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STEM CELL HOMING IN MUSCULOSKELETAL INJURY

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Abstract

The regenerative potential of injured adult tissue suggests the physiological existence of cells capable of participating in the reparative process. Recent studies indicate that stem-like cells residing in tissues contribute to tissue repair and are replenished by precursor bone marrow-derived cells. Mesenchymal stromal cells (MSC) are among the candidates for reparative cells. These cells can potentially be mobilized into the circulation in response to injury signals and exert their reparative effects at the site of injury. Current therapies for musculoskeletal injuries pose unavoidable risks which can impede full recovery. Trafficking of MSC to the injury site and their subsequent participation in the regenerative process is thought to be a natural healing response that can be imitated or augmented by enhancing the endogenous MSC pool with exogenously administered MSC. Therefore, a promising alternative to the existing strategies employed in the treatment of musculoskeletal injuries is to *reinforce the inherent reparative capacity* of the body by delivering MSC harvested from the patient's own tissues to the site of injury. The aim of this review is to inform the reader of studies that have evaluated the intrinsic homing and regenerative abilities of MSC, with particular emphasis on the repair of musculoskeletal injuries. Research that supports the direct use of MSC (without in vitro differentiation into tissue-specific cells) will also be reported. Based on accruing evidence that the natural healing mechanism involves the recruitment of MSC and their subsequent reparative actions at the site of injury, as well as documented therapeutic response after the exogenous administration of MSC, the feasibility of the emerging strategy of *instant stem-cell therapy* will be proposed.

Keywords

mesenchymal stromal cell; homing; trafficking; tissue engineering; bone; cartilage

Introduction

The ability of injured adult tissue to regenerate implies the existence of cells capable of proliferating, differentiating and/or functionally contributing to the reparative process [1].

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Recent evidence suggests that stem-like cells that reside in multiple tissues participate in the course of tissue repair and are replenished by precursor cells from the bone marrow [2]. Among the candidates for reparative cells from bone marrow are the non-haemopoietic progenitor cells that exhibit a multi-lineage differentiation capacity, being able to differentiate into the osteogenic, myogenic, chondrogenic and neurogenic lineages [3]. These cells are commonly referred to as multipotent mesenchymal stromal cells, mesenchymal stem cells or marrow stromal cells (MSC). Current knowledge about MSC is based almost entirely on their in vitro characteristics. The native identity, exact location and functions of MSC in vivo remain elusive [4]. Besides the bone marrow, MSC and MSC-like cells have been found to be harbored in various other sites including adipose tissue, periosteum, skeletal muscle, placenta, trabecular bone and others [5]. These tissue-specific stem cells differ in phenotype, morphology, proliferation potential and differentiation capacity, but possess many common features associated with those from the bone marrow, possibly implying that MSC-like populations share a similar ontogeny [5]. Emerging evidence suggests that the perivascular niche could be the anatomic location where MSC-like populations reside in tissues [6,7] and that pericytes may be integral to the origin of MSC [4,8]. Notably, the recent paper by Crisan and colleagues [8] suggests that an ancestor of MSC is natively associated with the blood vessel wall and belongs to a subset of perivascular cells. Whether the vascular setting is indeed the actual niche for pericytic MSC-like cells and is the main source of MSC in vivo remains to be established [9].

MSC appear to be reservoirs of reparative cells that lack tissue-specific characteristics and can potentially be mobilized and differentiate into cells of a connective tissue lineage under different signals, such as damage from trauma, fracture, inflammation, necrosis and tumors [10]. Recent studies [11,12] suggest that injury/trauma might initiate the mobilization of MSC into peripheral blood. These circulating stem cells are believed to home to the damaged or pathological tissues in a mechanism similar to leukocyte recruitment to sites of inflammation that involves adhesion molecules such as selectins, chemokine receptors and integrins. The migration of MSC from the circulation into injured or unhealthy tissues and resulting therapeutic response have been documented [13-16]. Increasingly, studies tend to conclude that the beneficial effects of MSC can be due to two possible mechanisms of reparative action [17]: not only the in situ differentiation of MSC to become normal constituents of the host cytoarchitecture and supporting stroma after recruitment to the injury site [18], but also to act via a paracrine mechanism. The latter is an emerging concept whereby MSC are believed to possess the capacity to home to the site of injury, and subsequently secrete a broad spectrum of paracrine factors that are both immunoregulatory and function to structure the regenerative microenvironment [19]. Caplan and colleagues have referred to the regenerative microenvironment created by the bioactive factors secreted by MSC as 'trophic activity' [19]. Effects of these bioactive factors secreted include inhibition of scarring and apoptosis, and stimulation of angiogenesis and mitosis of tissue-intrinsic stem or progenitor cells [19].

Surgical procedures to repair or replace injured musculoskeletal tissues are the current gold standard for lost or damaged bone, cartilage or skeletal muscle. In cases of extensive bone loss the reconstruction of large bone segments remains a significant clinical problem. Current therapeutic treatments include the use of particulate cancellous or bulk cortico-cancellous bone auto- or allografts, bone transport methods (Ilizarov technique), or implants made from natural or artificial materials. However, none have proven to be fully satisfactory [20]. Autologous bone graft is limited in supply and is often associated with significant donor site morbidity, while the use of allografts or xenografts poses the potential risk of infection and of an adverse immune response by the host tissue after implantation. In addition, while biomaterials have the advantage of unlimited availability and good osteoconductivity, their application is limited as they lack osteoinductivity. The Ilizarov and

related techniques take advantage of the regenerative potential of bone, however, these operations are painful and problematic for the patient and require weeks to months or longer before completion [20]. Current therapies presently employed for articular cartilage defects include autologous chondrocyte implantation (ACI), osteochondral autografts and allografts, microfracturing, mosaicplasty and in severe cases, total joint replacement [3]. ACI still faces several major challenges, including multiple surgical procedures, donor-site morbidity, chondrocyte de-differentiation during in vitro culture and fibrocartilage formation after cell implantation instead of defect healing [21]. These issues indicate that many current therapies for musculoskeletal repair have unavoidable risks which can have an impact on the patient's ability to fully recover after surgery [3].

Studies suggest that trafficking of native MSC to injured tissue and their subsequent participation in the regenerative process is a natural healing response, which can potentially be imitated or augmented by enhancing the endogenous MSC pool with exogenously administered MSC. Accordingly, based on this hypothesis, a promising alternative to the existing therapeutic strategies employed in the treatment of musculoskeletal injuries is to *reinforce the inherent reparative capacity* of the body by delivering MSC harvested from the patient's own tissues to the site of injury. Studies have already documented beneficial effects after the systemic or localized delivery of MSC for the repair of damaged cartilage [22-30], bone [13,31-34] and muscle [35-37].

The primary purpose of this review article is to inform the reader of studies that have evaluated the proposed intrinsic homing and regenerative abilities of MSC, with particular emphasis on the repair of musculoskeletal injuries. Research that supports the direct use of MSC (without in vitro differentiation into tissue-specific cells) will also be reported. Based on accruing evidence that the natural healing mechanism involves the recruitment of MSC and their subsequent reparative actions at the site of injury, as well as documented therapeutic response after the exogenous administration of MSC, the feasibility of the emerging strategy of *instant stem-cell therapy* will be proposed.

Mobilization and Homing of MSC

MSC are non-haematopoietic stromal cells that were first isolated from bone marrow and subsequently from other adult connective tissues [10]. They are a heterogeneous population of pluripotent progenitor cells that possess the capacity to differentiate into mesodermal and non-mesodermal cell lineages including osteocytes, chondrocytes, adipocytes, myocytes, cardiomyocytes, fibroblasts, myofibroblasts, epithelial cells and neurons [5]. MSC may be derived from bone marrow or other tissues and expanded in vitro with complete medium in culture plates or flasks, where they adhere, proliferate and form fibroblastic-like cell clusters (colony-forming unit-fibroblasts, CFU-F) [38].

At present, standard phenotypic criteria to characterize MSC do not exist due to the varying MSC markers that are utilized by different laboratories [39]. Attempts to define a unique phenotype characteristic of MSC have been impeded by the fact that these cells display significant heterogeneity [5], and can express a range of cell-lineage specific antigens that may differ depending on the culture preparation, culture duration, or plating density [40]. Furthermore, this task is complicated by the fact that MSC share features with other cell types including endothelial, epithelial and muscle cells [39].

Currently, MSC are defined by an array of characteristics in vitro, including a combination of phenotypic markers and multipotential differentiation and functional properties [5]. To address the problem of the lack of universally accepted criteria for defining MSC, the Mesenchymal and Tissue Stem Cell Committee of the International Society for Cellular Therapy have proposed the following minimal criteria [41]:

1. MSC must be plastic-adherent when maintained in standard culture conditions.
2. MSC must express CD105, CD73 and CD90, and lack expression of CD45, CD11b, CD34 or CD14, CD79 α or CD19 and HLA-DR surface molecules.
3. MSC must be able to differentiate into osteoblasts, adipocytes and chondroblasts in vitro.

The potential clinical utility of MSC is due to the relative ease with which they can be isolated, their lack of significant immunogenicity allowing allogenic transplantation without the need for immunosuppressive drugs, minor ethical controversy in their use, their capacity to differentiate into tissue-specific cell types, trophic activity and immunomodulatory properties [42]. The utility of MSCs in cellular therapy depends on the capacity of these cells to home and engraft long term into the appropriate target tissue [43].

Increasing evidence suggests that MSC are notably more important in regulating wound healing and inflammatory diseases than previously thought; host MSC appear to mobilize in response to injury and actively target specific tissues [42]. Several studies have reported the migratory capacity of MSCs to sites of injury such as traumatic brain injury [44], bone fracture [13], radiation-induced injuries [45,46], lung injury [14], liver injury [47], acute renal failure [48] and acute burns [11]. The recruitment of bone marrow-derived MSC to repair and regenerate injured tissue is a complex multi-step process involving mobilization, homing and reparative actions. It is believed that MSC are first released from their storage niche in the bone marrow into the circulation upon stimulation by specific signals arising from the remote injured tissue (mobilization). Subsequently, these circulating MSC are arrested within the vasculature of the tissue followed by transmigration across the endothelium (homing) where they proliferate and differentiate into mature, functional tissue [5] and possibly modulate the local environment of the injured tissue [49]. While the homing of leukocytes to sites of inflammation is well studied, the mechanisms that regulate MSC migration to injured tissue are still a poorly understood process. In the recruitment of inflammatory cells during inflammation, a coordinated multi-step sequence of adhesive and signaling events occurs, including selectin-mediated rolling, leukocyte activation by chemokines resulting in activation of integrins, integrin-mediated firm adhesion on endothelial cell monolayers, diapedesis through the endothelial cell monolayers and lastly, the migration/invasion in the extracellular matrix involving integrin-dependent processes and matrix-degrading proteases [50]. The process of homing, with reference to haematopoietic stem cells (HSC), has also been more extensively studied; in this context, homing is the active navigation of haematopoietic stem cells through the blood, across the endothelial vasculature to different organs as well as to their bone marrow niches [51]. In order to leave the bloodstream, mature leukocytes and haematopoietic progenitor cells have been reported to undertake a coordinated sequence of steps, initiated by tethering events which are largely mediated by selectins and their ligands. The captured cells then roll and chemokines activate integrins, leading to firm arrest and eventual transendothelial migration [52-54]. To elucidate the underlying mechanisms in each step of MSC migration, the well-established paradigms of leukocytes/HSC migration are good references for the investigation if it is assumed that MSC utilize similar mechanisms of recruitment.

On a side note, this review article is largely focused on our present understanding of *bone marrow – derived* MSC homing from the bone marrow to sites of injury because the trafficking of these cells have been widely studied while homing of MSC from other possible sources such as adipose tissue is less well understood. Also, one might question why MSC would transit from the bone marrow to remote sites of injury if they appear to be almost ubiquitous in the body as local stem cells. While it is known that local stem cells contribute to tissue healing, the relative contribution of local stem cells as compared to stem

cells homing from some other remote location to the injured site is currently still unclear and needs further investigation.

Factors Governing MSC Migration

The exact mechanism by which MSC are mobilized into the circulation, undergo recruitment and transmigrate across the endothelium is not yet fully elucidated. However, it is probable that injured tissue expresses specific receptors or ligands to facilitate trafficking, adhesion and infiltration of MSC to the site of injury, similar to the recruitment of leukocytes to sites of inflammation [43]. The well-characterized model of leukocyte migration, together with studies on haematopoietic cell migration which has been more widely investigated [1], may provide the platform needed to facilitate identification of factors critical for MSC homing [43].

Cytokines and chemokines are important factors in regulating mobilization, trafficking and homing of stem/progenitor cells [5]. Several studies aimed at investigating the different chemokine receptor profiles of human MSC and the chemotactic effect of particular cytokines on these cells have documented. Honczarenko et al. [55] examined human bone marrow MSC for chemokine receptor and function and showed that the cells expressed a distinct set of chemokine receptors, namely: CCR1, CCR7, CCR9, CXCR4, CXCR5 and CXCR6. It was also demonstrated that chemokines corresponding to these surface receptors induced cellular responses – specific chemotaxis as well as β -actin filament reorganization (CXCL12). Honczarenko et al. [55,56] highlighted that these findings support the belief that certain chemokines, CXCL12 in particular, are important in bone marrow MSC homing and localization within the bone marrow, as has been determined for haematopoietic cells. In another study that was aimed at elucidating chemokine receptor expression on human bone marrow MSC and their role in mediating migration to tissues, Sordi et al. [56] found that a small percentage of cells (2-25%) expressed a restricted set of chemokine receptors as well. However, though CXCR4, CXCR6, CCR1 and CCR7 were found to be expressed as well (consistent with the findings of Honczarenko et al.), receptors CCR9 and CXCR5 were not detected. Furthermore, cells were found to express CX3CR1 but this was not the case for the study by Honczarenko et al. Sordi et al. also showed that bone marrow MSC were capable of undergoing appreciable chemotactic migration in response to a restricted set of chemokines in vitro and that the attraction of these cells to an in vitro model of peripheral tissue was principally mediated by CX3CL1 and CXCL12. In addition to the chemokine receptors that have already been documented, Ringe et al. [57] further reported the MSC-expression of several others. Based on these studies, it is apparent that the reported chemokine receptor repertoire of MSC is not completely consistent. This could be due to the heterogenic nature of a typical MSC population which therefore prevents the profiling of a distinct chemokine receptor repertoire [17]. That MSC express a range of chemokine receptors suggests that these cells may possess the potential to home to different tissues [43].

Specifically, the CXC chemokine stromal derived factor-1 (SDF-1, also named CXCL-12) has been associated with the migration, proliferation, differentiation and survival of several cell types such as human and murine haematopoietic stem and progenitor cells [58]. CXCR4, the seven transmembrane G-protein coupled receptor of SDF-1, has been found to be exhibited by cell types including haematopoietic, endothelial, stromal and neuronal cells [58]. Taken together, SDF-1 and CXCR4 have been found to have an important role in migration as indicated by studies on engraftment of bone hematopoietic stem/progenitor cells [59] as well as tumor metastasis [60]. The SDF-1/CXCR4 axis also appears to regulate the migration of MSC. Using a transwell assay to investigate the response of the CXCR4 receptor to the ligand SDF-1, Wynn et al. [61] observed the dose-dependent migration of human MSC to SDF-1 and concluded that the receptor contributes to MSC migration.

Transplanting primary cultured MSC expressing luciferase into a stabilized tibial fracture murine model, Granero-Molto et al. [13] recently determined by bioluminescence imaging that the dynamic migration of MSC to the fracture site is exclusively CXCR4-dependent. On a similar note, using mouse segmental bone graft models, Kitaori et al. [62] demonstrated that the SDF-1/CXCR4 axis plays a critical role in the recruitment of MSC to sites of bone healing which contributes to endochondral bone repair. SDF-1 was observed to be highly expressed in the periosteum of the live bone grafts during the acute phase of healing post-surgery and blockage of the SDF-1/CXCR4 axis inhibited MSC migration to the site of bone injury and resulted in decreased callus formation.

In another study which evaluated the intravenous infusion of genetically modified MSC over-expressing CXCR4 after coronary occlusion/reperfusion in rats, CXCR4-MSC was observed to home towards the infarct region of the myocardium in greater numbers as compared to untransduced MSC [63]. With one of their objectives as the investigation of the possible physiological mobilization of MSC and the natural variation in stem cell homing factor SDF-1 in patients with ST elevation acute myocardial infarction (STEMI) treated with primary percutaneous coronary intervention, Wang et al. [12] found a decrease in circulating MSC within one week after STEMI, suggesting enhanced recruitment of these MSC to the damaged myocardium. Furthermore, it was documented that plasma SDF-1 concentration increased over time, and this was associated with the detection of receptor CXCR4 on a large proportion of MSC. The authors suggested that this increase in plasma SDF-1 concentration may be a pathologically relevant upregulation of a chemoattractant to augment the recruitment of circulating stem/progenitor cells into the damaged myocardium following STEMI. The SDF-1-CXCR4 axis has also been reported to play a role in mediating the trafficking of transplanted MSC to impaired sites in the rat brain [64]. Taken together, these studies provide evidence which suggest that the SDF-1/CXCR4 axis is critical in governing the migration of MSC to injured tissues.

To understand the growth factors/cytokines which can affect migration of MSC to injured tissues, Ozaki et al. [65] compared the effects of 26 growth factors/cytokines on the migration activity of rabbit and human MSC using a microchemotaxis chamber. It was observed that the following consistently enhanced the migration of MSC at appropriate concentrations – platelet-derived growth factor (PDGF)-BB, PDGF-AB, epidermal growth factor (EGF), HB-EGF, transforming growth factor (TGF- α), insulin growth factor (IGF-I), hepatocyte growth factor (HGF), fibroblast growth factor (FGF-2) and thrombin. In addition, as various combinations of these factors further enhanced the migration of MSC, it was suggested that combinations of growth factors may be important in eliciting the maximal chemotactic effect. In other studies, factors which have been shown to enhance the migratory capacity of MSC include IGF-1 [66], matrix metalloproteinase 2 (MMP-2), membrane type 1 MMP (MT1-MMP) and tissue inhibitor of metalloproteinase 1 (TIMP-1) [67], galanin [68], monocyte chemotactic protein-1 (MCP-1) [69,70] and monocyte chemotactic protein-3 (MCP-3) [49].

The extent to which MSC use specific adhesion mechanisms to egress from the bloodstream is at present, poorly understood [52]. Mature leukocytes and hematopoietic progenitor cells have been shown to undertake an orchestrated sequence of adhesion steps, initiated by tethering events, that are largely mediated by selectins and their ligands, in order to leave the bloodstream [52]. Thereafter, these captured cells roll and encounter chemokines, which activate integrins and lead to firm arrest and subsequent transendothelial migration [52]. To elucidate the underlying mechanisms in each step of MSC migration, the well-established paradigms of leukocytes/HSC migration are good references for the investigation if it is assumed that MSC utilize similar mechanisms. Schmidt et al. [71] performed co-culture experiments of MSC on an endothelial monolayer and demonstrated the capability of MSC

for transendothelial migration. Ruster et al. [52] investigated human MSCs under shear flow using a parallel plate flow chamber and by intravital microscopy in mice to examine the potential of MSC to undergo coordinated steps of interaction with endothelial cells. It was shown that human MSC home to different tissues and exhibit coordinated rolling and adhesion behaviour on endothelial cells. MSC was observed to bind to endothelial cells in a P-selectin-dependent manner in vitro and in vivo. In addition, it was also observed that rolling MSC engage VLA-4/VCAM-1 to mediate firm adhesion on the endothelial cells. Whilst the exact mechanisms that contribute to the mobilization, recruitment and transmigration of MSC still remain largely unclear, these studies have furthered our understanding of the factors that govern MSC migration, and much more has to be done to fully elucidate the entire process.

Bone

Fracture healing is a complex regenerative process that is initiated in response to injury and is characterized by a well-orchestrated series of biological events. Unlike other tissues that undergo repair by the formation of a poorly organized scar, bone is regenerated and pre-fracture tissue properties are largely regained [72]. From a classic histological perspective, fracture healing has been categorized into primary fracture healing and secondary fracture healing [72]. Primary healing or primary cortical healing involves a direct attempt by the cortex to bridge the fracture gap and rejoin the fracture fragments after it has been interrupted. This process appears to require anatomic restoration of the fracture fragments and minimization of inter-fragmentary strain which ensures the stability of fracture reduction. New haversian systems are reestablished by osteoclasts that perform a tunneling resorptive response to create pathways for the penetration by blood vessels and osteoprogenitors. Vascular endothelial cells and perivascular cells that accompany these new blood vessels become the osteoprogenitor cells that give rise to osteoblasts. Discrete remodeling units referred to as “cutting cones” are formed as a result of the events [72].

However, this mode of primary fracture healing is the rarer of the two types. More frequently, secondary fracture healing occurs, resulting in callus formation [73]. The process of secondary fracture healing involves a combination of intramembranous and endochondral ossification. In intramembranous ossification, bone is formed directly (without the prior formation of cartilage) from committed osteoprogenitor and undifferentiated mesenchymal cells that reside in the local periosteum, located away from the fracture site, leading to the histologically described ‘hard callus’ [74]. In endochondral ossification, undifferentiated mesenchymal cells are recruited, undergo proliferation and differentiation into cartilage, which calcifies and is eventually replaced by bone [74]. There are six temporal stages that characterize this process, including an initial stage of hematoma formation and inflammation, followed by a subsequent stage whereby angiogenesis occurs and cartilage begins to form, and then three consecutive stages of cartilage calcification, cartilage removal and bone formation and ultimately bone remodeling [72]. An early bridging callus, histologically described as ‘soft callus’ which functions to stabilize the fracture fragments, is formed via this form of fracture healing [74].

Underlying these histological events is a well-orchestrated series of biological events that involves the coordinated participation of several cell types and complex molecular signaling pathways. The review by Giannoudis et al. (2007) [74] provides a good overview of the signaling molecules involved in the mediation of bone repair. It is believed that the function of the fracture hematoma is to be a source of signaling molecules [72] in which different classes interact with both local and circulating cells to execute the healing cascade: proinflammatory cytokines (interleukin-1 (IL-1), interleukin-6 (IL-6), tumor necrosis factor alpha (TNF- α)), mitogens (transforming growth factor beta (TGF- β), insulin-like growth

factor (IGF), fibroblast growth factor (FGF), and platelet-derived growth factor (PDGF)), morphogens (bone morphogenetic proteins (BMPs)), and angiogenic factors (vascular endothelial growth factor (VEGF) and angiopoietins) [74,75]. Factors such as TNF- α , TGF- β , BMPs, FGF and PDGF have been shown to mediate the recruitment, proliferation or differentiation of MSC in the fracture healing process [76].

It is known that MSC reside in a number of tissues including the periosteum, bone marrow, synovium, trabecular bone and synovial fluid [75]. Cells that contribute to intramembranous bone formation seem to be derived from the underlying cortical bone and the local periosteum [77]. Periosteal MSC are required to undergo osteogenic differentiation and bone formation without the prior differentiation into cartilage and there is little recruitment of cells from other tissues [75]. However, the origins of the mesenchymal cells that contribute to the process of endochondral ossification in fracture healing remain unresolved. The periosteum, surrounding muscle tissue and the marrow space at the site of the damaged cortical tissue are three potential sources of mesenchymal stem cells that give rise to the endochondral components of fracture healing [77].

More than 30 years ago [78], it was believed that fracture repair tissue arose from specialized cells with a predetermined commitment to bone formation; these cells are what we call osteoprogenitor cells which are closely associated with the bone surface or the bone marrow. However, there was already in existence, at that time, another rival alternative theory that moved away from the concept that these cells involved in the reparative process are specialist cells which originate from the local periosteum. Rather, repair tissue was believed to arise from the activity of previously uncommitted fibroblasts possessing the potential for osteogenesis if provided with appropriate environmental stimuli. These cells are recruited from the surrounding soft tissue through a phenomenon termed osteogenic induction. The immediate development of an extraosseous blood supply following fracture which appears to function as the supply of external callus is suggestive that extraskeletal cells do in fact contribute to reparative tissue. It appeared that osteogenic cells residing in the marrow might have access to the circulation and are possibly responsible for extraskeletal bone formation. This concept persists today; these uncommitted fibroblasts are commonly referred to as MSC, and osteogenic induction is perhaps the observation of circulating MSC recruitment to the fracture site. Buckwalter et al. have shown that there is reduced capacity for fracture callus development if the periosteum is removed which suggests the importance of the periosteum as a primary and local source of skeletogenic stem cells [77]. If circulating MSC exist physiologically, the question that has to be addressed is whether these cells truly are a part of the 'global' reparative response - capable of being mobilized into the circulation in response to tissue damage signals, home into the fracture site and contribute functionally to fracture repair [75].

Intravenously administered MSC have been shown to preferentially engraft into bone marrow tissue and bone [79,80]. Recent studies have attempted to provide evidence of the ability of circulating MSC to migrate to a site of bone injury. In an interesting study that employed a parabiotic murine model, Muschler et al. [81] showed that circulating osteogenic connective tissue progenitor cells are capable of homing to the fracture site and contribute to skeletal repair. Parabiotic animals were formed by surgically conjoining transgenic mice constitutively expressing green fluorescent protein (GFP) in no erythroid tissue and syngeneic wild-type mice. After establishment of parabiosis, a transverse fibular fracture was made in the hind limb of the conjoined wild-type partner. Histomorphometric analysis at the fracture site showed significant increase of GFP+ cells after two (5.4%) and three (5.6%) weeks compared to non-fractured controls (1.7%). Furthermore, of the GFP+ cells, the percentage of cells expressing alkaline phosphatase activity (interpreted as osteogenic differentiation) at one (37.4%) and two (85.3%) weeks post-fracture time was

statistically higher than that in non-fractured controls (10.8%). These observations suggest that osteogenic CTPs are present in the systemic circulation and can be physiologically mobilized to contribute to fracture callus formation and fracture repair.

In another study aimed at studying the ability of MSC to traffic to a site of musculoskeletal injury, Biswal et al. (2008) [18] tracked a population of systemically administered reporter adipose-derived multipotent cells (ADMCs) in a murine model using molecular and small animal imaging technologies and observed that these cells were able to home to the fracture site and promote healing of the damaged tissue. In this experiment, harvested ADMCs were first transformed using a lentiviral vector or plasmid encoding luciferase and fluorescence protein and subsequently injected into animal femoral injury models via the tail vein. Bioluminescence imaging (BLI) was used to monitor the trafficking of the exogenously administered reporter ADMCs; injured animals consistently had higher BLI signal in the injured thigh than uninjured animals until post-injection day 15 and histological evaluation confirmed the presence of reporter ADMCs in the fracture callus. Furthermore, healing of the defect as measured by micro-computed tomography was observed to be faster in stem cell-injected animals as compared to non-injected controls.

In yet another study, using a stabilized tibia fracture mouse model, Granero-Moltó et al. [13] provided evidence of the homing ability of circulating transplanted MSC in response to a fracture injury cue into the endosteal niche. Transplanting MSC labeled with luciferase in combination with BLI analysis, migration of MSC to the fracture site was observed. This migration was reported to be both time- and dose-dependent and exclusively CXCR4-dependent (Figure 1). Furthermore, through biomechanical testing it was found that MSC transplantation enhances fracture healing by increasing the material toughness of the callus, causing it to be less brittle. Micro-computed tomography and histological analyses of an entire callus showed that animals that received MSC transplant demonstrated a significant increase in the total tissue volume, as well as total bone tissue, soft tissue, new bone and callus volumes and callus mineralization content compared with controls. Histological analyses confirmed that the fracture healing in animals that received MSC transplant progressed through more cartilage and newly mineralized bone than controls. Using Lac Z-tagged MSC, it was also found that transplanted MSC localize along the margins of woven bone where they assume the morphology of active osteoblasts, express osteocalcin and associate with the endosteal surface.

Other studies by Gang Li et al. [82] and Devine et al. [83] have also proven the ability of osteogenic cells to localize at the site of bone injury following systemic administration in animal models. Although the extent to which circulating osteogenic cells contribute to normal fracture repair is only beginning to be elucidated, these data suggest that stem cell homing may be an intrinsic biological process [84] whereby the therapeutic response can potentially be augmented by reinforcing the endogenous MSC pool with exogenously administered MSC.

Five to 10% of the fractures that occur annually in the United States demonstrate delayed healing or non-union [85]. Currently, the most effective bone graft is autogenous cancellous bone, which is obtained from one site and implanted in another site in the same patient; the graft not only provides the scaffolding to support the distribution of the bone healing response but also provides the connective tissue progenitor cells that form new cartilage or bone. However, the harvest of autogenous bone is associated with significant morbidity and cost, including pain, scars, blood loss, extended operative and rehabilitation time and risk of infection. Furthermore, autogenous bone grafts may be limited in supply depending on the volume of the graft site. Although fracture healing remains to a large extent, an unknown cascade of complex biological events [76], it is clear that MSC play a critical reparative role

in the bone regeneration process. Accordingly, a potential alternative therapeutic approach is to transplant autogenous MSC directly to the site of bone injury. It is hypothesized that in the appropriate microenvironment, MSC have the potential to effect rapid bone regeneration in critical-sized defects [86]. The beneficial effect of transplanting in vitro-expanded autologous MSC seeded onto porous ceramic scaffolds to improve the clinical outcome of large bone defects in pre-clinical and clinical experiments have been reported [31,33,87]. The general technique employed in studies like these involves aspiration of bone marrow, plating a suspension of bone marrow cells onto tissue culture dishes, culture-expanding the cells in a select medium until the number of MSC in the culture increases, and then detaching the cells from the culture dishes to obtain a cell suspension of an in vitro expanded population of MSC which is seeded onto the scaffold [86].

However, the disadvantage of this method of achieving the number of MSC desired for implantation is if the culture-expanded cells used are obtained from bone marrow aspirates drawn from the patient, the patient must undergo more than one invasive procedure (one to harvest the bone marrow and one at a later time to implant the prepared graft). Consequently, this approach may require the patient to undergo anaesthesia more than once [86]. Furthermore, in vitro expansion has been associated with decreased in vivo homing ability of MSC [88], and risks such as contamination with bacteria or viruses or depletion of the proliferative capacity of the connective tissue progenitors before implantation [89]. Honczarenko et al. [55] assessed the influence of long-term culture on human bone marrow MSC chemokine receptor expression and observed that long term culture of bone marrow MSC resulted in a marked decrease in chemokine receptor expression and abolished the stromal cell chemotactic responsiveness to chemokines. Chemokine receptors on bone marrow MSC are believed to be important for the trafficking of transplanted stromal cells. This loss of chemokine receptor expression and function was associated with a downregulation of other surface receptors characteristic for bone marrow MSC. The authors postulate that the loss of surface receptors could be an indicator of in vitro transformation of the bone marrow MSC into more differentiated cells.

Lastly, there is a need for MSC to be expanded in dedicated rooms with laminar flux flow for clinical utility, according to the same rules of good manufacturing practice used for drug production (which requires very strict conditions of sterility, specific reagents excluding heterologous proteins and various kinds of quality controls, including microbiological, immunological and functional tests) [38]. For a clinical application, sidestepping the expansion step of the cells would effectually reduce the costs involved and facilitate the use of MSC therapy in hospitals that are not equipped with the necessary laboratory facilities [90]. In view of this, there is a need for other therapeutic strategies that avoid the in vitro expansion step, and enables the efficient isolation of a sufficient number of MSC from the bone marrow aspirate via a concentration step within the operating room. The last section of this review paper addresses the topic of bone marrow aspirate concentration.

In an alternative therapeutic approach which is minimally invasive, several studies [91-95] have shown that delivering connective tissue progenitors in aspirated bone marrow aids bone healing in non-unions [96]. The implantation of bone marrow has the potential of effectively regenerating bone as marrow contains osteogenic progenitors [96]. As the first clinical study employing autologous marrow injection as a substitute for standard open-grafting techniques, Connolly et al. [93] demonstrated the efficacy of the marrow injection technique combined with adequate fracture immobilization, with eighteen of twenty tibial nonunions successfully treated. In a similar study, Goel et al. [97] conducted a prospective study to evaluate the efficacy of percutaneous bone marrow grafting in patients with established tibial non-union and minimal deformity and achieved clinical and radiological bone union in fifteen out of twenty patients. Because of its biological value and low risk,

this strategy has the support of other investigators and many surgeons [96]. Hernigou et al. reported the safety of this technique based on their experience, as well as the significantly diminished complications at the donor and recipient sites [93]. Other advantages of using autologous bone marrow injected percutaneously to stimulate osteogenesis of non-unions include its simplicity in execution as it can be done on an outpatient basis [93] and therefore it is cost effective. Furthermore, this strategy encourages early treatment of non-unions to minimize fracture disease as muscle and joint impairment could be a possible consequence of prolonged healing [93]. However, despite the effectiveness and safety of this method for the treatment of non-infected atrophic non-unions, percutaneous autologous bone marrow grafting has the following limitations: where there are pre-existing angular deformities or prior shortening which require direct access to the non-union, undertaking this approach is not possible. In addition, as the volume of callus achievable using this method is limited, the allowed gap between and displacement of the fragments for this method to be feasible should be restricted [96]. As such, percutaneous autologous bone marrow grafting may not be possible with large bone deficits.

Cartilage

Articular cartilage is a tissue with virtually no intrinsic reparative capacity as the cells within cartilage, the chondrocytes, have low mitotic potential in vivo [22], are isolated in their lacunae, adapted to a low metabolic rate and obtain functional information only through mechanical loading and diffusible humoral factors. As such, upon injury, these cells may not be able to sense the problem, are unable to migrate out of their territory through the dense matrix to fill the defect and are unlikely to increase their metabolic rate to regenerate the tissue [98]. Furthermore, as cartilage lacks both vascularity and innervation, the healing process is slow [21]. Because of its hypocellular nature, vulnerability to injury and poor capacity to undergo self-repair, treatment of articular cartilage injury still presents a major therapeutic challenge.

Articular cartilage injuries that do not penetrate the subchondral bone do not heal and typically progress to degeneration of the articular surface. There exists a short-lived tissue response to cartilage injury but this response does not provide sufficient cells and matrix to repair even small defects [99-102]. Depending on the species, the age of the animal, and the location and size of the injury, injuries that penetrate the subchondral bone undergo repair through the formation of tissues usually characterized as fibrous, fibrocartilaginous or hyaline-like cartilaginous [103-106]. However, these tissues, even those that resemble hyaline cartilage histologically, are biochemically and biomechanically inferior to normal hyaline cartilage. By 6 months, fibrillation, fissuring and extensive degenerative changes appear in the reparative tissues of about half of full-thickness defects. Likewise, the degenerated cartilage seen in osteoarthritis does not usually undergo repair but progressively deteriorates [107,108].

Orthopaedic surgeons have employed various therapeutic strategies aimed at inducing fibrocartilaginous repair tissue to manage patients with severe and persistent pain caused by osteochondral injuries; these include debridement, drilling and fixation, abrasion chondroplasty, microfracture and the use of carbon fiber pads [109]. Repair with hyaline cartilage is the desired outcome of other approaches such as articular cartilage autografting and the use of osteochondral allografts [109]. The current gold standard for cell-based repair of articular cartilage is implantation of autologous chondrocytes in the site of cartilage damage; this technique is currently used clinically to treat small cartilage lesions such as those caused by traumatic injury [110]. In 1994, the first cell therapy referred to as autologous chondrocyte transplantation (ACT) was reported. Brittberg et al. described the use of autologous cultured chondrocytes in the treatment of full-thickness chondral defects

of the knee. Healthy cells harvested from an uninjured region of the knee during arthroscopy were cultured and expanded in vitro, following which the cultured chondrocytes were injected into the prepared defect and sealed with a periosteal flap sutured around it [111]. The aim of injecting expanded autologous chondrocytes is to achieve rapid production of a cartilaginous extracellular matrix to regenerate the destroyed cartilage [98]. Subsequently, other studies have followed this procedure and reported encouraging results [112-114]. However, limitations associated with this procedure include the prerequisite injury of healthy cartilage tissue in a preceding surgery which presents additional trauma to an already damaged joint cartilage, that newly synthesized cartilage often consists of fibrous instead of hyaline tissue, and the in vitro de-differentiation of cultured chondrocytes to a fibroblast-like phenotype leading to a loss of their chondrogenic potential [115].

In 1959, Pridie introduced an approach based on in situ regeneration and self-healing, the aim of which was to recruit bone marrow cells to cartilage defects by drilling small holes into the subchondral bone marrow space underlying regions of damaged cartilage [98]. This marrow-stimulating procedure was later refined by Steadman who reduced the size of the perforations [116]. This approach is now referred to as the 'microfracture technique' which is aimed at enhancing chondral resurfacing by providing a suitable environment for tissue regeneration and by taking advantage of the body's own reparative ability. In this technique, multiple perforations, or 'microfractures' are made into the subchondral bone plate, which allows the outflow of marrow elements. The resulting roughened surface in the subchondral bone facilitates blood clot adherence [117] which is thought to develop into a favorable microenvironment called a superclot capable of stimulating attraction, proliferation and chondrogenic differentiation of MSC coming from the bone marrow [98]. The arthroscopic microfracture technique is frequently used as a first-line option and commonly serves as the standard technique against which other cartilage repair approaches are compared even though no validated treatment algorithm exists for treating articular cartilage lesions in the knee [118]. Although this marrow-stimulating technique is an easy, simple, minimally invasive, low morbidity, single-stage procedure, and is cost-effective with few associated complications with a high capacity for creation of durable cartilage repair tissue, there are associated drawbacks including age-dependent results [98], limited hyaline cartilage repair tissue, variable repair cartilage volume and possible functional deterioration [118]. Furthermore, a possible limitation of this marrow-stimulating technique is the expected low incidence of MSC in bone marrow; an approximate of only 7-10 MSC per million mononucleated cells can be isolated from bone marrow aspirates [98]. Therefore, an initial clot of several milliliters filling a large defect would contain less than 100 MSC (cartilage tissue in a 5 cm² defect of 4 mm thickness contains about 10-20 million chondrocytes) [98]. A possible therapeutic solution to this problem could be to augment the initial number of repair cells via exogenously delivered MSC. Overall, this technique appears to provide excellent short-term functional improvement of knee function. However, decreasing knee function after initial improvement and increasing failure rate over time observed in some patients highlight the long-term clinical limitations of this technique, which calls for further long-term studies to investigate the efficacy of this cartilage repair approach [118].

It is essential to understand natural healing responses in vivo in order to discern the value of biological or biophysical treatments designed to augment that repair [119]. The importance of the bone marrow as a source of repair cells for cartilage injury is indirectly confirmed by the lack of effectiveness in partial-thickness defects where damage is limited to the cartilage and does not traverse the subchondral bone [119]. Shapiro et al. [119] used a rabbit model of full-thickness defects of the articular cartilage to study the origin and differentiation of cells in the repair of full-thickness defects of articular cartilage. They showed that the repair was mediated wholly by the proliferation and differentiation of mesenchymal cells of the marrow. Autoradiography with ³H-thymidine labeling showed that the cartilage defect

contained undifferentiated mesenchymal cells 7 days post-surgery, of which dividing ones took up the label; the label progressively appeared in fibroblasts, articular chondroblasts, and osteoblasts at later time-periods. This observation conclusively indicated that the reparative cells involved in the healing process are derived entirely from undifferentiated mesenchymal cells of the marrow. The observation that marrow mesenchymal cells served as precursors for the entire range of fibrous, hyaline cartilage, and osseous tissues that were synthesized in this study suggests the critical role and therapeutic potential of MSC in facilitating cartilage repair.

Marrow-stimulating procedures are directed at mobilizing endogenous MSC to the site of cartilage injury, capitalizing on the intrinsic healing abilities of the body. In the microfracture technique, it is believed that MSC are attracted to the lesion and initiate repair of cartilage by proliferating and differentiating, thereby remodeling the blood clot formed during the perforation procedure [115]. However, this approach might be limited by the inadequate numbers of MSC recruited which could impede appropriate chondrocyte development since high cell density and condensation are required for successful chondrogenic differentiation [115]. In a study aimed at investigating whether precursor mesenchymal cells from the marrow that are expanded in culture can serve as long-lasting precursors of bone and other connective tissues after intravenous infusion into irradiated mice, Pereira et al. [79] reported that progeny of the donor cells exogenously delivered were present in cartilage and accounted for 2.5% of the isolated chondrocytes from xiphoid and articular cartilage. To the best of our knowledge, apart from this study, there is very little evidence suggesting that MSC (from the circulation) are capable of homing in response to cartilage injury, and this could be due to the lack of vascularity in cartilage. One potential therapeutic strategy based on an understanding of the natural healing response to cartilage injury could be to augment the number of reparative cells using exogenously delivered MSC.

At present, there are reports that have documented the beneficial effects of transplanting autologous expanded MSC for the repair of cartilage defects. Wakitani et al. [23] were amongst the first to report good results after the transplantation of autologous bone-marrow-derived MSC in a type-I collagen gel carrier to treat full-thickness defects of the articular cartilage in an animal model. This investigation demonstrated that the implantation of cultured autologous osteochondral progenitor cells resulted in the differentiation of these mesenchymal cells into chondrocytes that were eventually replaced by bone up to the bone-articular cartilage junction and led to the formation of articular cartilage on subchondral bone which resurfaced the condyle. Wakitani et al. also initiated the procedure in humans, whereby the reported cases include the treatment of patellar cartilage defects [120] and of osteoarthritic knees undergoing proximal tibial osteotomy [24]; these studies also demonstrated the therapeutic potential of autologous culture-expanded bone marrow mesenchymal cell transplantation in the regeneration of articular cartilage defects. Other studies have also investigated the efficacy of expanded MSC transplantation to treat cartilage defects. In these reports, MSC were either injected directly into the defect [29], or seeded into cell delivery vehicles prior to implantation into the defect. Some of these carriers include synthetic extracellular matrix consisting of hyaluronic acid and gelatin [26], bioceramic scaffold- β -tricalcium phosphate [27], fibrin glue [121], polylactic acid scaffold [30], hyaluronic gel sponge [122] and collagen gel [22]. In another approach [25], the possibility of using direct intra-articular injection of MSC suspended in hyaluronic acid as a minimally invasive technique for cartilage repair has also been explored. On a side note, the direct implantation of undifferentiated MSC has been observed to present complications such as the calcification of implanted cells, fibrogenesis, and heterotopic tissue formation in cartilage [123]. Co-culturing human MSC with chondrocytes as a preconditioning system

for enhancing chondrogenic differentiation is currently being investigated as a potential therapeutic strategy to circumvent this problem [123,124].

Skeletal Muscle

Skeletal muscle degeneration can result due to direct injury such as crushing, cutting, puncturing or freezing, ischemia, direct application of local anesthetics, exhaustive exercises or neuromuscular diseases [125]. At present, treatment options are dependent on the intrinsic self-healing properties of the injured muscle [37]. Skeletal muscles are capable of extensive regeneration. According to Fowler [125], the regeneration sequence is as follows: damage to muscle or fiber, intrinsic degeneration of the muscle fibers whereby myofibrils fragment into individual sarcomeric units, proliferation of satellite cells which are precursor muscle cells located between the cell membrane of the fiber and the basal lamina of the muscle fibers, invasion of macrophages and neutrophils to remove debris of the degraded damaged fiber, regeneration of a new fiber within the persisting basal lamina whereby activated satellite cells proliferate and differentiate into myoblasts capable of producing muscle contractile proteins, fusion of myoblasts into multinucleated fibers and lastly, formation of mature muscle fibers. The growth of these regenerating myofibers promotes healing of the muscle. However, functional deficiencies and the development of fibrosis may occur as a result of insufficient regeneration of muscle tissue after traumatic injury; in this case, the regeneration is imperfect [35].

The regenerative capacity of skeletal muscle, as mentioned, is usually attributed to resident satellite cells, which, upon activation by local or distant stimuli, initiate a myogenic differentiation program [126]. However, in regenerating muscle, the number of myogenic precursors exceeds that of resident satellite cells, suggesting migration or recruitment of undifferentiated progenitors from other sources [127]. To investigate whether myogenic progenitors could be physiologically recruited from the bone marrow and access a site of muscle regeneration from the peripheral circulation, Ferrari et al. [127] transplanted genetically marked bone marrow-derived cells from the C57/*MlacZ* line (H-2^b) into 12 irradiated immunodeficient scid/bg mice (H-2^d). Following transplantation, muscle regeneration was induced in the muscle of interest (tibialis anterior) of nine surviving mice. In five out of six reconstituted animals analyzed after 2 and 3 weeks after induction of muscle injury (via injection of cardiotoxin which induces death of differentiated myofibers and subsequent muscle regeneration), regenerating fibers were found to contain β -Gal⁺. This study demonstrated the existence of bone marrow-derived myogenic progenitors that can migrate into the damaged muscle from the circulation; this recruitment is possibly, in response to inflammatory signals originating from the degenerating tissue. These cells were also observed to participate in the regenerative process and give rise to fully differentiated muscle fibers.

To test the hypothesis that additional myogenic precursors may be recruited from an undifferentiated, permanently renewed cell pool to supply the permanent demand of myogenic progenitors during the longstanding course of muscular dystrophies, Bittner et al. [128] transplanted bone marrow cells from congenic male donor mice into muscle-dystrophic female mice, using the Y chromosome as a cellular marker. It was observed that bone marrow-derived cells were recruited by dystrophic regenerating skeletal muscle and underwent muscle specific differentiation. In another study designed to confirm the biological relevance of the observed contribution to mature adult multinucleate skeletal myofibers in bone marrow recipients by bone marrow-derived cells, LaBarge et al. [129] presented evidence which clearly demonstrates that adult bone marrow-derived cells (BMDC) contribute to muscle tissue in a biological stepwise progression from adult bone marrow to muscle-specific stem cell to differentiated muscle fiber. In this report, bone

marrow-derived cells were observed to respond to two temporally distinct biological cues. First, BMDC were capable of occupying the muscle stem cell niche after irradiation-induced damage, which resulted in the ablation of endogenous satellite cells in this niche. Second, as a result of exercise-induced damage, BMDC satellite cells participated in the regeneration of multi-nucleate muscle fibers at a frequency significantly greater than any previous reports for any bone marrow to muscle conversion. This stress-induced progression of BMDC to muscle satellite cell to muscle fiber is suggestive that BMDC could potentially be a previously unrecognized reservoir of cells that possibly can contribute to a tissue-specific stem cell pool, therefore serving as an alternative or back-up source of reparative cells for damaged adult tissues.

To date, however, the exact phenotype of the bone marrow-derived cells capable of contributing to muscle regeneration has yet to be conclusively determined. Studies that used whole bone marrow involved the transplantation of a complex mixture of several lineages including mature haematopoietic cells, mesenchymal cells and endothelial cells [130]. Several in vitro and in vivo studies have been carried out to resolve this controversy. Although the exact mechanism through which donor marrow cells contribute to myogenesis is unclear, on the basis that cell fusion occurs during normal muscle myogenesis, Shi et al. performed an in vitro experiment to test the capability of different human marrow cells to fuse with myotubes [131]. In this study, various subsets of human marrow cells were cocultured with differentiating mouse C2C12 myoblasts to investigate the ability of these human cells to contribute to myogenesis. After testing 4 populations of human cells in the haematopoietic lineage, it was found that these cells were not able to fuse with C2C12 and contribute to myogenesis in vitro. On the contrary, marrow-derived cells in MSC culture appeared to fuse with relatively high efficiency to myoblasts in vitro. A further investigation involving the direct injection of GRP-labeled MSC into injured mouse tibialis anterior muscle (via application of cardiotoxin) showed regenerating muscle fibers expressing GFP, indicating the positive contribution of human MSC to muscle regeneration. However, as the frequency of fusion was low, it was unclear whether the detection of donor-derived cells was attributed to fusion or transdifferentiation. Correspondingly, Sherwood et al. [132] reported that the only subset of donor-derived cells identified within the satellite cell niche with myogenic potential upon co-culture with differentiating myoblasts do not express the pan-haematopoietic marker, CD45. Furthermore, it was observed that this population is only generated following whole bone marrow transplantation and not after transplantation of highly purified haematopoietic stem cells, thus implying a mesenchymal rather than haematopoietic origin.

In another in vitro study [126], it was established that MSC differentiation to myogenic lineage is possible under the influence of factor(s) released by an injured muscle. Bone marrow-derived rat MSC were isolated, expanded and cultured in conditioned media obtained from rat tibialis anterior muscle damaged in vivo by the injection of barium chloride solution. It was found that there was a time-dependent morphological change of these cells from fibroblast-like into elongated multinucleated cells, a transient increase in the number of MyoD positive cells and the subsequent onset of myogenin, α -actinin and myosin heavy chain expression. It appears that local signals released after muscle damage are capable of promoting the myogenic commitment and differentiation of MSC.

A number of studies have sought to establish the therapeutic effect of locally transplanted MSC in traumatic muscle injury. In a particular study by Matziolis et al. [36], to examine whether regular muscle regeneration can be improved via the local application of autologous bone marrow-derived MSC in a rat model of blunt skeletal muscle trauma, cells were injected into the traumatized muscle of the animal following a standardized open blunt crush injury to the left soleus muscle. It was found that autologous MSC grafting significantly

restored contractile forces (increase of 14% in tetanic and 13% in fast twitch stimulation), demonstrating its potential to improve functional outcome after skeletal muscle crush injury. The effectiveness of MSC transplantation after severe skeletal muscle trauma has been confirmed by a similar study conducted by the same group [35] which reported that a correlation exists between the number of injected MSC and the resulting improvement of muscle contraction force. In another study by Natsu et al. [133], using half-stratum laceration on the tibialis anterior muscle of Sprague-Dawley rats as a skeletal muscle injury model, it was observed that transplanting bone marrow-derived MSC promoted maturation of myofibers and enabled the injured muscle to functionally acquire almost normal muscle power by 1 month after transplantation. Besides traumatic muscle injury, the beneficial effect of using human MSC in animal models of Duchenne muscular dystrophy has also been reported, using synovial membrane-derived [134] as well as adult adipose-derived MSC [135].

Ramirez et al. [136] were the first to report evidence of mobilization of MSC into the bloodstream following skeletal muscle damage. Such damage was not linked to damage in other tissues (especially the myocardium) in which MSC are thought to play a regenerative role. In this study, the number of circulating MSC in two models of skeletal muscle injury, acute and chronic, was compared with healthy controls. To model acute skeletal muscle injury, eleven healthy volunteers were studied before and two hours after running a long distance (21km) race, which involves eccentric muscle contractions in large skeletal muscle mass leading to an acute, reversible stage of rhabdomyolysis. Eleven patients diagnosed with McArdle's disease were selected for the study of MSC mobilization in the model of chronic skeletal muscle injury. Controls were healthy volunteers with no basal skeletal muscle damage. Serum creatine kinase activity, a well-established marker of muscle injury was measured in the peripheral blood samples of all subjects to quantify the degree of skeletal muscle damage. Flow cytometry analysis was used to determine the absolute counts of circulating MSC present in the peripheral blood samples. Patients afflicted with McArdle's disease had significantly more circulating MSC than healthy controls under basal conditions while the number of circulating MSC increased above basal levels in the peripheral blood samples obtained from healthy athletes two hours post-race. It was found that there exists a significant, positive correlation between the number of circulating MSC and creatine muscle damage in all samples, demonstrating that MSC are capable of being mobilized into the bloodstream in response to both acute and chronic muscle injury.

Taken together, these findings suggest a role for MSC in skeletal muscle regeneration. However, there is a possibility that another cell type, not MSC, which has a similar capacity to contribute to myogenesis, may exist in the bone marrow. Corbel et al. [137] showed that progeny of the classically defined single haematopoietic stem cell are capable of reconstituting both the haematopoietic system as well as contribute to muscle regeneration. Integration of these cells occurs at low frequency and is observed to increase with muscle damage. This study, however, does not exclude the possibility that a non-hematopoietic cell type, which has a similar capacity to participate in myogenesis, exists in the bone marrow. In another report, besides demonstrating that progeny of the single haematopoietic stem cell is capable of contributing to skeletal muscle, by transplanting single CD45⁺ haematopoietic stem cells, Camargo et al. [138] asserted that the entire circulating myogenic activity in bone marrow is derived from these cells and their progeny, and that the haematopoietic cells contributing to the repair of skeletal muscle are myelomonocytic in origin.

Findings thus far are conflicting; it remains a possibility that *both* haematopoietic and mesenchymal stem cells of the bone marrow possess myogenic potential and contribute to muscle regeneration. Burdzinska et al. [139] suggested a relationship between bone-marrow derived stem cells, multipotent muscle stem cells and satellite cells. In this proposed model,

both MSC and HSC are released from the bone marrow to peripheral blood in response to certain stimuli such as exercise or skeletal muscle injury. These cells then migrate according to gradients of chemokines or growth factors and home into skeletal muscles. Initially, these cells are located in interstitial tissue near blood vessels and are classified as multipotent muscle stem cells. Their phenotype gradually changes as they enter the myogenic pathway. Overtime, these multipotent muscle stem cells occupy the satellite cell niche and eventually become muscle precursor cells that express Pax 7. Perhaps, in order to account for these incongruent results from various studies, the existence of multiple sources of a circulating myogenic progenitor should indeed be considered [130].

Concentrating Bone Marrow - Derived MSC for Therapy

Evidence presented thus far supports the hypothesis of MSC mobilization, homing and recruitment, triggered by musculoskeletal injury - a phenomenon that is likely a part of the natural reparative response. It is postulated that stimulatory factors are released by the remote injured tissue which result in mobilization of MSC from their storage niche in the bone marrow into the circulation. These circulating MSC home to the site of injury; certain molecules presented on the endothelium leads to recruitment of MSC, where they transmigrate from blood vessels, proliferate in situ, and through differentiation and/or paracrine action, integrate into the injured tissue to effect the reparative response [5]. Transplanting autologous MSC to bypass the recruitment process and augment the local MSC population is one potential therapeutic strategy for use in musculoskeletal applications. Studies that have sought this cell-based therapy have been discussed.

With regard to the use of bone marrow aspirate as a source of MSC, one major problem faced is that it is often impracticable to acquire enough bone marrow with the required number of osteoprogenitor cells despite the successes that have been achieved using fresh marrow transfer [96]. The influence of cell-concentration on the osteogenic capacity of bone marrow grafts was first highlighted by Connolly et al. [140] who provided evidence for a positive correlation between the osteogenic capacity of bone-marrow and cell-concentration and proposed the development of an osteogenic bone marrow preparation. The observation that it is important to increase the number of progenitors in the graft after aspiration was later confirmed by a study conducted by Hernigou et al. [141]. In this study (Figure 2), the potential of percutaneous injection of an autologous MSC-enriched bone aspirate was affirmed with fifty-three out of sixty tibial non-unions successfully treated (Figure 3). In addition, it was observed that for the patients who did not achieve union, all had received a marrow graft with <1000 progenitors/cm³ (this mean concentration being significantly lower than the concentrations that were received by patients for whom the treatment did not fail). It appears that a graft needs to contain >1000 progenitors/cm³.

Consequently, Hernigou et al. suggested that the efficacy of percutaneous autologous bone-marrow grafting seems to be associated with the number of progenitors in the graft and the number of progenitors available in aspirated bone marrow from the iliac crest appears to be less than optimal without the concentration step because aspirated bone marrow (not concentrated) contains only a mean of about 600 progenitors/cm³. That the use of autologous MSC-enriched bone marrow aspirate is potentially more efficacious than the use of bone marrow aspirate (without concentration) was recently questioned by Cuomo et al. [86] who compared the osteogenic potential of human bone marrow aspirate with that of human MSC-enriched bone marrow aspirate in a critical-sized athymic rat defect. In this study, it was observed that there was a lack of an effect of MSC-enrichment on bone defect healing, bone volume and bone density. However, there are several explanations for the contradictory findings, some of which were addressed by the authors. Possible reasons include variability in the MSC sourced from the individual bone marrow donors, the use of

athymic rats which are immuno-compromised and therefore may have altered bone-healing properties that do not accurately reflect bone-healing in humans, and the suitability of the environment provided by the demineralized bone matrix carrier in supporting the growth of MSC injected. From these observations made, the authors highlighted that because of the potential pitfalls associated with the adapted clinical use of MSC, surgeons should therefore adhere to previous protocols that have been proven effective when using bone marrow aspirate, whether concentrated or not.

Methods of increasing the number of progenitor cells transplanted in the autologous graft include limiting the volume of bone marrow aspirated per site to 2ml or less [96] so as to avoid dilution with peripheral blood, as well as employing concentration techniques. Reported methods of concentrating bone marrow aspirates for osteogenic repair include the use of a conventional manual blood bag technique [142], the use of selected implantable matrices for selective cell attachment to rapidly concentrate osteoblastic progenitors from bone marrow aspirates [143] and running a volume reduction protocol on a closed system centrifuge to concentrate the MSC within the aspirated bone marrow [90]. Of note, Muschler et al. [143] showed that the efficacy of a bone marrow graft can be significantly improved using the surface of some porous implantable material (such as allograft bone) to rapidly concentrate marrow-derived connective tissue progenitors from bone marrow aspirates via selective cell attachment [89]. The main advantage of this method of preparing a composite bone graft containing an enriched population of connective tissue progenitor cells is that it can be performed intraoperatively (at the same time bone marrow is being aspirated from the patient). This minimizes the number of invasive procedures and exposure to anaesthesia that the patient would otherwise have to undergo [144]. Research aimed at improving the surface properties of the bone graft substitute material used to effect selective attachment of connective tissue progenitors and limit the concentration of non-osteogenic cells within the graft (which may hinder the survival and function of connective tissue progenitors by competing for nutrients and growth factors within the graft following implantation) is already underway [145].

Problems associated with the use of bone marrow as a source of therapeutic MSC could possibly be circumvented by exploring the use of human adipose-derived stromal cells isolated from the stromal vascular fraction of human lipoaspirate as a potential alternative cell source [146]. These cells have garnered research interest because they are a relatively abundant and easily accessible source of pluripotent cells [147]. Comparing MSC isolated from bone marrow and adipose tissue, Lee et al. reported that these cells have the same morphology, phenotype, and in vitro differentiation ability, and highly similar gene expression profiles [148]. Beyond in vitro studies, recent studies have demonstrated that adipose-derived mesenchymal cells have the capability to heal skeletal defects in both mouse and human, suggesting that these cells have a similar osteogenic ability with bone marrow – derived MSC [147]. Clinically, a German case study in 2004 reported the use of autologous adipose-derived stem cells and fibrin glue to treat calvarial defects in a seven-year-old child, where the post-operative course was uneventful and near complete calvarial continuity was achieved three months post-reconstruction [149]. With regard to the use of adipose-derived mesenchymal cells in cartilage regeneration, the chondrogenic capacity of these cells is still in the early stages of evaluation [147]. In an in vivo study, Nathan et al. investigated the use of adipose tissue as a stem cell source to repair osteochondral defects in the rabbit femoral condyle and obtained encouraging results where the repaired tissue approximated intact cartilage and was histologically and biomechanically superior to periosteal-derived or native repair mechanisms [150].

Conclusion

Injury is believed to trigger the mobilization of MSC into the circulation, and these cells contribute to healing by homing to damaged tissues in a mechanism yet elucidated. These MSC undergo differentiation and/or are involved in cytokine production upon arrival at the target tissue [18]. Such a response is thought to be an inherent one that can potentially be augmented by enhancing the endogenous MSC pool with exogenously administered MSC. Studies have demonstrated that the transplantation of MSC, either systemically or locally, have beneficial reparative effects on injured tissues. This suggests that normal tissue repair may indeed be limited by the endogenous population of these cells in local tissues. Thus, the study of MSC trafficking would enable novel therapeutic options to be designed, with the aim of compensating for a deficiency in the number or function of MSC, as may occur in regions of previous trauma, infection, irradiation, tissue defects, scar or compromised vascularity [89].

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Figure 1.

Figure obtained from Granero-Moltó et al. (2009) with permission. MSC migrate to the fracture site in a time- and CXCR4-dependent manner. BLI was performed at days 1, 3, 7 and 14 after fracture/transplantation in mice with tibia fracture that received a transplant of either 10^6 MSC- β -Act-Luc (MSC) (left panel), MSC- β -Act-Luc-CXCR4+ (CXCR4(+)) (middle panel), or MSC- β -Act-Luc-CXCR4-(CXCR4(-)) (right panel). Graded color bar indicates BLI signal intensity expressed as photons/seconds/cm²/steradian (sr).

Figure 2.

Figure obtained from Hernigou et al. (2005) with permission. Marrow aspirates drawn from the patient were pooled and concentrated using the centrifugation method. The resulting concentrated buffy coat was reinjected intraosseously. Figure shows a trocar used for the reinjection which was positioned both in the nonunion gap and around the bone ends with the help of an image intensifier. The marrow was injected slowly at a rate of 20ml/min.

Figure 3.

Figure obtained from Hernigou et al. (2005) with permission. Figure shows anteroposterior radiographs of a twenty-five-year-old patient who had sustained a type-I open fracture. The radiographs were made at the time of fracture (a) at the time of nonunion, before injection of autologous bone marrow (b) at one month after bone marrow injection, at which time the patient was allowed to begin partial weight-bearing (c) at two months after bone marrow injection (d) and at three months after bone marrow injection, at which time the external fixation was removed (e).

Table 1

Selected examples of stem cell homing to musculoskeletal injuries

Article	Type of Injury, Animal Model	Major Finding	Reference
BONE			
Circulating cells with osteogenic potential are physiologically mobilized into the fracture healing site in the parabiotic mice model	Transverse fibular fracture, mouse	Using the parabiotic murine model, GFP ⁺ cells from the donor mouse were observed to home through peripheral blood and contribute to the formation of fracture callus. In the fracture callus, these circulation-derived cells expressed alkaline phosphatase at a frequency equal to other cells. Results suggest that osteogenic connective tissue progenitors are present in the systemic circulation and are capable of being physiologically mobilized to contribute to fracture callus formation and fracture repair.	81
Stem cell-mediated accelerated bone healing observed with in vivo molecular and small animal imaging technologies in a model of skeletal injury	Femoral defect, mouse	Intravenously injected primary adipose-derived multipotent cells (ADMCs) were shown to traffic from the lungs then to the injury site. Accelerated bone healing was observed within a week of the injury in the injected group as compared to the controls. Results demonstrated the ability of ADMCs to home to a musculoskeletal injury and promote healing of the injured bone.	18
Regenerative effects of transplanted mesenchymal stem cells in fracture healing	Tibial fracture, mouse	Bone marrow-derived MSC were intravenously injected and found to migrate to the fracture site in a time- and dose-dependent manner. MSC transplantation was also associated with an improvement in the biomechanical properties of the fracture callus. Results demonstrated the dynamic migration of transplanted MSC to the fracture site and their regenerative contributions to the healing process.	13
CARTILAGE *			
Cell origin and differentiation in the repair of full-thickness defects of articular cartilage	Full-thickness articular cartilage defect, rabbit	Based on full-thickness defects extending into the marrow cavity, the origin and differentiation of cells in the repair of articular cartilage was studied. Results showed that the repair was mediated wholly by the proliferation and differentiation of mesenchymal cells of the marrow.	119
SKELETAL MUSCLE			
Recruitment of bone marrow-derived cells by skeletal and cardiac muscles in adult dystrophic <i>mdx</i> mice	Muscular dystrophy, mouse	Bone marrow cells from congenic male donor mice were transplanted into muscle-dystrophic female mice, with Y chromosome as cellular marker. Bone marrow-derived cells were recruited by dystrophic regenerating skeletal muscle and underwent muscle specific differentiation. Results suggest that skeletal muscles are capable of regeneration by recruitment of circulating bone marrow-derived myogenic progenitors. However, identity of the bone marrow-derived progenitor cells is unknown.	128
Biological progression from adult bone marrow to mononucleate muscle stem cell to multinucleate muscle fiber in response to injury	Irradiation-induced and exercise-induced muscle damage, mouse	Following irradiation-induced damage, transplanted GFP-labeled bone marrow-derived cells became satellite cells. After a subsequent exercise-induced damage, GFP-labeled multinucleate myofibers were detected. Results suggest that two temporally distinct injury-related signals first induce bone marrow-derived cells to occupy the muscle stem cell niche, and then assist in regenerating mature muscle fibers. Identity of the bone marrow-derived cells is unknown.	129

Article	Type of Injury, Animal Model	Major Finding	Reference
Mobilisation of mesenchymal cells into blood in response to skeletal muscle injury	Exercise-induced muscle damage and McArdle's disease, human	Number of circulating MSC in two models of skeletal muscle injury – acute and chronic – were determined and compared with those of healthy controls. Circulating MSC were found to be significantly higher in both models than in controls. Results suggest that MSC are mobilized into the bloodstream following skeletal muscle tissue damage.	136

* To the best of our knowledge, studies on MSC homing to cartilage injuries are few, if not none. Example given highlights a marrow-stimulating procedure aimed at mobilizing marrow cells to the site of cartilage injury.