In Situ Tissue Regeneration: Chemoattractants for Endogenous Stem Cell Recruitment

Wendy S. Vanden Berg-Foels, PhD^{1,2}

Tissue engineering uses cells, signaling molecules, and/or biomaterials to regenerate injured or diseased tissues. *Ex vivo* expanded mesenchymal stem cells (MSC) have long been a cornerstone of regeneration therapies; however, drawbacks that include altered signaling responses and reduced homing capacity have prompted investigation of regeneration based on endogenous MSC recruitment. Recent successful proof-of-concept studies have further motivated endogenous MSC recruitment-based approaches. Stem cell migration is required for morphogenesis and organogenesis during development and for tissue maintenance and injury repair in adults. A biomimetic approach to *in situ* tissue regeneration by endogenous MSC requires the orchestration of three main stages: MSC recruitment, MSC differentiation, and neotissue maturation. The first stage must result in recruitment of a sufficient number of MSC, capable of effecting regeneration, to the injured or diseased tissue. One of the challenges for engineering endogenous MSC recruitment is the selection of effective chemoattractant(s). The objective of this review is to synthesize and evaluate evidence of recruitment efficacy by reported chemoattractants, including growth factors, chemokines, and other more recently appreciated MSC chemoattractants. The influence of MSC tissue sources, cell culture methods, and the *in vitro* and *in vivo* environments is discussed. This growing body of knowledge will serve as a basis for the rational design of regenerative therapies based on endogenous MSC recruitment. Successful endogenous MSC recruitment is the first step of successful tissue regeneration

Introduction

THE GOAL OF tissue engineering is to orchestrate regeneration of injured or diseased tissue through the use of cells, signaling molecules, and/or biomaterials.^{1–3} The rational design of regenerative therapies is guided by lessons learned from embryonic development, adult homeostasis, and stem cell biology.^{1,3–7} Stem cell migration is required for morphogenesis and organogenesis during development and for tissue maintenance and injury repair in adults. Recruitment of mesenchymal stem cells (MSC) to an injury site is the first stage of a biomimetic therapy for tissue regeneration.

MSC are a cornerstone of tissue engineering therapies because they have multilineage potential. MSC have been isolated from bone marrow⁸ and many other tissues, including fat, periosteum, synovium, and dental pulp.^{9–11} Evidence suggests that MSC reside in perivascular niches throughout the body.^{12,13} MSC differentiation has been demonstrated along mesodermal,¹⁴ ectodermal,^{15,16} and endodermal¹⁷ lineages.¹¹ MSC also support regeneration through trophic actions.^{18,19}

Regenerative therapies have typically been based on *ex vivo* expanded bone marrow MSC (bMSC), delivered sys-

temically^{20,21} or as part of a cell-scaffold construct.^{22–24} However, there are drawbacks associated with MSC harvest and expansion, including cost, logistics, potential donor site morbidity, and regulatory hurdles. *Ex vivo* culture may also alter MSC signaling responses,²⁵ reduce homing capacity,^{25–27} and alter immunogenicity.²⁸ Recruitment of endogenous MSC avoids these drawbacks, and there is growing recognition that *in situ* regeneration by endogenous MSC recruitment is a viable alternative or adjunctive to MSC transplantation.^{29–32} Approaches under development use chemoattractants to enhance migration of endogenous MSC to accelerate wound healing and/or colonize biomaterial scaffolds.^{33–35} Recent proof of concept studies demonstrating tissue regeneration by endogenous MSC recruitment have motivated further development of this approach.^{36–38} The most effective chemoattractants for recruiting endogenous MSC may vary with tissue type and remain to be determined.

MSC are recruited to locations in adult tissues by homing through the vascular network and by interstitial migration within tissue.³⁹ The mode of recruitment exploited in *in situ* tissue regeneration is chemotaxis, which is directional migration in response to a gradient of soluble chemoattractants,

¹Department of Bioengineering, Clemson University, Clemson, South Carolina.

²Department of Craniofacial Biology, Medical University of South Carolina, Charleston, South Carolina.

such as growth factors or chemokines. A key requirement for therapy success is the recruitment of a sufficient number of MSC to the injury site to achieve regeneration.⁴⁰ The focus of this review is chemoattractants for MSC recruitment. The mechanisms that govern MSC migration to injury sites have not been fully elucidated^{27,41} and are beyond the scope of this review. Biomaterials and strategies for chemoattractant delivery are described elsewhere.^{31,42} In recent years, a substantial body of work has been published in which various chemoattractants have been tested for MSC recruitment *in vitro* and in several *in vivo* models. The objective of this review is to synthesize and evaluate evidence of recruitment efficacy by reported MSC chemoattractants.

Growth Factors

Growth factors are polypeptide extracellular signaling molecules that stimulate cell survival, migration, proliferation, and differentiation. Growth factors play critical regulatory roles in embryogenesis⁴³ and in guiding wound repair.⁴⁴ bMSC express a variety of growth factor receptors (Table 1).

PDGF-AA, PDGF-AB, PDGF-BB

Platelet-derived growth factor (PDGF) has important roles in the successive stages of embryogenesis and in wound healing.⁴⁵ PDGF is a polypeptide dimer with four homodimers, PDGF-AA, PDGF-BB, PDGF-CC, PDGF-DD, and one heterodimer, PDGF-AB. PDGF-CC and -DD are not discussed. PDGF has two receptor dimers, α and β . PDGF-AA binds α/α receptors, PDGF-AB binds α/α and α/β receptors, and PDGF-BB binds α/α , α/β , and β/β receptors.⁴⁶ *In vitro*, PDGF-AA,^{47,48} PDGF-BB,⁴⁷ and PDGF-AB^{47,49} were shown to be potent chemoattractants for human bMSC, with PDGF-BB generating the strongest response.⁴⁷ PDGF-AB also stimulated chemotaxis by ovine bMSC.⁵⁰ However, other studies reported only minimal recruitment of bMSC by PDGF-BB.^{51,52} The divergent conclusions may be due to MSC and/or culture condition differences. In a collagen gel invasion study, PDGF-BB induced minimal bMSC migration.⁵² Migration was enhanced when bMSC were cocultured with PDGF-BB activated fibroblasts in a modified scratch assay. Blocking basic fibroblast growth factor (bFGF) and epithelial neutrophil activating peptide-78 produced by the fibroblasts inhibited the enhanced migration. The coculture study un-

derscores the fact that migration may result from the integration of a number of chemotactic signals. In an *in vivo* tracking study in nude rats, PDGF-AA recruited nanoparticle-labeled human bMSC, which were injected into the proximal femoral medullary canal, to a fibrin scaffold in the distal femur, underscoring the promise of PDGF for regenerative therapies.⁵³

IGF-1, IGF-2, IGFBP-3, IGFBP-5

Insulin-like growth factors (IGF) are regulators of fetal and postnatal growth and development⁵⁴ and of bone growth, remodeling, and repair.⁵⁵ The IGF-1R receptor binds both IGF-1 and -2.⁵⁶ *In vitro*, IGF-1 and -2 stimulated migration by human bMSC in a dose-dependent manner.⁵⁷ IGF binding protein 5 (IGFBP-5) also induced migration. Adding IGFBP-5 to IGF-1 further enhanced IGF-1-stimulated migration; adding IGFBP-3 had no effect. Blocking the IGF-1R receptor inhibited migration completely. Other *in vitro* investigations have also reported IGF-1 to be a strong chemoattractant for human,^{49,58} rabbit,⁵⁸ and ovine⁵⁰ bMSC. Overexpression of IGF-1 in rat bMSC, resulted in enhanced recruitment to the infarcted heart and improved cardiac function in a rat model.⁵⁹ Overexpression also promoted expression of hepatocyte growth factor (HGF), bFGF, vascular endothelial growth factor (VEGF), and stromal cell derived factor-1 (SDF)-1 α by bMSC and promoted cell survival in culture. IGF-1 also promotes cell proliferation,⁶⁰ making it a strong candidate for tissue regeneration therapies.

BMP-2, BMP-4, BMP-7, TGF- β 1, TGF- β 3

The bone morphogenetic proteins (BMP) and transforming growth factors - β (TGF- β) are members of the TGF- β superfamily of signaling molecules. The BMPs and TGF- β s are regulators of embryogenesis with essential roles in gastrulation, establishment of the dorsal-ventral axis, angiogenesis, and organogenesis, particularly skeletal formation.⁶¹⁻⁶⁴ Postnatally, they play a prominent role in endochondral ossification, fracture healing, and bone remodeling.^{61,65} Receptors for the BMPs and TGF- β s are classified as type I and type II.^{66,67} Upon ligand binding, the receptors form a heterotetrameric complex composed of type I and type II receptor pairs. BMP-2, -4, and -7 are expressed during fracture healing.^{65,68,69} *In vitro*, BMP-2, -4, and -7 stimulated chemotaxis

TABLE 1. GROWTH FACTOR RECEPTORS EXPRESSED BY HUMAN BONE MARROW MESENCHYMAL STEM CELLS UNDER BASAL CULTURE CONDITIONS

Receptors ^a	Ligands	References
BMPR-1a (Alk3, CD292), -1b (Alk6, CDw293), BMPR-2	BMP-2, BMP-4	61,166
EGFR (ErbB)	EGF, HB-EGF, TGF α	49,167
FGFR-1 (CD331), -2 (CD332), -3 (CD333), -4 (CD334)	FGF-2	49,168
HGFR (c-Met)	HGF	49,93
IGF-1R (α IR3, CD221)	IGF-1, -2	49,60,169
PDGFR α/β (CD140a/b)	PDGF-AA, PDGF-AB, PDGF-BB	49,167,169,170
VEGFR-1 (Flt-1), -2 (Flk-1, CD309)	VEGF-A	170,171
TIE-2 (CD202b)	Ang-1	49,170

^aReceptors are listed for ligands that stimulated chemotaxis *in vitro*. The receptors evaluated varied among studies; receptor expression varied among donors.

BMP, bone morphogenetic protein; EGF, epidermal growth factor; HB-EGF, heparin-binding EGF; TGF, transforming growth factor; FGF, fibroblast growth factor; HGF, hepatocyte growth factor; IGF, insulin-like growth factor; PDGF, platelet-derived growth factor; VEGF, vascular endothelial growth factor.

by human bMSC,^{48,70} suggesting that their role in fracture healing may include bMSC recruitment. BMP-2 and -7 delivery have been studied extensively in animal models, and it has been approved as a clinical treatment for fracture non-unions and large segmental defects.^{71,72} TGF- β 1 and - β 3 are also expressed during fracture healing.⁶⁵ *In vitro*, TGF- β 1 stimulated chemotaxis by human adipose-derived MSC (aMSC),⁷³ but not by bMSC.⁷⁰ TGF- β 3 stimulated chemotaxis by human aMSC, synovium-derived MSC, and bMSC.⁷⁴ *In vivo*, sufficient cartilage was regenerated to cover the surface of a rabbit humeral head using only a scaffold infused with TGF- β 3.³⁷ This cartilage regeneration was achieved by endogenous MSC recruitment. TGF- β superfamily members have considerable promise for bone and cartilage regeneration therapies.

EGF, HB-EGF, TGF α

Epidermal growth factor (EGF; EGFR) has pleiotropic effects that include migration, proliferation, differentiation, and dedifferentiation.⁷⁵ EGFR is expressed by epithelial and stromal cells and by some glial and smooth muscle cells. EGFR is either the sole or the overwhelmingly predominant receptor for several ligands, including EGF, heparin-binding EGF (HB-EGF), and TGF- α . *In vitro*, EGF stimulated chemotaxis by human,^{58,76} rat,⁷⁶ rabbit,⁵⁸ and ovine⁵⁰ bMSC. Human aMSC also migrated in response to EGF.^{73,77} HB-EGF stimulated chemotaxis by human and rabbit bMSC.⁵⁸ TGF- α is structurally and functionally similar to EGF, sharing a 40% amino acid sequence identity.⁷⁸ TGF- α is upregulated in response to injury⁷⁹ and induces inflammation with ectopic expression.⁸⁰ It also induces production of VEGF by human bMSC.⁸¹ *In vitro*, TGF- α stimulated chemotaxis by human bMSC.⁵⁸ A significant advantage of EGF is the stability of its native and recombinant forms, even under harsh conditions.

FGF-2

FGF-2, or bFGF, is a member of the FGF family that includes twenty-two structurally related proteins.^{82,83} FGF-2 plays a role in limb development, wound healing, and angiogenesis,^{83,84} and it contributes to cell survival, migration, proliferation, and differentiation.^{82,84,85} Alternative splicing for FGF receptors (FGFR) 1-3 produces two isoforms, "b" and "c."^{82,86} FGF-2 preferentially activates the "c" isoforms (FGFR-1c, -2c, -3c) and has activity with FGFR-1b and FGFR-4.⁸⁶ The interaction of FGF with heparin and heparin sulfate plays an important role in FGF bioactivity.⁸² Heparin binding protects FGF from proteolysis and thermal denaturation, limits the diffusion of FGF in the interstitial space, and results in a store of FGF that is released upon heparin cleavage. *In vitro*, FGF-2 stimulated chemotaxis by murine,⁸⁷ rabbit,⁵⁸ ovine,⁵⁰ and human^{58,88} bMSC and human aMSC.⁷³ FGF-2 stimulated chemotaxis for rat bMSC at passage two, but not at passage zero,⁸⁹ emphasizing that the effects of cell culture must be considered when interpreting results of *in vitro* chemotaxis assays.

HGF

HGF (c-Met) is mitogenic and motogenic for hepatocytes and a variety of other cell types.⁹⁰ HGF plays a key role in

organogenesis during embryonic development⁹¹ and in wound healing.⁹² *In vitro*, HGF stimulated chemotaxis by human^{93,94} and murine⁹⁵ bMSC. However, HGF administration also resulted in marked inhibition of bMSC proliferation.^{93,95} Exposure of murine bMSC to HGF resulted in the loss of markers associated with the MSC phenotype and expression of markers typical of early myogenic differentiation.⁹⁵ Additional research will be required to determine if this differentiation also occurs *in vivo*.

VEGF-A

The VEGF family has five members: VEGF-A through -D and placental growth factor.⁹⁶ Here, I focus on VEGF-A, which binds to the receptors VEGFR-1 and -2.^{96,97} VEGFs are essential for vasculogenesis and angiogenesis in the embryo and angiogenesis in the adult. VEGF-induced angiogenesis plays a key role in wound healing.⁹⁷ There is considerable variation in the results of *in vitro* studies that have evaluated VEGF-A as a chemoattractant for bMSC. The chemotactic response has been strong,^{98,99} weak,^{88,100} or absent.^{58,87} In a study, in which VEGF stimulated a strong chemotactic response, the human bMSC were found to be negative for VEGFR.⁹⁸ Based on receptor blocking studies, the authors concluded that the VEGF-A-induced migration was mediated by the structurally similar PDGF receptors. Additional research will be required to fully elucidate the intricacies of VEGF signaling in bMSC recruitment. Exposure of murine bMSC to hypoxia increased expression of VEGFR-1 and enhanced the chemotactic response to VEGF-A *in vitro* after hypoxia treatment.⁹⁹ Delivery of VEGF from a collagen gel in endodontically treated human teeth, that were implanted subcutaneously in a murine host, resulted in regeneration of dental-pulp-like tissue.¹⁰¹ These results demonstrate the potential of VEGF for tissue regeneration based on endogenous stem cell recruitment.

Chemokines

Chemotactic cytokines, or chemokines, are a family of small molecules of approximately 8-10 kDa in size that induce leukocyte migration.¹⁰² They are classified into four groups based on the number and spacing of their cysteine residues: CC, CXC, C, and CX₃C.¹⁰³ Chemokines are key regulators of cell migration for tissue homeostasis, immune responses, and wound healing. bMSC express a variety of chemokine receptors (Table 2).

SDF-1

SDF-1 (CXCL12; CXCR4) is expressed during embryonic development,^{104,105} at injury sites,^{106,107} and in response to hypoxia.¹⁰⁸⁻¹¹¹ There are two similar SDF-1 isoforms, SDF-1 α and -1 β .¹¹² *In vitro*, SDF-1 β stimulated chemotaxis by human bMSC.^{88,113} In a rat hypoglossal nerve injury model, bMSC transplanted in the lateral ventricles migrated to injured tissue expressing SDF-1 and to the site of an intracerebral injection of SDF-1 α .¹¹⁴ In a murine bone fracture model, SDF-1 promoted bone regeneration by recruiting systemically delivered MSC to the fracture site.¹⁰⁷ Regeneration was inhibited by an anti-SDF-1 antibody and by a CXCR4 antagonist. In a murine model of myocardial infarction, systemically delivered bMSC migrated to infarcted

TABLE 2. CHEMOKINE RECEPTORS EXPRESSED BY HUMAN BONE MARROW MESENCHYMAL STEM CELLS UNDER BASAL CULTURE CONDITIONS

Receptors ^a	Ligands	References
CCR1 (CD191)	CCL3 (MIP-1 α), 5 (RANTES), 6 (MRP-1), 7 (MCP-3), 8 (MCP-2), 9 (MIP-1 γ), 13 (MCP-4), 14 (HCC-1), 15 (MIP-1 δ), 16 (HCC-4), 23 (MIP-3)	25,113,172-174
CCR2 (CD192)	CCL2 (MCP-1), 7 (MCP-3), 8 (MCP-2), 13 (MCP-4), 16 (HCC-4)	49,130,174
CCR3 (CD193)	CCL5 (RANTES), 7 (MCP-3), 8 (MCP-2), 11 (eotaxin-1), 13 (MCP-4), 15 (MIP-1 δ), 24 (eotaxis-2), 26 (eotaxis-3), 28 (MEC)	49,173,174
CCR4 (CD194)	CCL5 (RANTES), 17 (TARC), 22 (MDC)	49,172,174
CCR5 (CD195)	CCL3 (MIP-1 α), 4 (MIP-1 β), 5 (RANTES), 8 (MCP-2), 11 (eotaxin-1), 14 (HCC-3), 16 (HCC-4)	49,172-174
CCR6 (CD196)	CCL20 (MIP-3 α)	174,175
CCR7 (CD197)	CCL19 (ELC), 21 (SLC)	25,113,172-174
CCR8 (CDw198)	CCL1 (TCA-3)	173,174
CCR9 (CDw199)	CCL25 (TECK)	25,174
CCR10	CCL27 (CTACK), 28 (MEC)	172-174
CCR11	CCL19 (ELC), 21 (SLC), 25 (TECK)	173
CXCR1 (CD181)	CXCL6 (GCP-2), 7 (NAP-2), 8 (IL-8)	174
CXCR2 (CD182)	CXCL1 (GRO α), 2 (GRO β) 3 (GRO γ), 5 (ENA-78), 6 (GPC-2), 7 (NAP-2), 8 (IL-8)	174
CXCR3A/B (CD183)	CXCL4 (PF-4), 9 (MIG), 10 (IP-10), 11 (I-TAC)	130,173,174
CXCR4 (CD184)	CXCL12 (SDF-1)	25,49,113,130,173-175
CXCR5 (CD185)	CXCL13 (BLC)	25,49,174
CXCR6 (CD186)	CXCL16 (SR-PSOX)	25,113,130,173,174
CX ₃ CR1	CX ₃ CL1 (Fractalkine)	113,130,174
XCR1	XCL1 (SCM-1 α), 2 (SCM-1 β)	174

^aReceptors are listed for ligands that stimulated chemotaxis *in vitro*. The receptors evaluated varied among studies; receptor expression varied among donors.

MIP, macrophage inflammatory protein; RANTES, regulated on activation normal T-cell expressed and secreted; MCP, monocyte chemoattractant protein; TARC, thymus and activation-regulated chemokine; MDC, macrophage-derived chemokine; SLC, secondary lymphoid tissue chemokine; IL, interleukin; SDF, stromal cell derived factor.

tissue.¹¹⁰ Elevation of SDF-1 α expression by adenoviral gene delivery significantly increased MSC recruitment; a CXCR4 antagonist diminished recruitment. SDF-1 α expression did not increase MSC recruitment in the absence of infarction. SDF-1 has also been exploited to recruit endogenous bMSC to, and to promote their migration within, biomaterial scaffolds.^{115,116} Collectively, these studies suggest that SDF-1 is an important chemokine for MSC recruitment.

MCP-1, MIP-1 α , IL-8

Monocyte chemoattractant protein-1 (MCP-1, CCL2; CCR2), macrophage inflammatory protein-1 α (MIP-1 α , CCL3; CCR1,5), and interleukin-8 (IL-8, CXCL8; CXCR1,2) are expressed in rat ischemic cerebral tissue.^{117,118} Systemically infused bMSC migrated to ischemic cerebral tissue and reduced neurological functional deficits.¹¹⁹ *In vitro*, extracts of ischemic brain tissue and recombinant MCP-1,¹¹⁸ MIP-1 α ,^{113,118} and IL-8¹¹⁸ stimulated chemotaxis by human bMSC. MCP-1 is also transiently upregulated in the infarcted heart.¹²⁰ In a murine model with MCP-1 specifically expressed in the heart, systemically infused murine bMSC engrafted in the hearts of transgenic mice, but not of wild-type controls. CCR2 antibodies inhibited MCP-1 induced migration. In an *in vivo* tracking study, nanoparticle-labeled human bMSC were injected into the proximal femoral medullary canal of nude rats.⁵³ MCP-1 or PDGF-AA was released from a heparin-conjugated fibrin scaffold in a distal femoral osteochondral defect. Both chemoattractants recruited bMSC to the scaffold; however, recruitment by MCP-1 was weak relative to that of PDGF-AA. These studies

suggest that MCP-1, MIP-1 α , and IL-8 may be valuable chemoattractants for regenerative therapies; however, growth factors may induce stronger responses.

RANTES, MDC

RANTES (regulated on activation normal T-cell expressed and secreted, CCL5; CCR1, 3, 4, 5) is a chemoattractant for T-cells, monocytes, and eosinophils.^{121,122} Macrophage-derived chemokine (MDC, CCL22; CCR4) is a chemoattractant for monocytes, monocyte-derived dendritic cells, and natural killer cells.¹²³ *In vitro*, both RANTES and MDC stimulated chemotaxis by human bMSC.⁴⁹ Preincubation of human bMSC with tumor necrosis factor α (TNF- α), an inflammatory cytokine, substantially increased the chemotactic response. CCR5 and its ligands are transiently upregulated in murine skin wounds.¹²⁴ Wound healing was delayed in CCR5^{-/-} mice; however, systemic infusion of CCR5⁺ bone marrow-derived cells restored normal healing. MDC did not induce *in vitro* migration by human periosteum-derived MSC.¹²⁵ These studies suggest that chemokine-induced migration may depend on the systemic and local inflammatory state and that MSC receptors may vary with tissue source.

Fractalkine

Fractalkine (CX₃CL1; CX₃CR1) mediates chemotaxis and adhesion of inflammatory cells, pain sensation, and is involved in several inflammatory conditions.¹²⁶⁻¹²⁸ High levels of fractalkine and its receptor are expressed at wound sites.^{114,129} *In vitro*, fractalkine stimulated chemotaxis by

rat¹¹⁴ and human^{113,130} bMSC; however, the potential of fractalkine for *in vivo* MSC recruitment has not been investigated.

SLC, TARC

Secondary lymphoid tissue chemokine (SLC, CCL21; CCR7) is a potent chemoattractant for lymphocytes and some T-cell lines.¹³¹ Thymus and activation-regulated chemokine (TARC, CCL17; CCR4) is expressed by several cell types, including dendritic cells, endothelial cells, keratinocytes, and fibroblasts, and it is constitutively expressed in the thymus.¹³² SLC¹³³ and TARC¹³² are upregulated in several inflammatory skin conditions. *In vitro*, both SLC and TARC stimulated chemotaxis by murine bMSC.¹⁵ In a murine skin wound model, local injections of SLC recruited systemically infused bMSC to the wound and accelerated healing; however, TARC injections had no effect. The difference between the *in vitro* and *in vivo* results for MSC recruitment underscores the complexity of MSC recruitment in the *in vivo* environment.

Other Chemoattractants

In addition to the better known growth factors and chemokines, other molecules have also been identified as MSC chemoattractants. Four examples of these other chemoattractants are reviewed. bMSC express receptors for these molecules (Table 3).

LPA, S1P

Lysophospholipids, such as lysophosphatic acid (LPA) and sphingosine 1-phosphate (S1P), are bioactive signaling molecules that affect a variety of cell functions, including survival, migration, proliferation, and differentiation.^{134,135} LPA and S1P are released by activated platelets, and they may be as important as cytokines and growth factors in or-

chestrating tissue repair.¹³⁶ Thus far, there are five confirmed LPA receptors (LPA₁₋₅) and S1P receptors (S1P₁₋₅).¹³⁷ *In vitro*, LPA stimulated chemotaxis by human¹³⁸ and murine⁸⁷ bMSC. Human aMSC also migrated in response to LPA.¹³⁹ Migration was inhibited by Ki6425, an antagonist specific for LPA₁ and LPA₃. In murine¹⁴⁰ and rat¹⁴¹ cutaneous wound models, topical LPA application resulted in an accelerated healing response. S1P plays a key role in neovascularization for wound healing¹⁴² and for cancer tumor growth.¹⁴³ Binding S1P₁ and S1P₂ had opposing effects on tumor angiogenesis in a murine study, with S1P₁ stimulating angiogenesis and S1P₂ inhibiting angiogenesis.¹⁴⁴ *In vitro*, S1P stimulated a chemotactic response by murine bMSC.^{87,145} The migration response for S1P was stronger than that for LPA.⁸⁷ In a murine model of liver fibrosis, S1P was elevated in the liver and serum after liver injury, and it stimulated migration of intravenously delivered bMSC to the liver.¹⁴⁶ Receptors S1P₁₋₃ were abundant on the bMSC recruited. Use of an S1P₃ antagonist markedly reduced bMSC migration both *in vitro* and *in vivo*.¹⁴⁶ Additional studies will be required to better understand the regulation of MSC by lysophospholipids and how these signaling molecules can be harnessed for tissue regeneration.

HMGB-1

High mobility group box-1 (HMGB-1) is a nonhistone DNA-binding cytokine that stabilizes nucleosomes and facilitates transcription.¹⁴⁷ HMGB-1 is passively released by injured and necrotic cells, but not by apoptotic cells, and it is secreted by activated macrophages and monocytes.¹⁴⁸ HMGB-1 in the extracellular compartment is recognized as a signal of tissue injury and a trigger to initiate tissue regeneration. However, HMGB-1 also contributes to pathological conditions, such as sepsis, rheumatoid arthritis, lupus, and cancer. HMGB-1 binds to the receptor for advanced glycation end products and toll-like receptors -2 and -4.^{149,150} *In vitro*, HMGB-1 stimulated transmigration by mesoangioblasts, a vessel-associated stem cell, through an endothelial barrier.¹⁵¹ Beads containing HMGB-1 implanted in muscle resulted in recruitment of intravenously injected mesoangioblasts. HMGB-1 also stimulated migration by human bMSC *in vitro*.¹⁴⁹ Culture of bMSC with HMGB-1 suppressed proliferation in a dose-dependent manner and induced osteogenic differentiation. A better understanding of HMGB-1 signaling will be needed to harness its regenerative potential.

TNF- α

TNF- α is a proinflammatory cytokine produced by macrophages during acute inflammation of injured tissue.¹⁵² TNF- α binds two receptors, TNFR-1 and -2, that are thought to activate distinct signaling pathways.¹⁵² *In vitro*, TNF- α stimulated a strong dose-dependent migration response by rat bMSC¹⁵³ and by human aMSC⁷³ and bMSC.^{49,154} Preincubation of the aMSC with TNF- α enhanced the migration response to individual chemokines and growth factors.⁷³ However, preincubation of bMSC resulted in a stronger migration response to chemokines, but not to growth factors.⁴⁹ Incubation of human bMSC or aMSC with TNF- α stimulated production of VEGF, HGF, and IGF-1 by the MSC.¹⁵⁵ Neutralizing TNFR-2 significantly inhibited migration; neutralizing TNFR-1 had no effect on migration.¹⁵⁴ In a rat ischemia model,

TABLE 3. OTHER CHEMOATTRACTANT RECEPTORS EXPRESSED BY HUMAN BONE MARROW MESENCHYMAL STEM CELLS UNDER BASAL CULTURE CONDITIONS

Receptors ^a	Ligands	References
TNFR1 (CD120a), TNF- α 2 (CD120b)		49,130
RAGE; TLR2, 4	HMGB-1	149,159,176
LPA-1 (Edg2)	LPA	138
S1P-3 (Edg3)	S1P	138,146
TLR1 (CD281)	Lipoproteins, peptidoglycans	159
TLR2 (CD282)	Lipoproteins, peptidoglycans	159
TLR3 (CD283)	Viral dsRNA	159
TLR4 (CD284)	LPS, heat shock proteins, ECM molecules	159
TLR5	Flagellin	159
TLR6 (CD286)	Lipoproteins, peptidoglycans	159
TLR9 (CD289)	Bacterial and viral CpG-DNA	159

^aReceptors are listed for ligands that stimulated chemotaxis *in vitro*. The receptors evaluated varied among studies; receptor expression varied among donors.

TNF, tumor necrosis factor; RAGE, receptor for advanced glycation end products; HMGB, high mobility group box; LPA, lysophosphatic acid; S1P, sphingosine 1-phosphate; TLR, Toll-like receptor.

preincubation of rat bMSC with TNF- α before systemic infusion was associated with a greater bMSC accumulation in the ischemic muscle.¹⁵⁶ Additional research will be needed to determine if the migratory effects of TNF- α can be harnessed without introducing damaging inflammatory side-effects.

Ligands for toll-like receptors

Toll-like receptors (TLR) are a large family (TLR1-TLR11) of evolutionarily conserved receptors activated by intracellular components, such as heat shock proteins or RNA, that are released during wounding¹⁵⁷ and by invading pathogens.¹⁵⁸ These signals recruit immune cells to tissue injuries,¹⁵⁸ and it has recently been discovered that they may also recruit MSC.¹⁵⁹ *In vitro*, human bMSC migration was stimulated by ligands for TLR2-4 and TLR9.^{157,159} Preincubation with the ligands enhanced the migration response. However, in another study, TLR2 was reported to inhibit murine bMSC migration.¹⁵⁰ The discrepant results may be explained by the length of the preincubation period; a longer preincubation was shown to inhibit migration.¹⁵⁷ Interestingly, TLR signaling was also shown to regulate phenotype and differentiation down mesodermal pathways for human bMSC. TLR priming of a heterogeneous bMSC population resulted in a nearly homogeneous phenotype.¹⁵⁷ TLR3-primed bMSC expressed primarily immunosuppressive mediators; TLR4-primed bMSC expressed primarily proinflammatory mediators. TLR3 ligand exposure inhibited osteogenic, adipogenic, and chondro-genic differentiation.¹⁵⁷ TLR4 ligand exposure stimulated osteogenic, inhibited adipogenic, and did not affect chondrogenic differentiation. TLR2 ligand exposure inhibited differentiation down each of the three pathways.¹⁵⁰ The TLRs are novel targets for inducing a more homogeneous response from heterogeneous MSC populations and for exerting an additional level of control over MSC differentiation.

Discussion

A biomimetic engineering approach to *in situ* tissue regeneration by endogenous MSC requires the orchestration of three main stages: MSC recruitment, MSC differentiation, and neotissue maturation. The first stage must result in the recruitment of a sufficient number of MSC that are capable of effecting regeneration of the injured or diseased tissue. One of the challenges for engineering endogenous MSC recruitment is the selection of effective chemoattractant(s).

Reported chemotactic responses vary among MSC isolated from the same tissue type and among MSC isolated from different tissue types. Differences in culture conditions and cell passage numbers contribute to the variation. Both cell density and passage number correspond to changes in MSC morphologies and gene expression levels of some phenotype markers.^{25,89,160} Donor-related differences also contribute to the variance,¹⁶¹ as do species-related, and even strain-related, differences.^{89,162} Differences among MSC isolated from different tissues^{161,163} may reflect the diversity among MSC niches.¹⁶⁴

Exposure of MSCs to a chemoattractant may stimulate collateral responses in addition to the chemotaxis desired. Treatment with TGF α ,⁸¹ TNF- α ,¹⁵⁵ or overexpression of IGF-1⁵⁹ each stimulated the expression of other growth factors by the MSC. HGF inhibited proliferation and resulted in the loss of the MSC phenotype.^{93,95} HMGB-1 inhibited proliferation

and promoted osteogenic differentiation.¹⁴⁹ TLR priming changed MSC cytokine and chemokine expression patterns and differentiation responses; the changes were dependent on the TLR primed.¹⁵⁷

The *in vitro* and *in vivo* environments differ considerably; *in vitro* MSC chemotaxis may not translate to *in vivo* chemotaxis. *In vitro*, MSC are typically exposed to one chemoattractant, while *in vivo*, MSC are exposed to a multiplicity of signals that are influenced by the inflammatory state. *In vivo*, MSC are recruited from a tissue niche, while *in vitro*, MSC are recruited from a cell suspension or from a monolayer. *In vivo*, chemoattractants are subject to diffusion away from the regeneration site, enzymatic degradation, and/or deactivation.³¹ *In vivo*, chemoattractants interact with the extracellular matrix. Proteoglycans bind, store, and release growth factors and chemokines.¹⁶⁵ Matrix binding has been shown to enhance growth factor function over that of the soluble form.⁴² *In vivo*, other cells, such as fibroblasts, may also respond to a chemoattractant by migrating and/or by producing growth factors and chemokines.⁵⁸ Testing in animal models showed that lower doses of growth factor resulted in a greater chemotactic response⁵³ and that chemotaxis *in vitro* may not correspond to chemotaxis *in vivo*.¹⁵

Much remains to be discovered about mechanisms that govern MSC chemotaxis; however, proof-of-concept studies have demonstrated the promise of endogenous MSC recruitment for tissue regeneration. Each chemoattractant reviewed has been reported to induce MSC chemotaxis. The key will be to determine which chemoattractant(s) (1) recruit a sufficient number of MSC capable of effecting regeneration, (2) do not recruit cells that interfere with regeneration, and (3) do not interfere with subsequent stages of tissue regeneration. The rational design of MSC recruitment strategies will require a synergistic combination of *in vitro* and *in vivo* experiments. Novel cell labeling techniques⁵³ will be required to monitor the spatiotemporal dynamics of MSC chemotaxis *in vivo*. Successful endogenous MSC recruitment is the first stage of successful tissue regeneration.

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Disclosure Statement

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Address correspondence to:
Wendy S. Vanden Berg-Foels, PhD
Clemson-MUSC Joint Bioengineering Program
Department of Craniofacial Biology
173 Ashley Avenue
BSB 230G, MSC 507
Charleston, SC 29425

E-mail: wendyv@clermson.edu

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