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Stem cell recruitment after injury: lessons for regenerative medicine

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Abstract

Tissue repair and regeneration are thought to involve resident cell proliferation as well as the selective recruitment of circulating stem and progenitor cell populations through complex signaling cascades. Many of these recruited cells originate from the bone marrow, and specific subpopulations of bone marrow cells have been isolated and used to augment adult tissue regeneration in preclinical models. Clinical studies of cell-based therapies have reported mixed results, however, and a variety of approaches to enhance the regenerative capacity of stem cell therapies are being developed based on emerging insights into the mechanisms of progenitor cell biology and recruitment following injury. This article discusses the function and mechanisms of recruitment of important bone marrow-derived stem and progenitor cell populations following injury, as well as the emerging therapeutic applications targeting these cells.

Keywords

endothelial progenitor cell; hematopoietic stem cell; mesenchymal stem cell; stem cell recruitment; tissue regeneration; very small embryonic-like cell

Tissue repair and regeneration following injury demand the precise orchestration of complex signaling cascades to coordinate growth of spatially proximate, but physiologically distinct structures. While this process is facilitated in many cases by proliferation, migration and differentiation of local progenitor cells, the selective recruitment of bone marrow-derived stem and progenitor cells (herein referred to as bone marrow stem cells) is also thought to play a role.

The bone marrow acts as a reservoir for multiple stem cell populations, including hematopoietic stem cells (HSCs), mesenchymal stem cells (MSCs), endothelial progenitor cells (EPCs) and very small embryonic-like cells (VSELs), which are mobilized at varying

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degrees into the peripheral circulation following injury [1–3]. Subsets of these cells have also demonstrated the ability to home from the circulation to a variety of experimentally injured tissues, including muscle, heart, kidney, skin, bone, liver and brain [1,3–10], where they are thought to variably contribute to tissue repair and regeneration through paracrine effects and inconsistent levels of direct differentiation [1,3,11,12].

Despite this endogenous stem cell recruitment, the inability of most adult tissue to regenerate following injury suggests that these mechanisms are easily overwhelmed. Therapies attempting to augment bone marrow stem cell involvement following insult have therefore been developed, and have shown the ability to mitigate injury and enhance the regenerative capacity of adult tissue in a variety of preclinical models [8,13–20]. Effective clinical translation of these techniques, however, has thus far lagged behind [21–23]. Poor cellular retention within the harsh injury environment, as well as the use of incompletely defined or heterogeneous cellular populations are potential limiting factors to the clinical success of stem cell therapies [21,24], which has led to ongoing studies attempting to better understand the underlying biology of stem cell recruitment, as well as to identify methods to augment stem cell survival, signaling and function.

This article discusses the role of four of the most studied bone marrow-derived stem cell populations, HSCs, MSCs, EPCs and VSELs, in endogenous and experimental tissue repair and regeneration (Figure 1). We will define these populations, explore their molecular mechanisms of mobilization and homing, identify their role within the injury microenvironement and discuss experimental methodologies to enhance their number, function and therapeutic potential.

Hematopoietic stem cells

HSCs are self-renewing, multipotent bone marrow cells that are responsible for replenishing all cellular components of the blood, including leukocytes, erythrocytes and platelets. HSCs are relatively rare, comprising approximately 0.01–0.15% of nucleated bone marrow cells [1,25], and can be further characterized based on their capacity for sustained bone marrow reconstitution (long- versus short-term HSCs). HSCs are typically isolated based on surface antigen expression, and although these profiles are constantly evolving, commonly used definitions include lack of lineage-specific markers and positivity for CD45, c-kit and/or Sca-1 (murine), or CD34 and CD133 (human) [4,26]. Combinations of cell surface receptors from the SLAM family, including CD150, CD244 and CD48, have also been used for simplified murine HSC isolation and identification within tissue sections [27], but are not equally expressed in humans [28].

Clinically, HSCs have been shown to mobilize from the bone marrow into the circulation following a variety of injuries, including myocardial infarction [29], stroke [30], liver injury [31] and skin burns [32], although their contribution to tissue repair and regeneration is uncertain. It was initially thought, based on early preclinical studies, that HSCs could help repopulate injured tissue through direct differentiation [33,34]; however, the strongest current evidence for HSC plasticity is limited to rare differentiation events within the mesodermal lineage [4]. In fact, work from our laboratory showed that HSC recruitment and engraftment within murine ischemic tissue was minor compared to changes in bone marrow-derived MSCs (BM-MSCs) [1], casting doubt on the importance of endogenous HSCs within the wound environment.

Nonetheless, delivery of exogenous HSCs may still be therapeutic, as both systemic and local injection of HSCs has been shown to ameliorate experimentally induced injuries via hematopoietic lineage (myeloid) restricted differentiation and cytokine effects [12,35].

Endothelial progenitor cells

EPCs are rare circulating cells that have the ability to incorporate into foci of neovascularization. The mechanistic contribution of these cells to *de novo* postnatal neovascular formation is termed vasculogenesis, and represents a paradigm shift in adult vascular biology, as neovascularization was previously thought to occur through a strictly angiogenic mechanism, (whereby pre-existing endothelial cells undergo *in situ* proliferation and migration to form new blood vessels) [36]. First described in 1997[37], the definition of EPCs has evolved alongside new discoveries of their lineage, resulting in two proposed subpopulations (hematopoietic and non-hematopoietic EPCs) with distinct surface marker and functional characteristics [36].

Hematopoietic EPCs (including the alternatively described early EPC and circulating angiogenic cell populations) [38,39] may represent a vasculogenic subpopulation of bone marrow-derived HSCs [36]. While a unifying cell surface antigen profile does not exist, these cells are often described as CD34 (human) or c-kit/Sca-1 (mouse) positive, with co-expression of endothelial cell markers (CD31, vWF, VEGFR2), hematopoietic lineage markers (CD45) and inconsistent expression of monocyte markers (CD14 and CD163) [39–42]. Hematopoietic EPCs secrete high levels of cytokines, including VEGF, IL-8, HGF and G-CSF, and are thought to contribute to vascular repair mainly through paracrine mechanisms [39,41], but subsets of these cells have shown the ability to directly incorporate into the endothelium [43,44].

By contrast, non-hematopoietic EPCs (including late outgrowth cells and outgrowth endothelial cells, or EOCs) do not express CD45 or monocyte markers, and show a surface marker profile more closely resembling mature endothelial cells [39–41]. Non-hematopoetic EPCs exhibit low levels of cytokine production and are thought to contribute to vascular repair mainly through the direct formation of vessels [41]. The origin of non-hematopoetic EPCs remains unclear, but it is speculated that they derive from organ blood vessels or non-hematopoietic bone marrow cells [36].

While subpopulation delineations are often not made, it is assumed that EPCs are mobilized in response to ischemic injury [29,45], and contribute to neovascularization in small animal models through a combination of direct cellular differentiation and indirect production of cytokines and growth factors (VEGF, SDF-1, and IGF-1) to promote the migration of mature endothelial cells and resident progenitor cells [3,46]. The critical role of EPCs is suggested by their dysfunction and reduced levels in clinical disease states associated with poor wound healing, such as diabetes [47,48], and the observation that EPC transplantation can ameliorate injury and improve functional outcomes in models of stroke [13], myocardial infarction [14] and acute liver and lung injury [15,16].

Mesenchymal stem cells

MSCs are multipotent, non-hematopoietic stromal cells that can be isolated from various adult organs and tissues, including bone marrow [49], adipose tissue [50], peripheral blood [51], lung [52], brain [52] and skeletal muscle [53]. MSCs are thought to reside in a perivascular niche *in vivo* [52,54], and are capable of differentiating into various mesenchymal lineages *in vitro*, including bone, muscle, cartilage and fat [49], as well as forming cells from other germ layers, such as keratinocytes and neuron-like cells [55,56]. While there is no universally accepted definition, and surface antigen expression can vary by source tissue, a list of potential criteria for human BM-MSCs includes: plastic adherence under standard culture conditions; positive expression of CD105, CD73 and CD90, with absence of lineage-specific markers and CD34; and *in vitro* differentiation capacity to form osteoblasts, adipocytes and chondroblasts [57]. Murine BM-MSCs share these functional

characteristics, but are often isolated based on positive expression of Sca-1 and/or PDGFRα, with negative expression of hematopoietic or mature cellular markers [1,58].

BM-MSCs comprise approximately 0.001–0.08% of cells within the bone marrow [1,49], and have been shown to mobilize to the peripheral circulation following experimental injury [1,11]. Mobilized BM-MSCs home to sites of injury [1,11], where they are thought to contribute to tissue repair and regeneration mainly through paracrine support of injured cells (HGF, EGF, VEGF, sFRP-4) [59,60] and regulation of extracellular matrix remodeling [59,61,62], immune response (IL-1 antagonism, IL-10) [63,64] and local progenitor cell proliferation and differentiation [65]. Like EPCs, BM-MSCs are also thought to contribute to the restoration of vascular integrity and neovascularization following injury, as seen by their incorporation into almost 25% of new blood vessel endothelium in ischemic murine skin [1], as well as their ability to upregulate expression of pro-angiogenic factors, such as FGF, in response to environmental cues [66]. BM-MSCs have also been reported to undergo direct cellular differentiation and/or fusion to form a variety of other cell types following *in vivo* experimental injury, including myocardiocytes [67], kidney mesangial cells [68], osteoblasts [7], skeletal muscle cells [69] and neuron-like cells [5]; however, these events are rare, and likely less important than the aforementioned mechanisms of action.

The likely multifactorial role of BM-MSCs within the injury environment makes them especially appealing for cell-based therapies, as illustrated by their ability to support neovascularization, increase efficiency of cardiomyocyte mitochondrial oxidative phosphorylation and improve overall cardiac function in models of cardiac ischemia [67,70]. Further highlighting their therapeutic potential, transplantation of BM-MSCs has been shown to ameliorate experimental injury in almost all major organs, including the brain [17], liver [8], kidney [6] and lungs [19], and can even promote immune tolerance in tissue transplant models via cytokine activation of Tregs [71,72]. Given these diverse beneficial effects in preclinical models, an explosion of clinical trials involving BM-MSCs is currently underway to further evaluate these cells.

Very small embryonic-like cells

VSELs are a population of developmentally primitive pluripotent stem cells found in bone marrow and other adult organs [73–75]. These cells share several features typical for embryonic stem cells, including small size, a large nucleus surrounded by a narrow cytoplasmic rim, open-type chromatin and the ability to differentiate into all three germ layers [73]. VSELs comprise approximately 0.006% of all murine bone marrow cells [74], and are typically identified as being lineage- and CD45-negative, and CXCR4, Sca-1 (mouse), CD133 (human) and CD34 (human) positive [74,75]. Additionally, VSELs exhibit positive expression of pluripotency (Oct-4, SSEA-1) [73] and epiblast/germ line stem cell markers [76].

VSELs are hypothesized to be deposited in developing tissues and organs during early gastrulation, and play a role in the repopulation of more tissue specific stem cells under homeostatic conditions [77]. VSELs are also likely involved in tissue regeneration following injury, as they are mobilized into the peripheral circulation following both experimental insult and clinical cases of cardiac ischemia and stroke [2,30,78], and can improve cardiac function when delivered locally following induced myocardial infarction [20]. While a small proportion of VSELs may undergo direct cellular differentiation within the injury environment [20], their low long-term engraftment rate indicates the main beneficial effect of these cells is more likely due to paracrine mechanisms.

Mechanisms of bone marrow stem cell recruitment following injury

A complex signaling network likely underlies the selective recruitment of the aforementioned bone marrow stem cell populations following injury, which is best described for HSCs [79], but may be similar in other cell types [80,81]. Important steps in this process include cellular mobilization from the bone marrow into the circulation, homing to the injury site, vascular rolling and adhesion, endothelial transmigration and, finally, movement within the extracellular space to the injury site. Interactions of the cytokine SDF-1 with its receptor (CXCR-4) on bone marrow cells is one of the more well-described mechanisms underlying cellular mobilization and homing [82,83]; however, a variety of other molecules have been shown to affect each step of the recruitment process [12,84–87].

Cellular mobilization & homing

Under physiologic conditions, bone marrow stem cells are thought to be maintained within their niche through tightly controlled interactions of chemokines, cytokines and growth factors with cellular receptors, as well as through the presence of specific adhesion and extracellular matrix molecules [80,88]. Following injury, there is evidence that cytokine release by vascular endothelium and activated platelets, combined with local upregulation of growth factors, alters this homeostasis by providing a signal gradient for bone marrow stem cell mobilization and homing [89–91]. SDF-1 and other molecules implicated in this process are discussed below.

SDF-1—The cytokine SDF-1 is thought to play an important role in stem cell maintenance within the bone marrow, as well as cell mobilization and release following injury. SDF-1 is regulated in part by the transcription factor HIF-1α [89], and during homeostasis, SDF-1 is upregulated within discrete regions of hypoxia in the bone marrow, promoting stem cell tropism through interactions with its cellular receptor CXCR4 [83], and likely downstream modulation of adhesion molecule expression, cell proliferation and cell survival [92–94]. Following insult, SDF-1 is released by hypoxic endothelium and activated platelets at the injury site, creating a chemokine gradient that is thought to promote CXCR4-mediated bone marrow stem cell mobilization and recruitment [83,89,90]. Demonstrating the importance of this pathway, antibody blockade of SDF-1 in ischemic tissue, or CXCR4 on circulating cells, severely limits EPC recruitment to sites of experimental injury [83], and augmentation of SDF-1 expression in ischemic tissue models enhances HSC and EPC recruitment [84,95]. While the SDF-1/CXCR4 pathway is best described for HSCs and EPCs, it is also likely involved in the mobilization and recruitment BM-MSCs and VSELs, as both of these populations express CXCR4 [73,94].

Despite its demonstrated importance, the exact mechanism by which SDF-1 causes both tropism and mobilization of bone marrow stem cells is incompletely understood. There is evidence, however, that circulating SDF-1, as seen following injury, promotes cell mobilization from the bone marrow through CXCR4 receptor desensitization [83], as well as stromal cell upregulation of the protease MMP-9 [96]. Following cell mobilization, the increased binding capacity of immobilized SDF-1 found on or around ischemic blood vessels may then overcome CXCR4 desensitization to promote tissue specific adhesion and localization [83,97].

Nitric oxide—Nitric oxide (NO) is a gaseous signaling molecule that plays an important role in homeostatic vascular health. Interestingly, NO may also be involved in SDF-1/CXCR4-mediated bone marrow stem cell recruitment following injury, as endothelial nitric oxide synthase (eNOS) has been shown to increase SDF-1 expression through a cGMP-dependent mechanism in ischemic murine tissue [98], and experimental blockage of eNOS

inhibits SDF-1-mediated EPC homing [84]. Additionally, eNOS has been shown to play a crucial role in progenitor cell adhesion to the vascular endothelium through an ICAM-1- and CXCR4-dependent mechanism [99].

Jagged/Notch interactions—The Notch signaling pathway plays an integral role in embryonic development, but is also active in many adult processes, including regulation of stem cell self-renewal, expansion, survival and differentiation [100–102]. Notch1 interactions with its ligand Jagged have also demonstrated importance for murine BM-MSC and EPC recruitment and therapeutic effect following ischemic injury [85,86], with knockout models having particularly deleterious effects on neovascularization. While incompletely understood, the mechanism of this effect is likely due in part to modulation of CXCR4, as Notch knockout decreases CXCR4 expression in murine BM-MSCs [86], and Notch-mediated upregulation of CXCR4 has been reported in other bone marrow-derived cells [103].

MCP-1/CCR2 interactions—MCP-1 is a chemokine that is best know for its ability to recruit monocytes following injury. However, there is also evidence that MCP-1 contributes to bone marrow stem cell recruitment, as MCP-1 binding to its receptor CCR2 is required for efficient BM-MSC homing and engraftment in a murine model of cardiac ischemia [87], and CCR2 expression is important for mobilized murine HSC trafficking to sites of inflammation [12]. This pathway is thought to act in part by stimulating chemotaxis through promotion of asymmetric lamellipodia protrusions [87], but may not be as ubiquitous as the SDF-1/CXCR4 axis, since CCR2 expression was found to be low in human EPCs [104].

Growth factors—Growth factors, such as VEGF and G-CSF, may also contribute to bone marrow stem cell mobilization and recruitment following injury, as exogenous administration of G-CSF and VEGF has been shown to enhance the mobilization of specific stem cell populations, and promote neovascularization and tissue regeneration within ischemic or traumatic injury models [105–108]. Mechanistically, G-CSF administration has been shown to promote murine HSC and EPC mobilization by reducing SDF-1 expression in the bone marrow, as well as CXCR4 expression on HSCs [106,109]. VEGF, meanwhile, has been shown to cause divergent effects on murine bone marrow populations based on receptor profiles, inhibiting HSC mobilization through VEGF receptor 1 (VEGFR1), while stimulating EPC migration and survival through VEGFR2 [106]. Further supporting an endogenous cell recruitment role for these factors, VEGF and G-CSF are upregulated following specific types of human ischemic injuries [91,110], and VEGF is known to play a crucial role in HIF-1α-induced murine adult neovascularization [111].

Cellular adhesion, endothelial transmigration & extracellular migration

Once mobilized and homed to an area of injury, a variety of molecules have been implicated in stem cell vascular rolling and adhesion, endothelial transmigration and movement within the extracellular space. These include selectins (P-selectin, E-selectin) for cell rolling [112,113], protein/integrin interactions (VCAM-1/VLA-4, ICAM-1/β2 integrin) for adhesion [112–114], chemokines (CXCL9, CXCL16, CCL20, CCL25) for transendothelial migration [115] and matrix degrading enzymes/inhibitors (MMP-2, MMP-9, tissue inhibitor of metalloproteinase-2) for cellular migration within injured tissue [116,117]. Working together, it is thought that the coordinated expression of this complex molecular network enables bone marrow stem cells to mobilize and congregate at the original site of injury, facilitating the cell-specific cytokine and direct contributions previously described.

Strategies for enhancing stem & progenitor cell involvement following injury

Despite our growing mechanistic understanding of bone marrow stem cell recruitment, the reasons behind the relatively limited endogenous cell response following major injury remain unclear. Regardless of the efficacy seen in small animal models [6,8,12–17,19,20,35], therapies to enhance stem cell involvement following injury have only had muted clinical success thus far [21]. While this discrepancy may be partially due to variations in clinical study design [118], the effects of low cellular retention seen even in small animal models [119–121] may also be exacerbated by differences in physiology and stem cell phenotype between largely divergent species [122,123]. In support of this theory, a meta-analysis of stem cell therapies in large animal models of cardiac ischemia replicated the modest therapeutic efficacy of clinical trials [124]. This same work, however, provides potential insights for the improvement of cell-based therapies, as efficacy was increased in those studies using higher cell doses and more defined populations [124]. In fact, cellular heterogeneity is becoming increasingly recognized amongst even putatively homogenous stem cell populations [125,126], making further refinements in cell characterization and purification important areas of ongoing study.

The limited clinical efficacy of this field has also led to the development of a wide range of promising preclinical techniques to enhance stem cell function following injury. Mechanistically, these approaches can be divided into two main categories: enhancement of the endogenous stem cell response and augmentation of cell-based therapies (Figure 2).

Enhancement of the endogenous stem cell response

Enhancing a patient's endogenous stem cell response following injury is clinically appealing due to the elimination of time and costs associated with cell harvest, *ex vivo* processing and transplantation. A variety of experimental techniques have shown efficacy in this setting (Table 1).

Promoting bone marrow stem cell mobilization is a common strategy to augment the cellular yield of peripheral blood apheresis for clinical stem cell transplants [127], and a similar approach has been suggested to increase the number of circulating cells available for homing following injury. In fact, a variety of compounds have shown the ability to mobilize bone marrow-derived HSCs, MSCs, EPCs and VSELs [2,38,106,128], with differential mobilization of cellular populations seen depending on the agent [106].

Selected mobilizing agents have been tested for *in vivo* beneficial effects following experimental injury, with modulation of the SDF-1/CXCR4 axis being the most common strategy. As discussed, G-CSF decreases SDF-1 levels in the bone marrow [109], and systemic administration of G-CSF has been shown to mobilize HSCs, EPCs and BM-MSCs, and improve outcomes in models of brain, liver and blood vessel injury [5,108,129,130]. Similarly, plerixafor (a CXCR4 antagonist) can act alone or synergistically with G-CSF to mobilize HSCs and decrease hepatic injury in a rat model of acute liver failure [131]. The dual role of the SDF-1/CXCR4 axis in bone marrow retention and peripheral recruitment creates a potential logistical problem with this approach, however, as CXCR4 blockade presumably forces mobilized cells to rely on alternative homing mechanisms to reach injured tissue.

Targeting the other side of the SDF-1/CXCR4 axis avoids this problem, as seen with oral administration of the phosphodiesterase 3 inhibitor cilostazol causing mobilization of EPCs partly through increased SDF-1 expression at the injury site [132]. Interestingly, cilostazol also upregulates the expression of CXCR4, integrin $\alpha v\beta 3$ and VEGF in EPCs, and

significantly enhances EPC-mediated inhibition of neointimal formation and acceleration of re-endothelialization following experimental arterial injury [132]. Similarly, systemic administration of agents targeting the PI3K–Akt pathway, an important mediator of cell survival and upstream modifier of eNOS, has been shown to mobilize EPCs and enhance their *in vivo* regenerative role [133–135], although the exact mechanism of action requires further study.

Direct amplification of the cytokine signal within injured tissue is also possible, as local injection of molecules known to be involved in stem cell homing (SDF-1, E-selectin), has been shown to enhance bone marrow cell recruitment and beneficial effects following experimental ischemic and traumatic injuries of the heart, lungs and soft tissue [136–138]. However, the short-term nature of cytokine release following injury is thought to partially limit the endogenous stem cell response [139], and local injection of quickly degraded molecules does not address this concern.

Direct- or cell-based gene therapies have therefore been used to provide more sustained transgene expression at sites of injury, and localized amplification of HIF-1α and SDF-1 gene expression has been shown to enhance bone marrow cell recruitment and improve neovascularization in ischemic injury models [84,95,140,141]. Safety concerns regarding viral vector use and regulation of transgene expression at the end of the therapeutic window may limit the translational potential of *in vivo* gene therapies, but SDF-1 containing slow release biologics may provide a more regulated cytokine release at the injury site [139,142,143], increasing their clinical appeal.

Despite these experimental findings, selective modulation of only one aspect of endogenous stem cell signaling may not translate to a therapeutic effect in less controlled settings, as suggested by the disappointing results of clinical trials using stem cell mobilizing agents for cardiac repair [144]. While experimental models combining local cytokine delivery with systemic mobilization have shown synergistic effects of combined treatments [137,145–147], the intrinsic constraints in endogenous stem cell number may limit the efficacy of any therapy relying solely on native cells.

Enhancement of exogenous stem cell function

The other main experimental approach to augment stem cell involvement following injury is to bolster cellular engraftment and/or function following transplantation, and a variety of cellular or injury environment modifications have shown beneficial effects (Table 2).

Similar to studies focusing on endogenous recruitment, enhancement of SDF-1 signaling within injured tissue can also be used to augment cellular transplantation, as gene therapies, direct cytokine injection and low-energy shockwave treatments to increase SDF-1 concentration in ischemic injury models have been shown to improve the recruitment and neovascularization potential of intravenously infused EPCs [148–150].

The *ex vivo* modulation of cells prior to transplantation is another popular mechanism to enhance their therapeutic effect, with gene transfer and small-molecule modulation being commonly used techniques [151]. For example, the *ex vivo* transduction of BM-MSCs with genes encoding various kinases and anti-apoptotic proteins (e.g., Akt, Bcl-2, HSP-70, ILK and GSK-3β) has been found to improve vascularization and functional outcomes following induced myocardial infarction, likely due to enhanced BM-MSC survival [152–156]. Interestingly, GSK-3β transduction also promoted cardiomyocyte-specific BM-MSC differentiation and VEGF-independent improvement of cardiac function [153], suggesting that it may be possible to coordinate overexpression of specific genes with the promotion of organ-specific tissue regeneration.

Shifting targets, the genetic or pharmacologic (AVE9488) enhancement of eNOS signaling in EPCs has also been shown to improve transplanted cell survival and function within intimal or ischemic injury models [157–159]. While it is unclear if this effect is mediated by the previously discussed mechanisms, eNOS signaling in both damaged endothelium and EPCs is clearly important for EPC homing [84,160], making this approach particularly appealing for use in clinical disease states associated with reduced NO bioavailability, such as diabetes and coronary artery disease [161,162]. Similarly, the *ex vivo* transduction or small-molecule activation of growth factors, cytokines, integrins and cell receptors important for stem cell recruitment and function, such as of CXCR4, SDF-1, VEGF and HGF, has been shown to enhance transplanted BM-MSC and EPC homing and paracrine effects in ischemic or intimal injury models [163–173].

Perhaps not surprisingly, a combination of the aforementioned approaches may be even more efficacious than singularly focused therapies, as illustrated by the synergistic beneficial effects of VEGF transduction of EPCs delivered in combination with local SDF-1 injection in a murine model of peripheral ischemia [174]. Tempering the obvious potential of *ex vivo* manipulation for enhancing cell-based therapies, however, is the use of clinically unappealing viral vectors in many of these studies, as well as the presumably short modulatory effect of small molecules, which would need to be addressed prior to translational work.

Providing the appropriate environmental cues to delivered cells within the injury site is also thought to be a crucial aspect of tissue regeneration [175,176], and there has recently been an increased focus on alterations of the cellular microenvironment to not only enhance stem cell survival and engraftment, but also modulate cellular proliferation, paracrine activity and differentiation [177–179].

Bioscaffolds, in particular, are commonly used to control the microenvironment of exogenously delivered cells. Building upon earlier work suggesting that local delivery of BM-MSCs within a simple collagen matrix could support cellular engraftment following experimentally induced cardiac ischemia [180], more sophisticated methodologies have since utilized external BM-MSC seeding and directed collagen hydrogel contraction to form 3D cell-based constructs capable of augmenting contractile skin wound healing [181]. Additionally, BM-MSC seeding of a variety of scaffolds designed to mimic the microcomposition of native extracellular matrix has been used for the directive regeneration of a variety of tissues *in vitro* and *in vivo*, including bone [182–184], cartilage [179,185,186] and myocardium [187].

Similarly, our laboratory has shown that BM-MSC-seeded pullulan-collagen hydrogels not only improve BM-MSC survival and engraftment within the high-oxidative-stress environment of ischemic murine skin wounds, but also create a ‘stem cell niche’ that enhances cytokine secretion (VEGF, MCP-1, FGF-1 and MMPs), improves angiogenesis and accelerates wound healing [188,189].

Composite tissue & organ regeneration: an extension of stem cell therapies

The regeneration of composite tissues and organs is an obvious extension of stem cell-based therapies, but the complex cellularity and growth volume limitations in the absence of a functional perfusion system are significant barriers to the large-scale fabrication of engineered tissue. While advances in bioscaffold design have shown that spatial variance of mechanical and biochemical properties can be used to stimulate multilayer complex tissue from a single stem cell population [179,185], and the use of multiple stem cell populations can synergistically promote vascularization within engineered tissue [190,191], these constructs may still require complex vascular ingrowth when placed *in vivo*.

Explantable microvascular beds (EMBs) bypass these limitations by creating functional microcirculatory systems through the isolation and *ex vivo* manipulation of host tissue [192]. EMBs can be seeded with cells and subsequently re-planted with immediate circulatory integrity (direct vessel-to-vessel connections) [192]. Our laboratory has shown that EMBs can be maintained *ex vivo* for up to 24 h using a bioreactor, and intravascularly seeded with BM-MSCs, which remain viable following *in vivo* reimplantation [193]. Further illustrating the potential of this approach, ongoing work in our laboratory has found that EMBs seeded with BM-MSCs are also capable of directed differentiation *in vivo* [Gurtner GC, Unpublished Data].

Conclusion & future perspective

The evidence for endogenous bone marrow-derived stem cell contribution following injury varies by population, yet all four cell types discussed in this article have shown beneficial effects when applied to preclinical injury models. Our mechanistic understanding of this cellular behavior is rapidly evolving, and despite early clinical setbacks using cell-based therapies, advances in tissue engineering and cell manipulation have already begun to leverage our knowledge of stem cell–microenvironment interactions to enhance the regenerative potential of these cells following injury, while simultaneously laying the groundwork for neo-organ fabrication.

Looking towards the future, we expect that further characterization of bone marrow cellular mobilization, recruitment and function will continue to provide valuable insights for unlocking our innate regenerative potential, while providing additional targets for therapeutic modulation. Based on the synergism observed with the parallel use of multiple experimental manipulations [137,145–147,174], we believe that a combination of strategies, such as enhancing cell purity, intrinsic function and external microenvironment, will be the key to maximizing therapeutic effect and producing a clinically relevant therapy. Additionally, we anticipate that insights into the therapeutic action of exogenously delivered bone marrow-derived cells will be pertinent to more readily available sources of multipotent cells, such as those derived from adult adipose tissue [194–196]. The use of these alternative cell sources may accelerate the clinical translation of mesenchymal stem cell therapies by overcoming the limitation of obtaining adequate cell numbers without the need for *in vitro* expansion.

In summary, we believe that optimization of the fundamental mechanisms described herein has the potential to significantly increase the regenerative capacity of adult tissue following injury. As such, we expect to see the emergence of multiple clinically relevant cell-based therapies in the upcoming years, as the full potential of these cells is slowly realized.

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- ■■ of considerable interest

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Executive summary

- Tissue repair and regeneration involve resident cell proliferation, as well as the selective recruitment of stem and progenitor cell populations originating from the bone marrow:
  - Bone marrow stem and progenitor cell populations that are active following injury include hematopoietic and mesenchymal stem cells, endothelial progenitor cells and very small embryonic-like cells.
  - Recruited stem/progenitor cells are thought to promote tissue regeneration through some combination of cytokine release and direct cellular differentiation.
- Bone marrow stem and progenitor cells are mobilized and recruited to injured tissue through complex signaling and cytokine cascades, including the important SDF-1/CXCR4 cytokine-receptor axis:
  - Nitric oxide, Jagged/Notch and MCP-1/CCR2 interactions, as well as various growth factors, are also likely to contribute to this process.
  - A variety of molecules have been implicated in bone marrow stem cell vascular rolling and adhesion, endothelial transmigration and movement within the extracellular space, enabling homed cells to congregate within sites of injury.
- Cell-based therapies have shown the ability to augment tissue regeneration in animal models by increasing stem/progenitor cell involvement within the injury environment:
  - Clinical trials using stem cell therapies have shown mixed efficacy, partially due to poor cellular engraftment within the harsh injury environment.
- Preclinical techniques augmenting endogenous or exogenous bone marrow stem cell function, survival and homing have been developed to increase stem cell engraftment and the overall regenerative effects of stem cell therapies:
  - The synergism observed with combined therapies is particularly applicable to translational applications.
- Composite tissue and organ regeneration are natural extensions of stem cell therapies, with stem cell-seeded explantable microvascular beds showing promise for large-scale tissue engineering.
- Ongoing research into the actions of endogenous stem cells should continue to provide clues for the improvement of stem cell-based therapies.

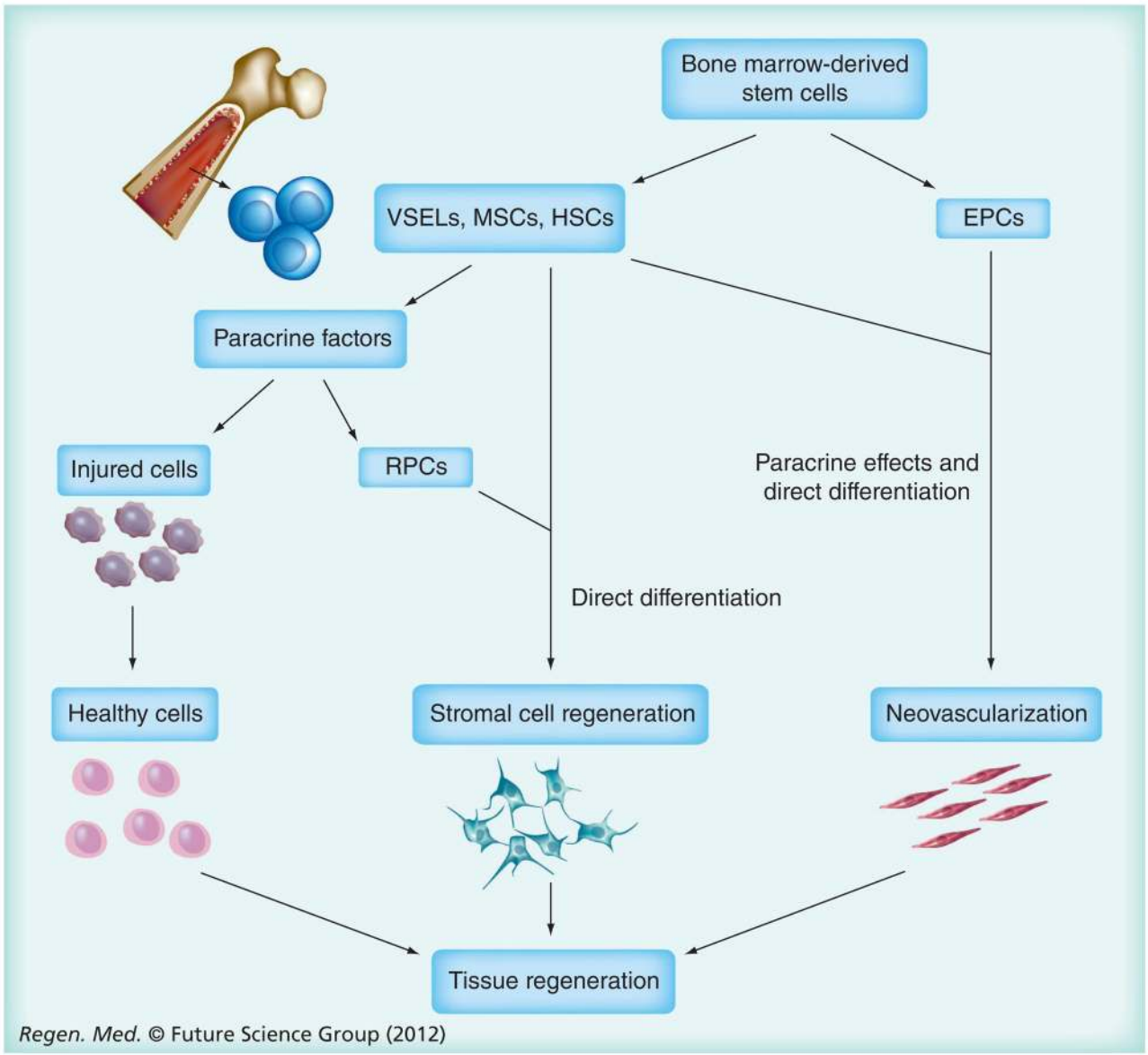

Figure 1. Proposed functions of recruited bone marrow-derived cellular subpopulations following injury

EPCs are thought to contribute mainly to neovascularization, while VSELs, MSCs and HSCs variably support neovascularization and tissue regeneration through paracrine effects on native cell survival and RPC proliferation, as well as infrequent direct cellular differentiation.

EPC: Endothelial progenitor cell; HSC: Hematopoietic stem cell; MSC: Mesenchymal stem cell; RPC: Resident progenitor cell; VSEL: Very small embryonic-like cell.

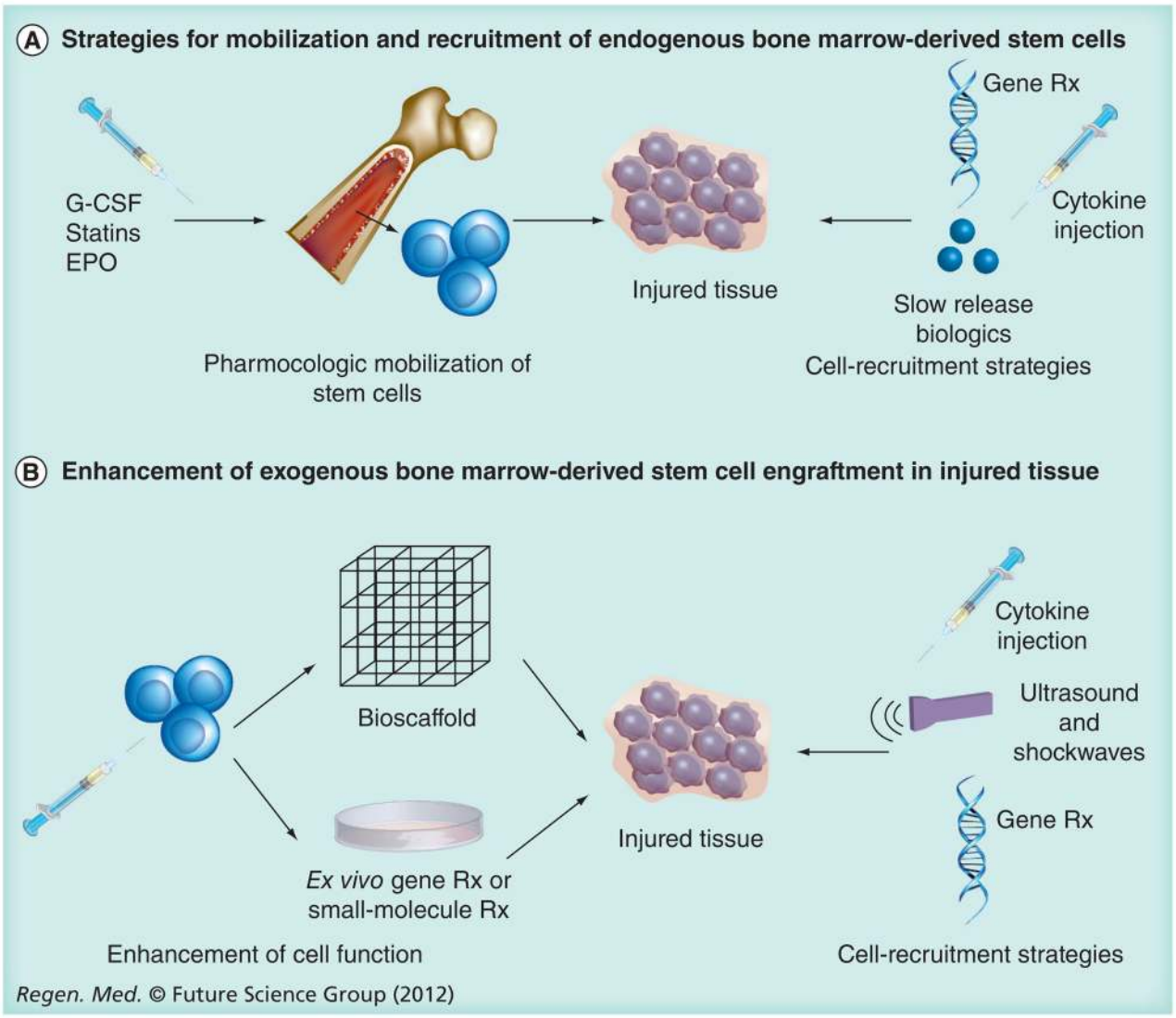

Figure 2. Stem cell enhancement strategies following injury

Clinical trials on stem cell therapies have shown mixed efficacy, but experimental approaches targeting the endogenous cellular response **(A)** or enhancement of cell delivery **(B)** can improve stem cell function, survival and/or homing, leading to improved outcomes following injury.

EPO: Erythropoietin.

Table 1

Preclinical methods for enhancing endogenous bone marrow stem and progenitor cell response after injury.

Molecules	Cell type	Injured tissue	Ref.
Increased cell mobilization			
Modulation of SDF-1/CXCR4 axis			
G-CSF	MSC, HSC, EPC, BMC	Brain, liver, artery	[5,108,129,130]
Plerixafor (CXCR4 antagonist)	HSC	Liver	[131]
Cilostazol (PDE-3 inhibitor)	EPC	Artery	[132]
Modulation of PI3K/Akt pathway			
Statins	EPC	Heart, kidney	[133,197]
EPO	EPC	Artery	[134]
Pioglitazone (PPARγ agonist)	EPC	Subcutaneous implant	[135]
Increased cell homing			
Local gene therapy			
HIF-1α	BMC	Heart	[140]
SDF-1	EPC, HSC	Heart, skeletal muscle	[84,95]
IGF-1	c-kit$^+$/CD34$^+$ cells	Heart	[141]
Local injection			
SDF-1	BMC	Lung, heart	[137,138]
E-selectin	EPC	Skeletal muscle	[136]
Slow release biologics			
SDF-1	MSC, sca1$^+$/c-kit$^+$ cells	Heart, *in vitro*	[139,142,143]
Combined mobilization and homing			
G-CSF with local SDF-1	BMC, c-kit$^+$ cells	Lung, heart	[137,147]
Substance P (mobilization) with local SDF-1	CD29$^+$/CD45$^-$ cells, c-kit$^+$ cells	Skeletal muscle implant	[145]
G-CSF with CXCR4 antagonist with local SDF-1	BMC	Brain	[146]

BMC: Bone marrow-derived cell; EPC: Endothelial progenitor cell; EPO: Erythropoietin; HSC: Hematopoietic stem cell; MSC: Mesenchymal stem cell; PDE-3: Phosphodiesterase-3.

Table 2

Preclinical methods for enhancing exogenous bone marrow stem and progenitor cell engraftment and function following injury.

Molecules/methods	Cell type	Injured tissue	Ref.
Increased cell homing/engraftment			
Enhancement of injured tissue homing/engraftment signal			
SDF-1 local injection	EPC	Skeletal muscle	[148]
SDF-1 local gene therapy	EPC	Skeletal muscle	[149]
U/S to upregulate local SDF-1, VEGF, ICAM-1, VCAM-1	MSC, BM-MNC	Heart	[198–201]
Low-energy shockwave to upregulate SDF-1	EPC	Skeletal muscle	[150]
Systemic coadministration of growth factors/cytokines			
G-CSF	BM-MNC	Brain, liver	[202,203]
HGF	BM-MNC	Liver	[204]
SDF-1	BM-MNC	Liver	[205]
***Ex vivo* modulation of cell function: gene therapies**			
Enhancement of cell homing/function			
eNOS, CXCR4	MSC, EPC	Artery, heart	[157,158,166–169,206]
Enhancement of cell survival			
TERT	EPC	Skeletal muscle	[207]
HSP-70, Bcl-2, Akt, GSK, ILK	MSC	Heart	[152–156]
Enhancement of cell survival/paracrine signaling			
HGF	EPC	Artery	[164]
VEGF	EPC	Skeletal muscle	[163]
SDF-1	MSC	Heart	[165]
IGF-1	EPC	Heart	[208]
***Ex vivo* modulation of cell function: small molecules**			
Enhancement of cell survival/function			
AVE9488 (eNOS enhancer)	EPC	Heart	[159]
PPARγ agonist	MSC	Heart	[209]
Inhibition of apoptosis			
p38 kinase inhibitor	EPC	Skeletal muscle	[210]
Activation of selectins/integrins			
Ephrin-B2-Fc, activating β2-integrin antibody	EPC	Skeletal muscle	[170,171]
Enhancement of paracrine signaling			
TGF-α, estradiol	MSC	Heart	[172,173]
Enhancement of differentiation capacity			
Angiotensin receptor blocker	MSC	Heart	[211]
Altered cellular microenvironment			
Bioscaffolds	MSC	Heart, skin, bone	[180–184,187–189]
Combined approaches			

Molecules/methods	Cell type	Injured tissue	Ref.
Ex vivo VEGF gene therapy with local SDF-1 delivery	EPC	Heart	[174]
Bioscaffold with SDF-1 pretreatment	EPC	Heart	[212]
Bioscaffold with IL-10 gene therapy	MSC	Heart	[213]

BM-MNC: Bone marrow-derived mononuclear cell; EPC: Endothelial progenitor cell; Ephrin-B2-Fc: Ligand for erythropoietin-producing human hepatocellular carcinoma receptor B4; MSC: Mesenchymal stem cell; U/S: Ultrasound.