circulation. 2004;110:2226–32.
30. Gerber HP, Vu TH, Ryan AM, Kowalski J, Werb Z, Ferrara N. VEGF couples hypertrophic cartilage remodeling, ossification and angiogenesis during endochondral bone formation. Nat Med. 1999;5:623–8.
31. Lee JY, Qu-Petersen Z, Cao B, Kimura S, Jankowski R, Cummins J, Usas A, Gates C, Robbins P, Wernig A, Huard J. Clonal isolation of muscle-derived cells capable of enhancing muscle regeneration and bone healing. J Cell Biol. 2000;150:1085–100.
32. Usas A, Huard J. Muscle-derived stem cells for tissue engineering and regenerative therapy. Biomaterials. 2007;28:5401–6.
33. Kuznetsov SA, Mankani MH, Gronthos S, Satomura K, Bianco P, Robey PG. Circulating skeletal stem cells. J Cell Biol. 2001;153:1133–40.
34. Zvaifler NJ, Marinova-Mutafchieva L, Adams G, Edwards CJ, Moss J, Burger JA, Maini RN. Mesenchymal precursor cells in the blood of normal individuals. Arthritis Res. 2000;2:477–88.
35. Wang Y, Johnsen HE, Mortensen S, Bindslev L, Sejersten Ripa R, Haack-Sorensen M, Jorgensen E, Fang W, Kastrup J. Changes in circulating mesenchymal stem cells, stem cell homing factor, and vascular growth factors in patients with acute ST elevation myocardial infarction treated with primary percutaneous coronary intervention. Heart. 2006;92:768–74.
36. Campagnoli C, Roberts IA, Kumar S, Bennett PR, Bellantuono I, Fisk NM. Identification of mesenchymal stem/progenitor cells in human first-trimester fetal blood, liver, and bone marrow. Blood. 2001;98:2396–402.
37. Erices A, Conget P, Minguell JJ. Mesenchymal progenitor cells in human umbilical cord blood. Br J Haematol. 2000;109:235–42.
38. Huss R, Lange C, Weissinger EM, Kolb HJ, Thalmeier K. Evidence of peripheral blood-derived, plastic-adherent CD34(-/low) hematopoietic stem cell clones with mesenchymal stem cell characteristics. Stem Cells. 2000;18:252–60.
39. Wu GD, Nolta JA, Jin YS, Barr ML, Yu H, Starnes VA, Cramer DV. Migration of mesenchymal stem cells to heart allografts during chronic rejection. Transplantation. 2003;75:679–85.
40. Lazarus HM, Haynesworth SE, Gerson SL, Caplan AI. Human bone marrow-derived mesenchymal (stromal) progenitor cells (MPCs) cannot be recovered from peripheral blood progenitor cell collections. J Hematother. 1997;6:447–55.
41. Wexler SA, Donaldson C, Denning-Kendall P, Rice C, Bradley B, Hows JM. Adult bone marrow is a rich source of human mesenchymal 'stem' cells but umbilical cord and mobilized adult blood are not. Br J Haematol. 2003;121:368–74.
42. Kuznetsov SA, Mankani MH, Leet AI, Ziran N, Gronthos S, Robey PG. Circulating connective tissue precursors: extreme rarity in humans and chondrogenic potential in guinea pigs. Stem Cells. 2007;25:1830–9.
43. Kumagai K, Vasanji A, Drazba J, Butler R, Muschler G. Circulating cells with osteogenic potential are physiologically mobilized into the fracture healing site in the parabiotic mice model. J Orthop Res. 2008;26:165–75.
44. Otsuru S, Tamai K, Yamazaki T, Yoshikawa H, Kaneda Y. Circulating bone marrow-derived osteoblast progenitor cells are recruited to the bone-forming site by the CXCR4/stromal cell-derived factor-1 pathway. Stem Cells. 2008;26:223–34.
45. Kitaori T, Ito H, Schwarz EM, Tsutsumi R, Yoshitomi H, Oishi S, Nakano M, Fujii N, Nagasawa T, Nakamura T. Stromal cell-derived factor 1/CXCR4 signaling is critical for the recruitment of mesenchymal stem cells to the fracture site during skeletal repair in a mouse model. Arthritis Rheum. 2009;60:813–23.
46. Zuk PA, Zhu M, Ashjian P, De Ugarte DA, Huang JI, Mizuno H, Alfonso ZC, Fraser JK, Benhaim P, Hedrick MH. Human adipose tissue is a source of multipotent stem cells. Mol Biol Cell. 2002;13:4279–95.
47. Bi Y, Ehirchiou D, Kilts TM, Inkson CA, Embree MC, Sonoyama W, Li L, Leet AI, Seo BM, Zhang L, Shi S, Young MF. Identification of tendon stem/progenitor cells and the role of the extracellular matrix in their niche. Nat Med. 2007;13:1219–27.
48. Lavoie JF, Biernaskie JA, Chen Y, Bagli D, Alman B, Kaplan DR, Miller FD. Skin-derived precursors differentiate into skeletogenic cell types and contribute to bone repair. Stem Cells Dev. 2009;18:893–906.
49. Shyu W, Lee Y, Liu D, Lin S, Li H. Homing genes, cell therapy and stroke. Front Biosci. 2006;11:899–907.
50. Liu ZJ, Zhuge Y, Velazquez OC. Trafficking and differentiation of mesenchymal stem cells. J Cell Biochem. 2009;106:984–91.
51. Fox JM, Chamberlain G, Ashton BA, Middleton J. Recent advances into the understanding of mesenchymal stem cell trafficking. Br J Haematol. 2007;137:491–502.
52. Miyasaka M, Tanaka T. Lymphocyte trafficking across high endothelial venules: dogmas and enigmas. Nat Rev Immunol. 2004;4:360–70.
53. Honczarenko M, Le Y, Swierkowski M, Ghiran I, Glodek AM, Silberstein LE. Human bone marrow stromal cells express a distinct set of biologically functional chemokine receptors. Stem Cells. 2006;24:1030–41.
54. Chamberlain G, Wright K, Rot A, Ashton B, Middleton J. Murine mesenchymal stem cells exhibit a restricted repertoire of functional chemokine receptors: comparison with human. PLoS One. 2008;3:e2934.
55. Karp JM, Leng Teo GS. Mesenchymal stem cell homing: the devil is in the details. Cell Stem Cell. 2009;4:206–16.
56. Burger J, Kipps T. CXCR4: a key receptor in the crosstalk between tumor cells and their microenvironment. Blood. 2006;107:1761–7.
57. Lataillade J, Clay D, Dupuy C, Rigal S, Jasmin C, Bourin P, et al. Chemokine SDF-1 enhances circulating CD34(+) cell proliferation in synergy with cytokines: possible role in progenitor survival. Blood. 2000;95:756–68.
58. Wynn R, Hart C, Corradi-Perini C, O'Neill L, Evans C, Wraith J, et al. A small proportion of mesenchymal stem cells strongly expresses functionally active CXCR4 receptor capable of promoting migration to bone marrow. Blood. 2004;104:2643–5.
59. Dar A, Goichberg P, Shinder V, Kalinkovich A, Kollet O, Netzer N, et al. Chemokine receptor CXCR4-dependent internalization and resecretion of functional chemokine SDF-1 by bone marrow endothelial and stromal cells. Nat Immunol. 2005;6:1038–46.
60. Kucia M, Ratajczak J, Reca R, Janowska-Wieczorek A, Ratajczak M. Tissue-specific muscle, neural and liver stem/progenitor cells reside in the bone marrow, respond to an SDF-1 gradient and are mobilized into peripheral blood during stress and tissue injury. Blood Cells Mol Dis. 2004;32:52–7.
61. Abbott J, Huang Y, Liu D, Hickey R, Krause D, Giordano F. Stromal cell-derived factor-1alpha plays a critical role in stem cell recruitment to the heart after myocardial infarction but is not sufficient to induce homing in the absence of injury. Circulation. 2004;110:3300–5.
62. Ceradini D, Kulkarni A, Callaghan M, Tepper O, Bastidas N, Kleinman M, et al. Progenitor cell trafficking is regulated by hypoxic gradients through HIF-1 induction of SDF-1. Nat Med. 2004;10:858–64.
63. Ji J, He B, Dheen S, Tay S. Interactions of chemokines and chemokine receptors mediate the migration of mesenchymal stem cells to the impaired site in the brain after hypoglossal nerve injury. Stem Cells. 2004;22:415–27.

64. Cheng Z, Ou L, Zhou X, Li F, Jia X, Zhang Y, Liu X, Li Y, Ward CA, Melo LG, Kong D. Targeted migration of mesenchymal stem cells modified with CXCR4 gene to infarcted myocardium improves cardiac performance. Mol Ther. 2008;16:571–9.
65. Granero-Moltó F, Weis JA, Miga MI, Landis B, Myers TJ, O'Rear L, Longobardi L, Jansen ED, Mortlock DP, Spagnoli A. Regenerative effects of transplanted mesenchymal stem cells in fracture healing. Stem Cells. 2009;27:1887–98.
66. Belema-Bedada F, Uchida S, Martire A, Kostin S, Braun T. Efficient homing of multipotent adult mesenchymal stem cells depends on FROUNT-mediated clustering of CCR2. Cell Stem Cell. 2008;2:566–75.
67. Wang L, Li Y, Chen X, Chen J, Gautam SC, Xu Y, Chopp M. MCP-1, MIP-1, IL-8 and ischemic cerebral tissue enhance human bone marrow stromal cell migration in interface culture. Hematology. 2002;7:113–7.
68. Hung SC, Pochampally RR, Hsu SC, Sanchez C, Chen SC, Spees J, Prockop DJ. Short-term exposure of multipotent stromal cells to low oxygen increases their expression of CX3CR1 and CXCR4 and their engraftment in vivo. PLoS One. 2007;2:e416.