Pericytes, mesenchymal stem cells and their contributions to tissue repair

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Abstract

Regenerative medicine using mesenchymal stem cells for the purposes of tissue repair has garnered considerable public attention due to the potential of returning tissues and organs to a normal, healthy state after injury or damage has occurred. To achieve this, progenitor cells such as pericytes and bone marrow-derived mesenchymal stem cells can be delivered exogenously, mobilised and recruited from within the body or transplanted in the form organs and tissues grown in the laboratory from stem cells. In this review, we summarise the recent evidence supporting the use of endogenously mobilised stem cell populations to enhance tissue repair along with the use of mesenchymal stem cells and pericytes in the development of engineered tissues. Finally, we conclude with an overview of currently available therapeutic options to manipulate endogenous stem cells to promote tissue repair.

Introduction

The concept of regenerative medicine and tissue repair has in recent years garnered considerable public attention due to the potential of returning tissues and organs to a normal, healthy state after injury or damage has occurred. This area of translational medical research draws on the fields of molecular biology, cell biology and tissue engineering to devise methods of stimulating the natural ability of the body to heal itself, with the end result of a return to full function in an organ that would normally be beyond repair. This can be achieved through the delivery of culture-expanded stem cells from a donor (the allogenic or exogenous route), through the mobilisation and recruitment of stem cells from within the body of the patient (the autologous or endogenous route) or through the transplantation of organs grown in the laboratory from stem cells (tissue engineering).

Building on 50 years of success and knowledge in the field of haematopoietic stem cell transplants to treat diseases such as lymphoma and leukaemia, advances have recently been made in efforts to repair solid organs using mesenchymal stem cells (MSCs). Adult stem cells of the mesenchymal lineage have been identified in a number of organs, such as the bone marrow, adipose tissue and dental pulp (Main et al., 2014); moreover, there is evidence for an MSC niche associated with the vasculature throughout the body in the form of pericytes (Birbrair et al., 2015). These cell types display remarkable similarities in terms of their marker expression, their ability to self-renew, and their potential to differentiate into multiple mesenchymal cell types such as adipocytes, chondrocytes, osteocytes and myocytes in culture (Figure 1). As the use of cultured mesenchymal stem cells in regenerative medicine has been exhaustively reviewed elsewhere, this review will rather focus on endogenous mesenchymal stem cell populations, their recruitment to sites of injury, their utility in tissue repair and tissue engineering and currently available pharmacological means to support and enhance these processes. Moreover, given the recent publication of excellent review articles describing the role of pericytes (Birbrair et al., 2015) or MSCs (Farini et al., 2014) separately in tissue regeneration and repair, we have designed this review to include both of these cell types as a side-by-side comparison in terms of their differentiation capacity, mechanisms of mobilisation and contributions to the inflammatory response during tissue repair, as well as the availability of pharmacological interventions targeting MSCs and pericytes to enhance tissue regeneration.

Bone marrow-derived mesenchymal stem cells and pericytes – definitions and characteristics

Mesenchymal stem cells are prime candidates in the field of regenerative medicine with considerable interest in characterising these cells as well as optimising harvesting and expansion techniques for their use in injury and tissue repair (Murray et al., 2014). While most of our understanding of MSCs is based on *ex vivo* cultured cells, their *in vivo* counterparts, along with their origin, localisation, potential and roles in disease have remained elusive (Pontikoglou et al., 2011). The existence of non-haematopoietic stem cells in the bone marrow was first demonstrated by Friedenstein *et al.* in the late 1960s and 1970s. In a series of publications, it was shown that these cells are able to adhere to plastic and differentiate into osteoblasts and stromal cells. In addition, these cells can give rise to colonies, with each colony arising from a single cell, termed a colony forming unit-fibroblast, or CFU-F (Friedenstein et al., 1970; Friedenstein et al., 1974; Owen and Friedenstein, 1988). These characteristics have since been used extensively to isolate MSCs from the bone marrow as well as other tissues to further characterise and define these cells (Bouacida et al., 2012).

The ability of MSCs to adhere to plastic has been essential in their isolation from bone marrow, leading to extensive work on *in vitro* MSC cultures. However, due to the vast amount of research in this area, a standard definition was required to enable the comparison of results from different groups (Murray et al., 2014). In 2006, the International Society of Cellular Therapy (ISCT) developed a minimum criteria of characteristics required to define human MSCs (Feng et al., 2010). This definition states that MSCs must be plastic adherent, express the cell surface antigens CD105, CD73 and CD90 uniformly (in over 95% of the cell population), in the absence of CD45, CD34, CD14, CD11b, CD79a, CD19 and HLA-DR. Lastly, to be considered a true MSC, isolated cells must be able to undergo tri-lineage differentiation into osteoblasts, chondrocytes and adipocytes under the correct culture conditions (Murray et al., 2014). While MSCs have been shown to express other markers and to differentiate into other cell types, such as myocytes, these aspects are not considered requirements to define cells as MSCs (Dimarino et al., 2013). Murine MSCs must fulfil similar criteria, with positive expression of CD106 and Sca1, and the absence of CD45, CD11b and CD31 (Li and Ikehara, 2013).

Isolated bone marrow-derived MSCs remain a heterogeneous population due to the lack of *in vivo* knowledge of these cells. In order to isolate a pure MSC population, several new markers have been suggested to further define MSC. These markers include Stro-1, GD2, SSEA4, CD49a, CD146, CD200, CD271, Sca1, CD24, CD140a (PDGFRα) and CD140b (PDGFRβ) (Pontikoglou et al., 2011). As well as using these markers to define MSC as a whole, these will also be essential in understanding how this heterogeneous population may be subdivided into discrete groups which fulfil specific roles. One

such example is the expression of CD146, which is now believed to be indicative of greater pluripotency and self-renewal potential in MSC. Indeed, CD146+ cells have been isolated from the bone marrow and shown to possess many MSC-like qualities, and when transplanted into mice, could generate bone and support haematopoiesis, a known role for MSC (Sacchetti et al., 2007).

In recent years, there has been an increasing body of evidence to suggest that MSCs may reside in a perivascular niche *in vivo* (Bautch, 2011; Crisan et al., 2012; Lin and Lue, 2013). The idea that MSC are found near the vasculature explains why these cells can be isolated from most tissues around the body. Furthermore, perivascular cells, namely pericytes, have been shown to possess stem cell-like qualities, and have thus been hypothesised to be the *in vivo* counterparts, or perhaps precursors, of MSCs (Caplan, 2008; Crisan et al., 2012; Feng et al., 2010). In contrast to research on MSCs, pericytes have been predominantly studied based on their *in vivo* location and morphology. Pericytes form close associations with endothelial cells (ECs) with essential roles in the maintenance of blood vessels as well as angiogenesis (Diaz-Flores et al., 2009). In addition, pericytes have also been shown to play a role in niche maintenance for haematopoietic stem cells in the bone marrow (Kunisaki et al., 2013). Physical interactions, by way of adhesion plaques, 'peg-and-socket' and gap junctions are key to maintaining pericyte-endothelium attachment. In addition, paracrine signalling from ECs recruits pericytes by the secretion of platelet derived growth factor (PDGF)-BB, which binds to the PDGFRβ receptor expressed on pericytes (Bjarnegard et al., 2004). In addition, the secretion of angiopoietin 1 (Ang-1) by pericytes mediates pericyte-endothelial attachment via the Tie2 receptor, which is expressed by ECs (Cai et al., 2008; Sundberg et al., 2002). Disruptions in pericyte-endothelial interactions can result in the loss of pericyte coverage from the vessels and lead to a leaky vasculature, resulting in haemorrhage and oedema (Hellstrom et al., 1999; Soriano, 1994).

Pericytes are a heterogeneous population of mesenchymal cells associated with the microvasculature which vary greatly in morphology and marker expression in different tissues. As such, pericytes residing in different tissues have been termed according to their function and morphology, such as hepatic stellate cells in the liver and glomerular mesangial cells in the kidney (Lin et al., 2008; Mederacke et al., 2013). The morphology of pericytes can be stellate or spindle-like, with finger-like projections surrounding the vessels which are now believed to have a role in regulating blood flow (Hall et al., 2014) and inflammatory cell trafficking (Proebstl et al., 2012). The phenotype of pericytes not only varies based on the resident tissue and vessel size, but also on whether they are active or quiescent; active pericytes tend to shorten their processes and increase their somatic volume (Diaz-Flores et al., 1992). Furthermore, pericytes are closely related to vascular

smooth muscle (VSM) cells, which are mural cells found on larger blood vessels, with a phenotypic continuum existing between these cells (Armulik et al., 2005).

While there are several known markers of pericytes, these are not uniquely found on pericytes and are often dynamically expressed (Armulik et al., 2005; Crisan et al., 2008). Furthermore, some pericyte markers are also expressed on other cell types, most notably endothelial and smooth muscle cells (Armulik et al., 2005). Research into the role of pericytes has therefore been hampered, and only recently, with technological advances in the fields of microscopy, multi-coloured fluorescence activated cell sorting (FACS) and genetic lineage tracing, has there been significant insight into the identity and potential of pericytes (Crisan et al., 2008). The use of a combination of exclusion markers for other cell types and positive expression of known pericyte markers has enabled a more thorough understanding of these cells. As such, Crisan *et al.* have defined a FACS gating strategy for human pericytes based on negative expression of haematopoietic and endothelial cell markers (CD45 and CD31/CD34, respectively), and positive expression of CD146 (Crisan et al., 2008). Additional markers commonly used to define pericytes include PDGFRβ, neural glial antigen 2 (NG2), desmin, regulator of GTPase signalling 5 (RGS5), 3G5, CD13, alkaline phosphatase and alpha smooth muscle actin (αSMA) (Crisan et al., 2008). Immunohistochemical analysis of human samples has revealed that expression of NG2 and αSMA on pericytes varies depending on the type of vessel they surround. Pericytes surrounding capillaries are NG2+/αSMA-, on venules NG2-/αSMA+ and on arterioles NG2+/αSMA+, with CD146 and PDGFRβ expression found throughout (Crisan et al., 2012). Identification of pericyte markers in mice is based on the use of lineage tracing techniques in transgenic mice, including nestin-GFP, NG2-DsRed and coll1α1-GFP mice in models of tissue fibrosis (Birbrair et al., 2013d; Johnson et al., 2015; Lin et al., 2008; Rock et al., 2011). These studies have confirmed the validity of pericyte markers used in humans by showing co-localisation between transgenic markers used and αSMA, PDGFRβ and CD146 (Johnson et al., 2015; Lin et al., 2008; Rock et al., 2011).

The identification of pericytes has been based on *in vivo* location while MSC isolation has been based on *ex vivo* cultures, which could explain why it has only recently been hypothesised that these two cell types may in fact be the same, or at least very closely related. In accordance with the ISCT definition of MSC, Crisan *et al*. demonstrated that pericytes isolated from human skeletal muscle, pancreas, adipose tissue, placenta and bone marrow all express CD105, CD73 and CD90 as well as other known MSC markers, both *in vivo* and in long-term culture. In addition, these cells are plastic adherent and could undergo the classic MSC tri-lineage differentiation into bone, cartilage and fat (Crisan et al., 2008) as well as muscle (Birbrair et al., 2013a). Moreover, some studies have

demonstrated that pericytes are also neural cell precursors (Birbrair et al., 2013b; Dore-Duffy et al., 2011; Montiel-Eulefi et al., 2012). Not only are known MSC markers expressed on pericytes, but many pericyte markers, including NG2, 3G5, PDGFRβ and αSMA, have been found to be expressed on isolated MSC (Feng et al., 2010), highlighting the similarity between these two cell types.

As previously mentioned, a subset of bone marrow-derived MSC which are CD146+ have been postulated to retain more stem cell-like potential, with a greater degree of pluripotency. CD146 is one of the defining markers of pericytes, and thus could indicate that these cells are precursors to MSCs throughout the body. In a study by Bouacida *et al*., bone marrow mononuclear cells were harvested from patients and cultured in either mesenchymal cell medium or pericyte medium. Both cell groups were similar in terms of proliferation, CFU-F numbers and could undergo tri-lineage differentiation. However, cells cultured in pericyte medium showed greater potential to differentiate into neuronal cells under neurogenic conditions, while this was not observed in mesenchymal cell medium-cultured cells. Furthermore, pericyte-like cells showed greater stemness, with higher expression of the stem cell markers OCT4 and SOX2 and markedly lower levels of proteins associated with osteo-, chondro- and adipogenic differentiation (Bouacida et al., 2012). There is accumulating evidence to support pericyte stem cell potential, with studies showing increased proliferation and migration in response to hypoxic stress and injury in multiple tissues (Feng et al., 2010).

Due to the heterogeneity of MSC and pericytes, it is difficult to conclusively determine whether these cells represent *in vitro* and *in vivo* counterparts of the same cell population. Pericytes fulfil the criteria required to define an MSC, and show greater differentiation potential, and more stem cell-like qualities and thus may represent a source of MSC which can be found throughout the body, with reservoirs found in a perivascular niche (Diaz-Flores et al., 2009). While differences exist between pericytes and bone-marrow derived MSC, this may be the result of tissue-specific influences, which can also be observed within isolated pericyte and MSC populations themselves (Feng et al., 2010).

Mechanisms of MSC and pericyte recruitment to sites of injury

Mechanical, chemical or disease-mediated tissue injury is followed by a sequence of events to restore tissue integrity and homeostasis. The site of injury produces a number of soluble mediators that influence resident-cell fate and initiate tissue- and injury-specific immune responses. These processes are critical for tissue regeneration and repair (Eming et al., 2007; Godwin and Brockes, 2006). In this section, we review the recruitment of MSCs and pericytes to the site of injury, and the soluble mediators responsible for this process.

Studies reporting on the recruitment of endogenous BM MSCs are rare, whilst studies reporting on the recruitment of culture-expanded BM MSCs are plentiful. This is due to the reliance of culture methods to isolate and identify MSCs in early work, and their emerging use in cell therapy, as described previously. *Ex vivo* expansion of MSCs has been shown to generate genetic, phenotypic and morphological changes (Mosna et al., 2010; Wang et al., 2011) and thus result in questioning whether cultured MSCs would behave the same way as endogenous MSCs. Despite this, studies on the recruitment of cultured MSCs still provide valuable information. These studies subsequently drove innovations in target modifications which aim to achieve enhanced recruitment during therapy. MSCs express CXCR4, a chemokine receptor found predominantly on cells residing in the bone marrow. The CXCR4/CXCL12 chemokine axis has been described as the main stem cell retention system which ensures that stem cells are firmly held in the bone marrow (Rankin, 2012). Numerous studies have investigated this relationship as the mechanism involved in the recruitment of cultured BM MSCs to a site of injury. Almost all of these studies have shown upregulation of CXCL12, in some form, in and around the site of injury. Some examples of injury include skin burns (Hu et al.; Xu et al., 2013; Yang et al., 2013), brain lesions (Wang et al., 2008b) and skeletal fracture injury (Kitaori et al., 2009). In one of these studies, CXCL12 production reached peak levels at day 7 post injury (Hu et al.), whilst in another, the chemokine displayed bimodal upregulation, with levels peaking at day 1 and then again at day 5 post wounding (Xu et al., 2013). Despite differences in the kinetics involved, blocking of CXCR4 on MSCs inhibits their recruitment and impaired wound healing (Hu et al.; Xu et al., 2013). However, *in vitro* expansion of BM MSCs may result in the downregulation of CXCR4 expression (Karp and Leng Teo, 2009). To guarantee robust recruitment of MSCs to the site of injury for the most effective therapeutic results, several studies have genetically modified MSCs to overexpress CXCR4, either by viral transfection (Liu et al., 2013; Yang et al., 2013) or through a deficiency in negative transcription factor regulators (Xie et al., 2013). These studies reported enhanced recruitment and improved tissue repair (Liu et al., 2013; Xie et al., 2013; Yang et al., 2013).

It is now generally accepted that endogenous bone marrow-derived stem cells circulate in the peripheral blood after insult on a distant organ (Orlic et al., 2001; Takahashi et al., 1999). Whether bone marrow (BM) MSCs circulate in humans has been the subject of debate (Hoogduijn et al., 2014; Mansilla et al., 2006; Wang et al., 2006). Despite this, recent studies conducted in animal models of injury have provided robust evidence that endogenous bone marrow-derived stromal cells can circulate and localise in injured tissue (Chen et al., 2010; Gao et al., 2014; Hong et al., 2009; Shu et al., 2013). In a study by Gao et al., endogenous Nestin+ MSCs were recruited to the lungs of *Nes-GFP*-transgenic mice challenged intranasally with cockroach allergen. Histological studies showed activated transforming growth factor (TGF)-β1 signalling in challenged airways, and TGF-β1 was

found to enhance the chemotactic activity of MSCs using *in vitro* chemotaxis assays (Gao et al., 2014). The authors suggested that MSCs recruited to the site of injury would exert their anti-inflammatory properties and alleviate pathogenesis. It is worth noting that this study failed to show whether circulating *Nes*-GFP MSCs were present in peripheral blood, nor did the authors eliminate the possibility that tissue resident MSCs simply proliferated, thereby contributing to MSC accumulation (Gao et al., 2014). On the contrary, Chen and colleagues detected circulating MSCs in the peripheral blood of mice that had undergone acute liver injury. To demonstrate the recruitment of bone marrow-derived MSCs, the authors delivered cultured EGFP+ MSCs via intra-bone marrow injection. Recruitment of EGFP+ MSCs was reported to be mediated by CXCR4/CXCL12, since CXCR4 neutralising antibody studies suppressed the migration of MSCs toward serum collected from mice that had undergone liver injury. MSCs were thought to alleviate fibrosis by decreasing collagen I deposition (Chen et al., 2010). In another study investigating fibrotic injury, MSC recruitment was actually reported to assist in fibrogenesis. Studies were conducted in a parabiosis model where GFP+/CD44+/CD45-/CD105+ MSCs originating from the saline-treated parabiont were detected by flow cytometry in the lungs of the bleomycin/radiation treated parabiont. Robust upregulation in serum and BAL CXCL12 levels following injury highlighted that the CXCR4/CXCL12 axis mediates MSC recruitment. Contrary to the previous study, inhibition of MSC recruitment with a CXCR4 antagonist attenuated radiation-induced fibrosis (Shu et al., 2013).

Another mediator of stem cell migration is the sphingolipid sphingosine-1-phosphate (Bendall and Basnett, 2013; Christopherson, 2012). This molecule has been implicated in the recruitment of cultured MSCs after chemically-induced liver injury. Li *et al.* showed that recruited MSCs supplied a new source of myofibroblasts and thus supported the process of fibrosis (Li et al., 2009). Further signalling studies by Kong and colleagues identified that S1P-mediated migration of MSCs occurred via S1P receptor 1 and 3, initiating G_i-dependent activation of ERK1/2 signalling (Kong et al., 2014). Other studies reporting on the recruitment of cultured MSCs in bleomycin-induced lung injury have supported opposing roles of these cells in terms of promoting fibrosis (Rojas et al., 2005; Song et al., 2010).

Unlike most published studies on MSCs homing to sites of injury, pericyte recruitment is based on *in vivo* data, mainly in support of their recruitment from the local vasculature. It is interesting to note that studies which report the bone marrow as the source of pericytes in recruitment may in fact be replicating studies investigating BM MSCs (Pi et al., 2011). PDGFRβ/PDGF-BB is the main signalling pathway mediating pericyte recruitment during vessel development (Hellstrom et al., 1999). This same pathway has been reported in the recruitment of pericytes to injured tissue, where they eventually contribute to fibrosis in organs such as the lung (Hung et al., 2013) and kidney

(Humphreys et al., 2010; Lin et al., 2011; Schrimpf et al., 2012). Activated pericytes have been shown to detach from local capillaries, migrate to the site of injury and differentiate into myofibroblasts (Goritz et al., 2011; Lin et al., 2011; Ren et al., 2013). However, the recruitment of pericytes following injury and their collagen-producing capacity may be dependent on both the type of injury and the tissue under investigation (Birbrair et al., 2014a; Nakagomi et al., 2011). Activated pericytes have been shown to upregulate genes associated with migration, for example ADAMTS1 and TIMP3 (Hung et al., 2013; Schrimpf et al., 2012). Furthermore, the increased motility of these cells is coordinated by the upregulation of ADAMTS1 peptidase and downregulation of TIMP3 (Schrimpf et al., 2012). A study conducted by Lin *et al.* showed detachment of pericytes at day 1 post injury. In the same study, VEGF was reported to work alongside PDGF-BB in regulating pericyte recruitment (Lin et al., 2011). Another cytokine that has been identified to mediate pericyte recruitment in the lung (Liu et al., 2010), retina (Pi et al., 2011) and kidney (Ren et al., 2013) in response to injury is ciliary neurotrophic factor (CNTF). CNTF is a neurotrophic factor which primarily functions in the differentiation, survival and regeneration of neurons (Sendtner et al., 1994). In a study investigating kidney fibrosis, CNTF produced at the site of injury activated a migratory phenotype and recruited pericytes via LRP-6/WNT ligand and JNK-dependent signalling (Ren et al., 2013). The mediators and pathways responsible for pericyte recruitment may be targeted for treatment to prevent severe fibrogenesis in a number of organs and tissues; for example, the scar formation and fibrosis seen in spinal cord injury have been associated with pericyte recruitment. Goritz *et al.* observed that Type A pericytes (PDGFRβ+/CD13+/desmin-) detach from the vascular basal lamina, invade the surrounding tissue and contribute to scar formation in spinal cord injury. Despite not showing the mediator responsible in this recruitment, this study used powerful techniques such as genetic labelling as well as electron and fluorescent microscopy to visualise the process of detachment, recruitment and invasion (Goritz et al., 2011).

Promoting angiogenesis during tissue repair *in vivo*

Angiogenesis

While much research has been performed using exogenous sources of pericytes in artificial *in vivo* models, the innate role played by pericytes in tissue regeneration and repair is still unclear (Crisan et al., 2011). However, one indisputable function of pericytes is their contribution to angiogenesis in early development, tissue regeneration and pathological conditions. Angiogenesis requires complex coordination between anti- and pro-angiogenic growth factors, extracellular matrix proteins, proteases and adhesion molecules interacting with multiple cells and tissues for vessel sprouting and

growth – processes in which pericytes play a leading role. ECs detach from the vasculature, proliferate and migrate to line new blood vessels, while macrophages and other inflammatory cells infiltrate tissues following hypoxia and, together with other cells such as fibroblasts, secrete VEGF, PIGF, FGF2 and PDGF, which are well-known growth factors necessary for constructing new blood vessels and restoring circulation (Figure 2).

Following injury, in the initial stages of angiogenesis, the hypoxic state activates pericytes and pericytes develop a bulky cytoplasm with shortened cytoplasmic processes which lead to a highly proliferative status with a pluripotent ability to differentiate into smooth muscle cells, matrix-forming cells, adipocytes or other progenitors. Pericytes project into perivascular spaces, thereby disrupting the basement membrane, and dissociate from the wall of the vasculature; thus, they are the first cells to invade newly vascularised tissue. This activity is similarly observed in early development where individual pericytes can be found at the tip of angiogenic sprouts, making pericytes the first cells to invade the corpus luteum (Gerhardt and Betsholtz, 2003). The pericyte located at the front of the growing vessel guides the endothelial sprout and determines the formation and location of the new vessel, which in a damaged environment is thought to be guided by a graded distribution of VEGF-A levels (Gerhardt and Betsholtz, 2005). As previously mentioned, Ang-1 is produced by pericytes and smooth muscle cells, which activates endothelial Tie-2, increasing the strength of the peg and socket interaction between EC and pericytes. Ang-2 is expressed by ECs, particularly during tissue regeneration (Hu et al., 2014); it binds competitively with Ang-1 for Tie-2 and acts as an opposing factor in pericyte-EC interactions. Furthermore, increased Ang-2 expression dissociates pericytes from vessels (Ziegler et al., 2013). It has been shown transgenic mice overexpressing Ang-2 develop dense vascular networks with drastically reduced pericyte numbers (Feng et al., 2007).

In addition to anchoring and providing structural integrity to pericytes, the basal lamina has been suggested to promote pericyte quiescence and control their function. When activated, pericytes migrate through the basal lamina and it is suggested that this migration is necessary for pericytes to activate their stem cell capacity (Dore-Duffy and Cleary, 2011). Pericytes then differentiate into smooth muscle cells or other progenitor cells, which then suppresses the growth and migration of endothelial sprouts which cease vessel tube formation (Gerhardt and Betsholtz, 2005). Strong evidence shows that the newly formed microvessels are unstable without the formation of a pericyte matrix and subsequent tight interactions including the peg and socket interactions and gap junctions (containing N-cadherin, β-catenin and other adhesion molecules) between ECs with pericytes that appear during vessel stabilisation (Ozerdem and Stallcup, 2003). The mechanisms by which pericytes mediate vessel stability in injury are still unclear, but it is likely that both pericytes

and endothelial cells contribute to influence vessel stability by the engagement with matrix molecules such as fibronectin and integrins, and/or by the release and activation of PDGF-BB, VEGF-A and Ang-1 that promote EC survival (Jain and Booth, 2003). As a strong indication of the necessity for pericytes, depletion of pericytes in PDGFRβ$^{-/-}$ animals leads to the rupture and collapse of newly formed vessels (Bjarnegard et al., 2004). The sites of pericyte/endothelial interactions are altered in pathological disease. In diabetes and cerebral oedema, gap junctions are substantially decreased in the retina and disrupted in retinal pericytes (Yamanishi et al., 2006), indicating the importance of pericyte- endothelial cell communication for adaptability to injury.

Using endosialin+ (CD248) pericytes (a marker associated with new sprouts but not mature quiescent vessels), a recent study showed that the loss of endosialin+ pericytes results in EC apoptosis and detachment in the retina and in tumours (Simonavicius et al., 2012). Whether or not the loss of EC cells lead to vessel regression or vessel regression leads to EC apoptosis is still being debated, although both scenarios are possible. In a human study using eccentric contractions to cause skeletal muscle injury, early expression of NF-κB, a strong inducer of angiogenesis, was shown to be induced primarily in muscle-residing pericytes (Hyldahl et al., 2011).

It is also known that the interaction of pericytes with ECs induces TGF-β1, which stimulates perivascular cell proliferation (Jo et al., 2013). MSCs are also strong promoters of angiogenesis and produce basic FGF and VEGF-A which further promotes proliferation, recruitment and stabilisation of the microvasculature (Lozito et al., 2009b). BM-derived MSCs have been shown to enhance vascular formation *in vivo* in many injury models and represent a promising target for stem cell therapies (extensively reviewed elsewhere).

Recent studies have further investigated the role of pericytes in angiogenesis and showed a distinction between pericyte populations by examining nestin-GFP-/NG2-DsRed+ (termed Type 1) and nestin-GFP+/NG2-DsRed+ (termed Type 2) pericytes. While both subpopulations are present in vessel vasculature, Birbrair et al. showed that only Type 2 pericytes induce ECs to form new blood vessels in vivo and participate in tumour angiogenesis in vivo (Birbrair et al., 2014b). Ultimately, the damaged environment determines the distinction process as to whether multipotent pericytes become vascular smooth muscle cells, differentiate into progenitors or undergo both processes. In disease states, pericyte differentiation into osteoblasts has been linked to ectopic calcification (Collett and Canfield, 2005), and in fibrotic skin conditions, pericytes have been identified as the primary source of scar-forming fibroblasts (Rajkumar et al., 2005). The end-point tissue required for regeneration determines the quality and levels of specific inducers present or secreted in the tissue for the differentiation process (Crisan et al., 2012).

Muscle injury

Studies have shown that muscle-specific stem cells called satellite cells located between the basal lamina and sarcolemma of individual myofibres participate in skeletal muscle repair in response to injury, along with a range of other cells with myogenic potential, such as the muscle side population cells, ECs, interstitial cells and pericytes (Birbrair et al., 2013a). There is controversy as to the origins of satellite cells, and there are studies which suggest that they are completely distinct from pericytes and do not share any phenotypic or functional similarities (Caplan, 2008). However, *in vitro*, pericytes have been shown to be myogenic, and when delivered exogenously, they have been shown to induce a higher regenerative muscle index (Crisan et al., 2008). The differences between these studies may be due to quantitative results from the expansion of pericytes isolated from different stages of life (early foetal or adult tissue), which may express different markers. Plausibly, pericytes residing in the skeletal muscle have a bipotent capacity and contribute to both the smooth muscle layer of blood vessels as well as to skeletal muscle fibres by forming satellite cells post-injury in adult life (Dellavalle et al., 2011). Using tracking studies with alkaline phosphatase (AP) as a marker of a subset of pericytes in the striated muscles of Cre-ER mice, Dellavalle et al. showed a dramatic rise in the AP+ pericyte contribution to skeletal myogenesis, corresponding to about a five-fold increase in the area of acute regeneration following cardiotoxin assault and a three-fold increase in dystrophic muscle (Dellavalle et al., 2011). These results suggest that AP+ pericytes respond to skeletal muscle regeneration in response to chemical injury and may be of interest in muscle repair therapies. With regards to adipogenesis, Uezumi et al. showed that PDGFRα+ MSCs, completely distinct from PDGFRβ+ pericytes, are responsible for adipogenesis in skeletal muscles and a balance between satellite cell-derived myofibres and PDGFRα+ cells maintain muscle homeostasis (Uezumi et al., 2010). However, differentiation of pericytes into adipocytes has been documented (Birbrair et al., 2013a; Farrington-Rock et al., 2004; Tang et al., 2008), and the differences between studies may well be dependent on the muscle condition and the subsequent interaction between cell types in the context of muscle injury.

Further work distinguishing pericyte populations by Birbrair et al. showed that Type 2, but not Type 1, pericytes form myotubes in culture, and only Type 2 pericytes contribute to muscle regeneration by forming myofibres *in vivo* after injury, indicating that only Type 2 pericyte subpopulation is myogenic and of use in stem cell therapy. Further studies investigating whether the absence of Type 2 pericytes may compromise skeletal muscle regeneration endogenously may be of use to advance therapies utilising pericytes for tissue repair (Birbrair et al., 2013a). Of note, the host microenvironment for cell therapy appears to be crucial as it has been shown that regenerative capacity of pericytes decreases when transplanted into older recipients (Birbrair et al., 2013d).

Neurological injury

The response of pericytes to neural injury is largely unclear, and currently a large amount of research is dedicated to investigating the role of pericytes in neuromuscular junction denervation. The loss of nerve supply due to trauma, injury, disease or ageing can cause significant progressive skeletal muscle degeneration. It is known that peripheral nerves have the ability to regenerate due to the capacity of peripheral axons to reoccupy neuromuscular junctions in denervated muscle fibres and that even without injury, nerves are constantly renewed during the regeneration of muscles (Li and Thompson, 2011).

To gain insight into the dynamics of the pericyte contribution to angiogenesis in a model of spinal cord injury, R26R-YFP+ labelled pericytes were shown be increased in number at the lesion at days 3-5 post injury with a correlated increased in the density of blood vessels (Goritz et al., 2011). Activated pericytes upregulate NG2, and in human stroke, pericytes have also been shown to upregulate RGS5 and co-express the macrophage/microglial marker galectin-3 (GAL-3) (Berger et al., 2005). A recent paper by Ozen et al. showed that in mice subjected to focal brain ischaemia GFP+ pericytes (driven by the RGS5 promoter) migrate into the ischaemic brain parenchyma, adopt an active morphology and express microglial markers IBA-1, CD11b and GAL-1 at one week post-ischaemia. Consistent with this, human pericytes exposed to hypoxic conditions *in vitro* similarly upregulate several microglial genes (CD11b, GAL-2, IBA1, TNF-α and MHC11), demonstrating the ability of pericytes to acquire a microglial phenotype (Ozen et al., 2014). Further work has shown that Type 2 pericytes form oligodendrocytes under *in vitro* culture conditions and differentiate into mature oligodendrocytes and Schwann cells (Birbrair et al., 2013b), indicating that pericytes may constitute a previously unknown vascular source of microglial cells in stroke and may therefore play a role in the pathogenesis of tissue damage after injury (Ozen et al., 2014). Again, fate mapping looking at both pericytes and satellite cells after skeletal muscle denervation would be helpful to identify whether pericytes form peri-/endoneural cells and Schwann cells or whether satellite cells are the ones that differentiate into Schwann cells, neural cells or even pericytes, ECs and smooth muscle cells to replenish the damaged peripheral nerve niche.

Tissue fibrosis

Fibrosis is caused by an excessive accumulation of extracellular matrix components such as collagen and occurs in injury and chronic disease. Fibrous tissues may prevent normal tissue recovery, particularly in the muscle where fibrosis has been shown to lead to progressive skeletal muscle injury, for example in Duchenne's muscular dystrophy (Acuna et al., 2014). Previous work has suggested that PDGFRβ+ pericytes contribute to scar formation (Arimura et al., 2012) and express

fibroblast-associated markers (such as collagen IV, fibronectin and fibroblast surface proteins). Using a *coll1a1* (collagen-1, α1) reporter mice to determine where scar-associated myofibroblasts generate extracellular matrix collagen type 1, Lin et al. demonstrated that pericytes and perivascular muscle cells are a major source of myofibroblasts in a unilateral ureteral obstruction (UUO) kidney fibrosis model. The authors go on to show that the differentiation of pericytes into myofibroblasts is driven by SnaiI and Id1 transcripts (Lin et al., 2008). Further studies employing fate mapping in mouse models of UUO have established that pericytes, not ECs, undergo proliferative expansion and differentiate into myofibroblasts during UUO (Humphreys et al., 2010). Further analysis into Type 1 and 2 subpopulations of pericytes show that pericytes react differently to injury depending on the organ affected. In nestin-GFP/NG2+-DsRed transgenic mice, Type 1 pericytes are shown to accumulate in surrounding blood vessels and produce collagen in the aging skeletal muscle and in pulmonary fibrosis following bleomycin treatment, but do not in renal and cardiac fibrosis post-injury (Birbrair et al., 2014a). Therefore, the potential for therapeutic use of pericytes will gain a significant boost from understanding the roles of specific pericyte subsets in pathological conditions and in aging.

In contrast, MSCs are known to be immunomodulatory once activated by the inflammatory cytokines IFN-γ, TNF-α and IL-1β, and their capability to regulate invading leukocytes and control T-cell recruitment, proliferation and activity has been well-studied (Ren et al., 2008). There is also evidence that MSCs suppress the proliferation of B cells and NK cells, thereby attenuating the acute immune response in the injured environment. In contrast to pericytes, this would suggest that MSCs would likely reduce the pro-fibrotic response in wound healing. In addition, MSCs have been shown to upregulate paracrine factors such as integrin α7 and downregulate ICAM, VCAM and MCP11, which would favour specific wound healing by resident dermal fibroblasts which more closely represent the surrounding dermal tissue, thereby preventing fibrotic scar formation (Smith et al., 2010). *In vitro*, MSCs have also been shown to differentiate into different dermal cell types in the wound environment, such as epidermal cells, keratinocytes and microvascular ECs, which may present an additional mechanism by which MSCs prevent fibrosis (Lozito et al., 2009a).

Pericytes have also been shown to play a role in the innate immune response by controlling leukocyte trafficking to inflamed tissue (Stark et al., 2013). Following the induction of sterile inflammation, NG2+ pericytes have been shown to express ICAM-1, allowing engagement with monocytes and neutrophils, and to secrete chemoattractants such as MIF (macrophage migration-inhibitory factor). The resulting change triggers the migration of neutrophils and macrophages from postcapillary venules mediated by ICAM-1 and MIF (Stark et al., 2013). Using 4D real-time microscopy, Proebstl et al. observed that pericytes actively support neutrophil migration by changes

in morphology allowing for larger gaps whereby neutrophils enter into the circulation (Proebstl et al., 2012). Furthermore, neutrophils were shown to crawl along pericyte processes towards the spaces between pericytes. Inhibition of this crawling, by blockade of ICAM-1 and its leukocyte integrin ligands Mac-1 and LFA-1, prevented breaching of neutrophils through the pericyte layer. Again, the interaction between ECs and pericytes play a role where neutrophil adhesion to both cell types was found to be Mac-1 dependent, and increasing neutrophil Mac-1 surface expression increased the ability of neutrophils to transverse pericytes. In contrast, transduction of ICAM-1 alone on pericytes increased neutrophil adhesion but not extravasation, indicating the importance of both ECs and pericytes in the leukocyte trafficking cascade (Ayres-Sander et al., 2013). In a recent paper using cultured pericyte-derived ECM, Sava et al. showed that pericytes respond by altering their basement membrane composition, favouring neutrophil extravasation and increasing the ratio of fibronectin to collagen I in response to inflammatory stimuli (Sava et al., 2015). In conclusion, the importance of pericytes, their interactions with the ECM and their altered expression of adhesion molecules and cytokines should be considered when investigating immunomodulation in tissue injury.

Cancer

One of the most studied areas of angiogenesis is in cancer research. Here, focus has shifted to pericytes and their interactions with ECs to better understand how to control angiogenesis with regards to preventing tumourigenesis and metastasis (Raza et al., 2010). In healthy tissue, as mentioned earlier, pericytes have a stellate morphology and an elaborate system of extensions which protect ECs, providing mechanical stability to fragile blood vessels (Diaz-Flores et al., 2009). Unlike those on corresponding normal vessels, pericytes on tumour vessels uniformly express αSMA on capillary-size vessels, are loosely associated with ECs, have cytoplasmic processes that project into the tumour parenchyma, and form a sleeve around endothelial sprouts that is longer than the sprouts themselves (Morikawa et al., 2002). Indeed, pericyte deficiency may be responsible for the abnormalities seen in tumour blood vessels and may contribute to increased tumour cell permeability into the vasculature. To look into this further, depleting PDGFRβ+ pericytes in a mouse model led to the suppression of tumour growth but a corresponding increase in the metastatic potential of the tumour due to leaky unstabilised vessels from poor pericyte coverage (Cooke et al., 2012). Indeed there are concerns regarding targeting pericytes in cancer treatment, as pericyte depletion triggers HGF/Met activation, a key promoter for EMT and metastasis, and is often a poor prognosis in disease progression. Again, further understanding of the subpopulations of pericytes might be beneficial, for example by only targeting Type 2 pericytes which have been shown to contribute to angiogenesis in tumour models (Birbrair et al., 2014b). In other studies targeting

pericyte-EC connections, Jain et al. used an anti-angiogenic therapy to destabilise pericyte-EC socket junctions by targeting ECs using tyrosine kinase inhibitors to block PDGF receptor function and enhance the inhibition of VEGF (Jain and Booth, 2003). Tumours were shown to regress and vessel normalisation was demonstrated, indicated by normalised pericyte coverage, tumour perfusion and increased chemotherapy sensitivity. The treatment both removed pericytes which were loosely connected to the diseased vasculature, causing vessel collapse, while simultaneously did not affect normal vessels, thereby enabling the delivery of cytotoxic drugs to the tumour (Baranowska-Kortylewicz et al., 2005; Jain and Booth, 2003). Therefore, targeting pericyte-EC interactions by inhibiting PDGF signalling with treatments such as Gleevec (imatinib) (Wilkinson-Berka et al., 2004) have strong potential in cancer therapy by disrupting pericyte support of the tumour vasculature.

Contribution of MSCs and pericytes to *ex vivo* tissue engineering

Ultimately, tissue engineering aims to replicate the process of organogenesis. Commonly, cells are cultured *in vitro*, seeded onto bio-scaffolds, and grafted into the site of injury, where the implanted cells endeavour to repair and replace the damaged tissue. Efforts, very much in their infancy, are also underway to generate entire organs in the laboratory that can be used for transplantation in the clinic. Central to the success of both therapeutic strategies is the creation of a sufficiently vascularised tissue; therefore, the main role of pericytes in tissue engineering is that of angiogenesis and neo-vascularisation. Moreover, the progenitor cell potential and secretion of pro-repair factors by pericytes means that their recruitment to sites of injury is highly desirable.

Repair occurs via two mechanisms: regeneration, where damaged cells are replaced, or repair through granulation, where MSCs differentiate in conjunction with angiogenesis and macrophage recruitment (Diaz-Flores et al., 2012). Generally, tissues with the capacity to repair via granulation are located close to perivascular niches (Diaz-Flores et al., 2012). For *in vivo* bone and cartilage repair, pericytes and/or MSCs are recruited to the site of injury, where they proliferate and differentiate, produce matrix and growth factors, and integrate with native tissue (Diaz-Flores et al., 2012). Generation of a clot induces angiogenesis and a wound healing response, stimulating recruitment, proliferation and differentiation of perivascular MSCs (Diaz-Flores et al., 2012).

Previously, sources of MSCs for the regeneration of mesenchymal tissue have included the bone marrow, adipose tissue and the stromal vascular fraction (SVF) of adipose tissue (Chung et al., 2014). However, these cell types are fraught with disadvantages; it is necessary to expand bone marrow-derived MSCs in culture, and they exhibit limited cellular activity in elderly patients; adipose MSCs

are immunogenic, and genetically unstable; and SVF MSCs are notoriously heterogeneous, leading to inefficient and unreliable tissue formation (Chung et al., 2014). Therefore, it is desirable to utilise specific cells with MSC capacity from the perivascular niche which are, in theory, obtainable from any microvascularised tissue.

As discussed earlier, pericytes can be isolated from tissue using culture-based methods, or using flow cytometry-based sorting on the basis of CD146, NG2 and/or PDGFRβ expression. Pericytes can also be differentiated from human induced pluripotent stem cells (hiPSC) by the generation of embryoid bodies or, more reproducibly, by culturing in standardised mTesR1 medium, replacing with mesoderm induction medium, and differentiating magnetically-isolated CD31- cells in 10% FBS DMEM supplemented with TGF-β3 and PDGF-BB (Orlova et al., 2014b). Another perivascular population located in the tunica adventitia of larger vessels (and therefore commonly referred to as adventitial cells) are, unlike other MSC, CD34+, and have also been shown to participate in tissue regeneration; many studies combine pericytes and adventitial cells for tissue engineering strategies (Askarinam et al., 2013; James et al., 2012a; James et al., 2012b; James et al., 2012c). This section reviews the current therapeutic potential for these perivascular populations in the fairly novel field of cell-based tissue engineering.

In vitro tissue models

There is an onus to develop *in vitro* 3D models of tissues for drug toxicity and efficacy testing and to mimic disease states, as an ethical alternative to animal models. Moreover, a significant hurdle with generating tissue thicker than 200 μm is the necessity for a microvascular system for molecular transportation, as diffusion ceases to be a viable mechanism.

Pericytes co-cultured with HUVECs form tubular structures akin to blood microvessels, with pericytes tightly associated on the abluminal surface, closely recapitulating the *in vivo* structure. This phenomenon has been utilised in tissue-engineered microenvironment systems to develop microfluidic-chip models of angiogenesis, the blood brain barrier and cancer cell extravasation (Tourovskaia et al., 2014). In these models, EC-pericyte interactions form extracellular matrices, demonstrate angiogenic sprouting, barrier function, and extravasation of migratory cells, suggesting that they are functionally representative models. Endothelial cells and fibroblasts co-cultured in endothelialised reconstructed connective tissue (ERCT) constructs lead to the formation of capillary-like tubes, with NG2+/αSMA+ pericyte-like cells embedded in the capillary basement membrane (Zehendner et al., 2013); these constructs could be used to model angiogenesis in cancer. These *in vitro* models provide vital clues into the signalling pathways and molecules involved in vascularisation, and pave the way for replicating these mechanisms *in vivo*.

Vascularisation

As previously discussed, pericyte-EC crosstalk is paramount for angiogenesis; pericytes co-cultured with ECs *in vitro* demonstrate endothelial sprouting (Orlova et al., 2014a) (co-culture study). This has been demonstrated using pericytes derived from hiPSC and adventitial sorted cells from human saphenous vein digests (Campagnolo et al., 2010; Orlova et al., 2014a). Recapitulation of these phenomena in *in vivo* implantations would be highly advantageous. Promisingly, injection of adventitial cells sorted from the human saphenous vein improved blood flow recovery following mouse hind limb ischemia and led to the formation of a denser network of capillaries and arterioles as compared to vehicle controls, suggesting the presence of a proangiogenic environment (Campagnolo et al., 2010). In addition, type 2 (nestin+/NG2+), but not type 1 (nestin-/NG2+) pericytes facilitated partial recovery following hind limb ischemia, and were found to form part of the new vessel wall (Birbrair et al., 2014b). Furthermore, Matrigel implantation of human endothelial and pericyte co-culture results in neo-formation of the microvasculature, with perfusion and anastomoses with murine blood vessels (Dar et al., 2012). In this study, pericytes were derived from differentiated human induced pluripotent stem cells (hiPSC) from embryoid bodies, and defined, somewhat controversially as compared to traditional methods, as CD105+/CD31- cells (Dar et al., 2012).

Neo-vascularisation is too protracted a process to allow for perfusion within implanted tissue; therefore, implants containing pre-vascular constructs are desirable. Verrier's group (Duttenhoefer et al., 2013) have shown that human BM MSC isolated via density gradient separation and co-cultured with human endothelial progenitor cells (EPC) (further sorted from BM MSC isolates on basis of CD34 and CD133 expression) seeded on 3D polyurethane scaffolds form complex capillary-like structures, with expression of endothelial markers (CD3, von Willebrand factor) and pericyte markers (NG2, αSMA, CD146). This method of MSC isolation yields a highly heterogeneous population, inclusive of the EPC population; indeed, only a portion of the MSC culture participated in tube formation, and these cells were shown to be CD146+. Moreover, culture of MSCs alone did not result in tube formation, suggesting that enrichment of the EPC population is necessary. Further characterisation of MSC and EPC revealed expression of PDGFRβ, αSMA, CD146 and NG2 mRNA, and some expression of CD31 mRNA in both cell types. It seems that the phenotype of these EPC closely mirrors that of adventitial cells described earlier in that they are CD34/CD133+, and also express pericyte markers. It is possible that they are in fact one and the same, although adventitial cells generally do not express CD146 (Loibl et al., 2014). During three days of culture, whilst PDGFRβ mRNA was downregulated, CD146 and NG2 expression increased (Loibl et al., 2014). These studies suggest that MSCs in culture differentiate toward a pericyte-like phenotype and it is these pericyte-

like cells that participate in vascularisation. Therefore, enrichment of EPC and pericyte-like MSC might be advantageous for pre-vascularisation in tissue implants.

Pericyte-seeded scaffolds such as these have shown promise for use as vascular grafts following implantation in immunocompetent Lewis rats. Cultured flow cytometry-sorted pericytes from human skeletal tissue (CD146/NG2/PDGFRβ+, CD45/CD56/CD34-) seeded onto poly(esterurethane) urea (PEUU) scaffolds have been used to create small-diameter tissue engineered vascular grafts (TEVG) (He et al., 2010). Following implantation, TEVG displayed vascular remodelling as evidenced by collagen and elastin deposition, as well as the presence of αSMA and calponin (He et al., 2010). Despite an intact immune system, implants were not rejected, supporting the notion that MSC-derived implants are less immunogenic, perhaps due to the secretion of anti-inflammatory factors (He et al., 2010). It was suggested that pericytes reduce obstruction in vascular grafts and show promise for use in arterial conduits (He et al., 2010). Of concern however, the grafts did not seem to fully integrate with the host, as the majority of the tissue was rat-derived, rather than human-derived. A comprehensive understanding of the role of perivascular cells in angio- and vasculogenesis as well as vascular stabilisation will provide crucial knowledge on the vascularisation of engrafted tissue.

Bone, fat and cartilage

In vitro tri-lineage differentiation of pericytes into mesenchymal cell types has been well-documented (Crisan et al., 2012; Crisan et al., 2008; Farrington-Rock et al., 2004), and efforts to translate into *in vivo* transplantation models are underway. CD146+/CD34-/CD45-/CD56- pericytes from human skeletal tissue have exhibited osteo-, adipo-, chondro- and myogenic potential. Adventitial cells sorted from human saphenous vein digests, (PDGFRβ+, CD146-) are also capable of clonogenic growth, and osteo, adipo and myogenesis, but do not display chondrogenic potential. In a separate study, adventitial cells that were CD146+ were found to be capable of chondrogenic differentiation, although this might have simply been due to the tissue of origin, as the latter were derived from the meniscus, and therefore may have chondrogenic properties prior to isolation (Osawa et al., 2013).

Pericytes (CD146+/CD34-) and adventitial cells (CD146-/CD34+) comprise 40% of the stromal vascular fraction (SVF) of adipose tissue, which is commonly used in tissue regeneration models. These populations may be combined to increase cell yield, and have been shown to undergo osteogenesis both *in vitro* as assessed by positive alkaline phosphatase staining and formation of bone nodules, and *in vivo*, whereby intramuscular insertions of perivascular cells on collagen sponges induced spontaneous mineralisation (James et al., 2012a; James et al., 2012c). Cultured,

osteogenically-stimulated hiPSC–pericytes have also demonstrated mineral deposition and the formation of bone-like structures when implanted *in vivo* (Dar et al., 2012). Perivascular cells isolated from the SVF have been shown to generate greater ectopic bone formation in a human-mouse xenograft using biological scaffolds as compared to unsorted SVF, suggesting that these are the crucial SVF cells involved in osteogenesis (James et al., 2012a; James et al., 2012c). This is in line with the literature claiming that the quintessential MSC derives from the perivascular niche (Crisan et al., 2008). Their ability to contribute to osteogenic repair has been validated in four distinct models: rat spinal fusion (Chung et al., 2014), murine muscle pouch, murine femoral segmental defect and rat calvarial defect, the latter two of which will not display self-healing due to their critical size (James et al., 2012b). However, although perivascular cells are capable of differentiation into osteoblasts and osteocytes, the majority of neo-bone is of host origin, suggesting that they contribute to bone formation via the secretion of trophic factors (Chung et al., 2014). Furthermore, greater vascularisation occurs with the use of perivascular cells compared to SVF; they did not differentiate into ECs, but rather resume their natural role as vascular mural cells (Askarinam et al., 2013). Thus, the role of the perivascular cell seems three-fold: osteogenic differentiation resulting in new bone formation, participation in vascular support, and secretion of osteogenic factors.

Studies prior to 2012 investigating MSC-based cartilage repair were reviewed by Diaz-Flores et al. (Diaz-Flores et al., 2012). In brief, cultured MSC of a range of phenotypes have shown promise in combination with type II collagen hydrogel scaffolds and delivery of appropriate growth factors (Diaz-Flores et al., 2012). Pericytes isolated using culture-based methods from bovine retina (αSMA+) could be induced to an adipogenic and chondrogenic phenotype, exhibiting oil red O+ droplet formation and expression of PPAR-γ2 mRNA or demonstrating expression of aggrecan, type II collagen and sox2 mRNA, and secreting a type II collagenous matrix *in vitro*, respectively (Farrington-Rock et al., 2004). When injected into athymic mice via a diffusion chamber, mineralised and fibrocartilage were formed, suggestive of a cartilaginous matrix, and small clusters of adipocytes were identified morphometrically (Diaz-Flores et al., 2012; Farrington-Rock et al., 2004). This early study did not determine pericyte marker expression and therefore the pericyte subtypes involved are not clear, although the crude method of extraction suggests a rather heterogeneous population. In a rat model of meniscus tear, cultured adventitial cells (CD146+) from human foetal menisci enhanced cartilage repair compared to PBS and CD34-/CD146- controls (Osawa et al., 2013). The number of pericyte-specific studies in the field of cartilage and connective tissue engineering are limited, and although the studies discussed suggest promising results, it will be necessary to determine whether pericytes from all vascularised tissue can be utilised, or if better results are to be obtained using pericytes derived from a cartilaginous environment.

Skeletal muscle and cardiac repair

The myogenic potential of perivascular cells has been repeatedly demonstrated *in vivo.* As mentioned previously, Birbrair et al. (Birbrair et al., 2013c) has demonstrated that Type 2 pericytes (nestin+/NG2+/CD146+) are myogenic, whilst their nestin- counterparts are not. Induced pericytes are also capable of myogenesis; in the presence of TGF-β and PDGF-BB, hiPSC-derived pericytes co-cultured with hiPSC-derived ECs are capable of differentiation into contractile smooth muscle cells *in vitro*, as demonstrated by expression of the contractile markers SM22 and CNN1 (Orlova et al., 2014a). Moreover, CD105+ pericytes isolated from hiPSC-derived embryoid bodies have been found to aid in ischemic muscle recovery and display direct myogenic differentiation, as indicated by desmin expression, in addition to providing vascular support by integrating with the host vasculature (Dar et al., 2012).

Adventitial cells from the human saphenous vein engrafted into infarcted mouse hearts retained their pericytic phenotype, as suggested by expression of NG2 and PDGFRβ and their perivascular localisation along perfused vessels (Katare et al., 2011). Transplantation of CD146+/CD34-/CD45-/CD56- pericytes isolated from human skeletal muscle into mice with ischemic heart disease has been found to improve cardiac function, likely due to a combination of increased vascularity, reduced fibrosis and immunomodulation (Chen et al., 2013). Moreover, pericytes integrated with host cardiac tissue, surviving and proliferating in addition to differentiating into cardiomyocytes, which was consistent with the loss of αSMA expression (Chen et al., 2013).

An advantage of using autologous cell therapy is of course the acceptance of the tissue by the host immune system. However, use of autologous material carries an inherent limitation in that the progenitor capacity of pericyte-like cells is compromised with age; therefore, in elderly populations, tissue grafts composed of host tissue will require additional considerations. The use of polyethylene-glycol (PEG)-fibrinogen scaffolds has shown promise in restoring myogenic and vasculogenic potential, and pericytes isolated from adult pigs grafted into mice on PEG-fibrinogen scaffolds have shown increased myogenic potential and promoted ectopic tissue formation comparable to similar to changes driven by young pig-derived pericytes (Fuoco et al., 2014).

It is clear that pericytes from a range of sources, isolated in numerous ways, and of various phenotypes, show bioengineering potential. However, lack of standardisation regarding perivascular marker expression and that of their subtypes renders comparison between studies and overall conclusions difficult. Common limitations seem to be the lack of integration of grafts with host tissue, although further understanding of the roles of the various perivascular subtypes will help overcome this issue. It is unclear whether perivascular cells from any tissue can contribute to the

regeneration of bone, fat, muscle, cartilage and neuronal, or if, for a more specialised tissue, they must be obtained from a similar niche. Further understanding of the various mechanisms by which perivascular cells contribute to repair, such as direct differentiation, secretion of pro-angiogenic and immunomodulatory growth factors, participation in vasculogenesis and stabilisation of microvasculature, will propel this field forward.

Pharmacological interventions to enhance tissue repair

The concept of MSCs being present in the peripheral blood under normal physiological conditions is divisive; some studies have identified low frequencies of MSCs within the circulation, whereas other studies have been unable to detect their presence (He et al., 2007). However, in response to injury, cytokines, chemokines and neuroendocrine modulators are released, initiating processes thought to mobilise and recruit regenerative cells. This hypothesis is substantiated by an observed increase in putative MSC mobilisation post-injury, correlating with an increase in VEGF and G-CSF levels in the blood (Wang et al., 2008a). This artefact is presumed to reflect that the MSCs are en route to the site of damage to promote tissue repair. Furthermore, injury-induced peripheral blood MSCs can be cultured up to ten passages and can differentiate into osteocytes and adipocytes *in vitro* (Wang et al., 2008a).

Several pre-clinical and clinical trials have looked at the therapeutic benefits of systemic infusion of *ex vivo* isolated and expanded MSCs, but there are problems with the consistency, heterogeneity and delivery of these cells (Main et al., 2014). Consequently, identifying the intrinsic signals involved in MSC mobilisation could allow for targeted pharmacological intervention (Figure 3) to exploit this system and induce the mobilisation of endogenous MSCs as a therapy, with the goal of improving tissue regeneration and/or managing inflammation; unfortunately, there are currently few examples of this phenomenon.

It is therefore of note that the exposure of rats to chronic hypoxia (3 weeks) increases peripheral blood colony forming unit fibroblasts (CFU-Fs) by almost 15-fold (Rochefort et al., 2006). Subsequently, it was discovered that there is a significant but comparatively low level CFU-F mobilisation after only 2 days of hypoxia that steadily increases over time (Liu et al., 2011). These hypoxia-derived blood CFU-Fs display tri-lineage differentiation capacity and express CD29, CD44, CD54, CD73 and CD90, and are CD31, CD34 and CD45 negative; an expression profile directly comparable to bone marrow-derived MSCs (Liu et al., 2011; Rochefort et al., 2006). Hypoxia-induced MSC mobilisation is dependent on increased expression of hypoxia inducible factor 1 (HIF-1);

consequently, the HIF-1α target genes VEGF and SDF-1α have been assessed. VEGF expression was found to be elevated in the bone marrow and correlated with an increase in sinusoidal vessels. In addition, VEGF is known to increase vascular permeability and blood flow, which may play a role in MSC mobilisation. CXCL12 (SDF-1α) levels are also increased in the peripheral blood of hypoxic rats and could stimulate MSC migration *in vitro* under hypoxic conditions, suggesting that MSCs react to a CXCL12 gradient *in vivo*. Moreover, pre-treatment of mice under normoxic conditions with VEGF for 4 days in combination with the acute CXCR4 inhibitor (AMD3100) treatment also mobilises CFU-Fs, which were characterised as having an expression profile analogous to bone marrow-derived MSCs and can undergo tri-lineage differentiation (Pitchford et al., 2009). This treatment programme appears to mimic the physiological response observed in hypoxic conditions and reinforces the idea that VEGF signalling and disruption of the CXCR4-CXCL12 axis are important factors for MSC mobilisation.

It is well-known that granulocyte-colony stimulating factor (G-CSF) pre-treatment mobilises haematopoietic stem and progenitor cells (HSPCs) and is used clinically for bone marrow transplantation (Tatsumi et al., 2008); its effect can be further enhanced using a CXCR4 antagonist (AMD3100). In addition, acute treatment with AMD3100 alone can mobilise HSPCs, although in comparably fewer numbers. However, the ability of G-CSF or AMD3100 to also mobilise MSCs is contentious, with several studies claiming to be able to demonstrate this, but there has been a consistent failure to establish that mobilised peripheral blood cells fulfil the basic requirements to be characterised as MSCs. In contrast, other studies have actively discounted the ability of G-CSF or AMD3100 to mobilise bone marrow MSCs. Most notably, Pitchford et al. showed that, in uninjured mice, there was no increase in the number of circulating CFU-Fs with either of these treatments alone or in combination (Pitchford et al., 2009).

The complete mechanism of G-CSF HSPC mobilisation is still unclear, but G-CSF mediated disruption of the CXCR4-CXCL12 axis appears to be an important step (Ehninger and Trumpp, 2011). Thus, the presence of G-CSF and VEGF simultaneously, as observed post-injury, may synergise to promote MSC mobilisation. Interestingly, the vitamin E analogue γ-tocotrienol (GT3) induces the endogenous production of both G-CSF and VEGF, and leads to mobilisation of CFU-Fs after 24 h with the effect lasting for up to 72 h (Ray et al., 2013). CFU-F mobilisation was further enhanced when GT3 treatment was combined with the CXCR4 antagonist AMD3100. However, further studies are required to properly characterise these CFU-Fs to demonstrate whether or not they represent MSCs.

Identification of other endogenous signalling factors released in response to injury will help further delineate the complex mechanisms of MSC mobilisation. For instance, the concentration of

substance P, a neuropeptide involved in pain perception, has been shown to increase in the circulation in response to corneal injury (Hong et al., 2009). This was associated with the appearance of stromal-like cells in the circulation, which was determined to be due to higher circulating levels of substance P, since i.v. injection of substance P in uninjured mice, rats and rabbits elicited the same response. The mobilised CD29-positive stromal cells were comparable to bone marrow MSCSs and were able to differentiate into cartilage *in vitro* and bone when transplanted *in vivo* (Hong et al., 2009).

Transforming growth factor β1 (TGF-β1) is a signalling molecule thought to act as a molecular sensor, converting into its active form in response to injury to aid tissue regeneration. Using a model of arteriole damage, stromal cells characterised as expressing Sca1, CD29 and nestin that were CD11b and CD45 negative were mobilised post injury, correlating with an increase in the level of peripheral blood TGF-β1. It was indirectly demonstrated that this cell expression profile selected for cells that had mesenchymal tri-lineage differentiation potential. Accordingly, treatment of uninjured mice with active TGF-β1 led to a ~2.5-fold increase in the circulation of these cells after 24 hours (Wan et al., 2012). Since there is a lack of definitive MSC markers, subsequent research would benefit from observing whether or not TGF-β1 treatment can increase the number of peripheral blood CFU-Fs, which would allow for direct characterisation of individual clones from these cell colonies.

In the research field of pharmacologically induced MSC mobilisation, the data regarding the characterisation of MSCs and the kinetics of their egress are not consistent, and in some cases may need confirmation. Future studies need to focus on using consistent methods to establish the existence of peripheral blood MSCs. In the absence of a clear expression profile with which to define MSCs, it is crucial that at the very least they must meet the criteria for defining MSCs as proposed by the International Society for Cellular Therapy (Dominici et al., 2006). This will allow for clear and easy comparison between studies to establish a central dogma on MSC mobilisation.

Pharmacological modification of pericyte mobilisation has also been investigated in recent years. It is well-understood that pericytes are mobilised in response to PDGF-BB during the process of angiogenesis; PDFG-BB signalling via PDGFRβ stimulates the p44/p42 MAP kinase, stress-activated protein kinase/c-Jun N-terminal kinase (SAPK/JNK), PI3-kinase and PKC pathways to induce vascular smooth muscle cell/pericyte migration (Pukac et al., 1998; Zhan et al., 2003). Recently, it has also been shown that PDGF-BB mediated pericyte migration can also be suppressed *in vitro* by inhibiting adenosine monophosphate-activated protein kinase (AMPK); this strategy was applied both pharmacologically and using siRNA to inhibit the activity of AMPK in cultured rat A10 pericytes. Pharmacological inhibition was found to suppress pericyte migration in a Boyden chamber assay,

while the compound AICAR, used to stimulate AMPK activity, was found to enhance pericyte migration toward PDGF-BB *in vitro*, suggesting that exploiting this pathway could be a promising target to manipulate pericyte migration (Iida et al., 2013).

A number of therapeutic strategies have also been investigated *in vivo* to inhibit pericyte mobilisation (Figure 4), most notably as a means by which to disrupt the blood supply in tumours and induce tumour regression. Tyrosine kinase inhibitors such as sunitinib, nilotinib and imatinib have been used to target PDGFRβ signalling in pericytes in mouse models of cancer as a strategy to inhibit pericyte migration to the developing tumour vasculature. Ruan et al. showed that treatment of human lymphoma-bearing mice with imatinib inhibited pericyte proliferation and PDGF-BB signalling, and led to the depletion of pericytes and endothelial cells in the tumour, with the end results of decreasing tumour size (Ruan et al., 2013). Onoyama et al. expanded on this strategy by combining a tyrosine kinase inhibitor (nilotinib) with an mTOR inhibitor (everolimus) in an orthotopic gastric cancer mouse model, and demonstrated that this combined intervention was able to reduce pericyte coverage of the tumour microvasculature and inhibited tumour growth, whereas treatment with nilotinib alone had effects on tumour pericyte coverage without affecting tumour growth (Onoyama et al., 2013). However, the success of targeting tumour pericytes may be highly dependent on the characteristics of the tumour as well as the delivered dose, as shown by Welti et al., who demonstrated that sunitinib treatment of mice injected with 4T1 and RENCA tumour cells, which both form lung metastases, did not inhibit the growth of 4T1 lung metastases, but was able to block the growth of RENCA lung metastases. The differential effect was related to the degree of vascularisation of tumour nodules, as sunitinib treatment led to profound devascularisation of only RENCA tumours. However, a high dose of sunitinib led to pericyte depletion in the lung, which enhanced the seeding of metastases (Welti et al., 2012). Taken together, these studies support further research into the use of drugs to impair pericyte mobilization in cancer therapy, with the proviso that additional pathways may also need to be blocked, and keeping in mind that impairing pericyte function in healthy tissues may have unexpected negative side effects.

The role of pericytes in driving tissue fibrosis has also been targeted therapeutically. In a recent study, curcumin, a major component of the South Asian spice turmeric, was investigated as a means by which to impair pathological tissue remodelling in a carbon tetrachloride-mediated rat model of hepatic fibrosis. It was found that oral curcumin delivery in carbon tetrachloride-exposed rats was able to dose-dependently inhibit organ fibrosis and vascularisation; the mechanism of action of curcumin was postulated to be related to PDGFRβ transrepression through the activation of peroxisome proliferator-activated receptor gamma (PPAR-γ) (Zhang et al., 2014). Pericytes have also been targeted in a number of mouse models of kidney fibrosis, in which the pericyte-to-

myofibroblast transition has been shown by several groups to contribute to tissue fibrosis (Lin et al., 2008; Scharpfenecker et al., 2013). One recent study used thalidomide, known to have anti-inflammatory and angiogenesis-modulating properties, in a radiation-induced mouse model of kidney fibrosis in an attempt to normalise the kidney microvasculature and inhibit the development of fibrosis. While this intervention was found to improve vessel perfusion, likely by promoting pericyte retention on the vasculature due to increased expression of PDGFRβ, thalidomide caused significant tubular damage and was not able to inhibit tissue fibrosis (Scharpfenecker et al., 2014). However, the most promising results suggesting that pericytes can be targeted in organ fibrosis have focused on inhibiting growth factor receptor signalling in these cells. Using adenoviral vectors to overexpress the soluble ectodomains of PDGFRβ and VEGFR in the UUO model of kidney fibrosis, it was shown that blocking either PDGFRβ signalling in pericytes or VEGFR2 signalling in endothelial cells attenuated tissue fibrosis and capillary rarefaction following kidney injury (Lin et al., 2011). Despite these promising results, it is important to consider that targeting growth factor receptor pathways to inhibit fibrosis in one organ may have unwanted effects on the healthy vasculature in other organs.

Concluding remarks

It is hoped that further advances in the field of regenerative medicine will improve our ability to control the function and differentiation of mesenchymal stem cells and pericytes, direct their migration from the niche to sites of injury and harness their ability to build new organs to replace damaged or exhausted tissues. To achieve the goal of replacing or regenerating damaged organs, there are a number of roadblocks that need to be addressed and surmounted, such as the integration of stem cells into the tissue, understanding the immunomodulatory effects of these cells at the site of injury, determining the most suitable populations of stem cells for the repair of a given tissue and optimizing the differentiation of stem cells following mobilisation or transplantation. Furthermore, translation of preclinical data from animal and cell culture experiments into viable treatments in humans will require considerable effort on the part of researchers, physicians, pharmaceutical and biotech companies and regulatory bodies. Most importantly, the design and reporting of clinical trials and rigorous post-treatment patient follow-up will be vital to reaching a consensus on the therapeutic efficacy of mesenchymal stem cell therapies.

Conflict of Interest

The authors declare that there are no conflicts of interest.

Figure legends

Figure 1. The schematic diagram illustrates the capacity for MSC and pericyte differentiation into separate lineages (grey and pink arrows) and for self-renewal (red arrows). The unique and shared markers of both cell types are shown.

Figure 2. In a quiescent state (top), pericytes have a stellate morphology and a complex system of extensions which protect ECs and reinforce the vasculature. The balance between ECs and pericytes are tightly controlled by signalling pathways operating in a paracrine manner, such as PDGF-BB. In injury, disruption of PDGF-BB expression dissociates pericyte-EC connections, which leads to detachment of ECs and corresponding vessel collapse (bottom). Pericytes are then activated, such that they develop a bulky cytoplasm with shortened processes which allow migration through the basement membrane to invade newly vascularised tissue.

Figure 3. Therapeutic interventions to increase MSC mobilisation from the bone marrow niche have primarily focused on inhibiting CXCR4 signalling on bone marrow MSCs, thereby releasing them from their niche. This strategy can be combined with other systemic stimuli, such as hypoxia, VEGF and substance P. The arrow indicates the gradient of CXCL12 expression.

Figure 4. Therapeutic interventions to modulate pericyte mobilisation have attempted to inhibit the migration of these cells to the vasculature by impairing PDGFRβ signalling through the use of soluble receptors, tyrosine kinase inhibitors (sunitinib, nilotinib, imatinib, etc.), PPAR-γ activation and AMPK inhibition. The arrow indicates the gradient of PDGF-BB expression.

References

Acuna MJ, Pessina P, Olguin H, Cabrera D, Vio CP, Bader M, Munoz-Canoves P, Santos RA, Cabello-Verrugio C and Brandan E (2014) Restoration of muscle strength in dystrophic muscle by angiotensin-1-7 through inhibition of TGF-beta signalling. *Human molecular genetics* **23**:1237-1249.

Arimura K, Ago T, Kamouchi M, Nakamura K, Ishitsuka K, Kuroda J, Sugimori H, Ooboshi H, Sasaki T and Kitazono T (2012) PDGF receptor beta signaling in pericytes following ischemic brain injury. *Current neurovascular research* **9**:1-9.

Armulik A, Abramsson A and Betsholtz C (2005) Endothelial/pericyte interactions. *Circulation research* **97**:512-523.

Askarinam A, James AW, Zara JN, Goyal R, Corselli M, Pan A, Liang P, Chang L, Rackohn T, Stoker D, Zhang X, Ting K, Peault B and Soo C (2013) Human perivascular stem cells show enhanced osteogenesis and vasculogenesis with Nel-like molecule I protein. *Tissue engineering Part A* **19**:1386-1397.

Ayres-Sander CE, Lauridsen H, Maier CL, Sava P, Pober JS and Gonzalez AL (2013) Transendothelial migration enables subsequent transmigration of neutrophils through underlying pericytes. *PloS one* **8**:e60025.

Baranowska-Kortylewicz J, Abe M, Pietras K, Kortylewicz ZP, Kurizaki T, Nearman J, Paulsson J, Mosley RL, Enke CA and Ostman A (2005) Effect of platelet-derived growth factor receptor-beta inhibition with STI571 on radioimmunotherapy. *Cancer research* **65**:7824-7831.

Bautch VL (2011) Stem cells and the vasculature. *Nature medicine* **17**:1437-1443.

Bendall LJ and Basnett J (2013) Role of sphingosine 1-phosphate in trafficking and mobilization of hematopoietic stem cells. *Current opinion in hematology* **20**:281-288.

Berger M, Bergers G, Arnold B, Hammerling GJ and Ganss R (2005) Regulator of G-protein signaling-5 induction in pericytes coincides with active vessel remodeling during neovascularization. *Blood* **105**:1094-1101.

Birbrair A, Zhang T, Files DC, Mannava S, Smith T, Wang ZM, Messi ML, Mintz A and Delbono O (2014a) Type-1 pericytes accumulate after tissue injury and produce collagen in an organ-dependent manner. *Stem cell research & therapy* **5**:122.

Birbrair A, Zhang T, Wang ZM, Messi ML, Enikolopov GN, Mintz A and Delbono O (2013a) Role of pericytes in skeletal muscle regeneration and fat accumulation. *Stem cells and development* **22**:2298-2314.

Birbrair A, Zhang T, Wang ZM, Messi ML, Enikolopov GN, Mintz A and Delbono O (2013b) Skeletal muscle neural progenitor cells exhibit properties of NG2-glia. *Experimental cell research* **319**:45-63.

Birbrair A, Zhang T, Wang ZM, Messi ML, Enikolopov GN, Mintz A and Delbono O (2013c) Skeletal muscle pericyte subtypes differ in their differentiation potential. *Stem cell research* **10**:67-84.

Birbrair A, Zhang T, Wang ZM, Messi ML, Mintz A and Delbono O (2013d) Type-1 pericytes participate in fibrous tissue deposition in aged skeletal muscle. *American journal of physiology Cell physiology* **305**:C1098-1113.

Birbrair A, Zhang T, Wang ZM, Messi ML, Mintz A and Delbono O (2015) Pericytes at the intersection between tissue regeneration and pathology. *Clinical science* **128**:81-93.

Birbrair A, Zhang T, Wang ZM, Messi ML, Olson JD, Mintz A and Delbono O (2014b) Type-2 pericytes participate in normal and tumoral angiogenesis. *American journal of physiology Cell physiology* **307**:C25-38.

Bjarnegard M, Enge M, Norlin J, Gustafsdottir S, Fredriksson S, Abramsson A, Takemoto M, Gustafsson E, Fassler R and Betsholtz C (2004) Endothelium-specific ablation of PDGFB leads to pericyte loss and glomerular, cardiac and placental abnormalities. *Development* **131**:1847-1857.

Bouacida A, Rosset P, Trichet V, Guilloton F, Espagnolle N, Cordonier T, Heymann D, Layrolle P, Sensebe L and Deschaseaux F (2012) Pericyte-like progenitors show high immaturity and engraftment potential as compared with mesenchymal stem cells. *PloS one* **7**:e48648.

Cai J, Kehoe O, Smith GM, Hykin P and Boulton ME (2008) The angiopoietin/Tie-2 system regulates pericyte survival and recruitment in diabetic retinopathy. *Investigative ophthalmology & visual science* **49**:2163-2171.

Campagnolo P, Cesselli D, Al Haj Zen A, Beltrami AP, Krankel N, Katare R, Angelini G, Emanueli C and Madeddu P (2010) Human adult vena saphena contains perivascular progenitor cells endowed with clonogenic and proangiogenic potential. *Circulation* **121**:1735-1745.

Caplan AI (2008) All MSCs are pericytes? *Cell Stem Cell* **3**:229-230.

Chen CW, Okada M, Proto JD, Gao X, Sekiya N, Beckman SA, Corselli M, Crisan M, Saparov A, Tobita K, Peault B and Huard J (2013) Human pericytes for ischemic heart repair. *Stem cells* **31**:305-316.

Chen Y, Xiang LX, Shao JZ, Pan RL, Wang YX, Dong XJ and Zhang GR (2010) Recruitment of endogenous bone marrow mesenchymal stem cells towards injured liver. *Journal of cellular and molecular medicine* **14**:1494-1508.

Christopherson KW, 2nd (2012) PBSCs Sphingoling along S1P/S1P1 axis. *Blood* **119**:645-646.

Chung CG, James AW, Asatrian G, Chang L, Nguyen A, Le K, Bayani G, Lee R, Stoker D, Zhang X, Ting K, Peault B and Soo C (2014) Human Perivascular Stem Cell-Based Bone Graft Substitute Induces Rat Spinal Fusion. *Stem cells translational medicine*.

Collett GD and Canfield AE (2005) Angiogenesis and pericytes in the initiation of ectopic calcification. *Circulation research* **96**:930-938.

Cooke VG, LeBleu VS, Keskin D, Khan Z, O'Connell JT, Teng Y, Duncan MB, Xie L, Maeda G, Vong S, Sugimoto H, Rocha RM, Damascena A, Brentani RR and Kalluri R (2012) Pericyte depletion results in hypoxia-associated epithelial-to-mesenchymal transition and metastasis mediated by met signaling pathway. *Cancer cell* **21**:66-81.

Crisan M, Corselli M, Chen CW and Peault B (2011) Multilineage stem cells in the adult: a perivascular legacy? *Organogenesis* **7**:101-104.

Crisan M, Corselli M, Chen WC and Peault B (2012) Perivascular cells for regenerative medicine. *Journal of cellular and molecular medicine* **16**:2851-2860.

Crisan M, Yap S, Casteilla L, Chen CW, Corselli M, Park TS, Andriolo G, Sun B, Zheng B, Zhang L, Norotte C, Teng PN, Traas J, Schugar R, Deasy BM, Badylak S, Buhring HJ, Giacobino JP, Lazzari L, Huard J and Peault B (2008) A perivascular origin for mesenchymal stem cells in multiple human organs. *Cell Stem Cell* **3**:301-313.

Dar A, Domev H, Ben-Yosef O, Tzukerman M, Zeevi-Levin N, Novak A, Germanguz I, Amit M and Itskovitz-Eldor J (2012) Multipotent vasculogenic pericytes from human pluripotent stem cells promote recovery of murine ischemic limb. *Circulation* **125**:87-99.

Dellavalle A, Maroli G, Covarello D, Azzoni E, Innocenzi A, Perani L, Antonini S, Sambasivan R, Brunelli S, Tajbakhsh S and Cossu G (2011) Pericytes resident in postnatal skeletal muscle differentiate into muscle fibres and generate satellite cells. *Nature communications* **2**:499.

Diaz-Flores L, Gutierrez R, Madrid JF, Varela H, Valladares F, Acosta E, Martin-Vasallo P and Diaz-Flores L, Jr. (2009) Pericytes. Morphofunction, interactions and pathology in a quiescent and activated mesenchymal cell niche. *Histology and histopathology* **24**:909-969.

Diaz-Flores L, Gutierrez R and Varela H (1992) Behavior of postcapillary venule pericytes during postnatal angiogenesis. *Journal of morphology* **213**:33-45.

Diaz-Flores L, Jr., Gutierrez R, Madrid JF, Acosta E, Avila J, Diaz-Flores L and Martin-Vasallo P (2012) Cell sources for cartilage repair; contribution of the mesenchymal perivascular niche. *Frontiers in bioscience* **4**:1275-1294.

Dimarino AM, Caplan AI and Bonfield TL (2013) Mesenchymal stem cells in tissue repair. *Frontiers in immunology* **4**:201.

Dominici M, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini F, Krause D, Deans R, Keating A, Prockop D and Horwitz E (2006) Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. *Cytotherapy* **8**:315-317.

Dore-Duffy P and Cleary K (2011) Morphology and properties of pericytes. *Methods in molecular biology* **686**:49-68.

Dore-Duffy P, Mehedi A, Wang X, Bradley M, Trotter R and Gow A (2011) Immortalized CNS pericytes are quiescent smooth muscle actin-negative and pluripotent. *Microvascular research* **82**:18-27.

Duttenhoefer F, Lara de Freitas R, Meury T, Loibl M, Benneker LM, Richards RG, Alini M and Verrier S (2013) 3D scaffolds co-seeded with human endothelial progenitor and mesenchymal stem cells: evidence of prevascularisation within 7 days. *European cells & materials* **26**:49-64; discussion 64-45.

Ehninger A and Trumpp A (2011) The bone marrow stem cell niche grows up: mesenchymal stem cells and macrophages move in. *The Journal of experimental medicine* **208**:421-428.

Eming SA, Krieg T and Davidson JM (2007) Inflammation in wound repair: molecular and cellular mechanisms. *The Journal of investigative dermatology* **127**:514-525.

Farini A, Sitzia C, Erratico S, Meregalli M and Torrente Y (2014) Clinical applications of mesenchymal stem cells in chronic diseases. *Stem cells international* **2014**:306573.

Farrington-Rock C, Crofts NJ, Doherty MJ, Ashton BA, Griffin-Jones C and Canfield AE (2004) Chondrogenic and adipogenic potential of microvascular pericytes. *Circulation* **110**:2226-2232.

Feng J, Mantesso A and Sharpe PT (2010) Perivascular cells as mesenchymal stem cells. *Expert Opin Biol Ther* **10**:1441-1451.

Feng Y, vom Hagen F, Pfister F, Djokic S, Hoffmann S, Back W, Wagner P, Lin J, Deutsch U and Hammes HP (2007) Impaired pericyte recruitment and abnormal retinal angiogenesis as a result of angiopoietin-2 overexpression. *Thrombosis and haemostasis* **97**:99-108.

Friedenstein AJ, Chailakhjan RK and Lalykina KS (1970) The development of fibroblast colonies in monolayer cultures of guinea-pig bone marrow and spleen cells. *Cell and tissue kinetics* **3**:393-403.

Friedenstein AJ, Chailakhyan RK, Latsinik NV, Panasyuk AF and Keiliss-Borok IV (1974) Stromal cells responsible for transferring the microenvironment of the hemopoietic tissues. Cloning in vitro and retransplantation in vivo. *Transplantation* **17**:331-340.

Fuoco C, Sangalli E, Vono R, Testa S, Sacchetti B, Latronico MV, Bernardini S, Madeddu P, Cesareni G, Seliktar D, Rizzi R, Bearzi C, Cannata SM, Spinetti G and Gargioli C (2014) 3D hydrogel environment rejuvenates aged pericytes for skeletal muscle tissue engineering. *Frontiers in physiology* **5**:203.

Gao P, Zhou Y, Xian L, Li C, Xu T, Plunkett B, Huang SK, Wan M and Cao X (2014) Functional effects of TGF-beta1 on mesenchymal stem cell mobilization in cockroach allergen-induced asthma. *Journal of immunology* **192**:4560-4570.

Gerhardt H and Betsholtz C (2003) Endothelial-pericyte interactions in angiogenesis. *Cell and tissue research* **314**:15-23.

Gerhardt H and Betsholtz C (2005) How do endothelial cells orientate? *Exs*:3-15.

Godwin JW and Brockes JP (2006) Regeneration, tissue injury and the immune response. *Journal of anatomy* **209**:423-432.

Goritz C, Dias DO, Tomilin N, Barbacid M, Shupliakov O and Frisen J (2011) A pericyte origin of spinal cord scar tissue. *Science* **333**:238-242.

Hall CN, Reynell C, Gesslein B, Hamilton NB, Mishra A, Sutherland BA, O'Farrell FM, Buchan AM, Lauritzen M and Attwell D (2014) Capillary pericytes regulate cerebral blood flow in health and disease. *Nature* **508**:55-60.

He Q, Wan C and Li G (2007) Concise review: multipotent mesenchymal stromal cells in blood. *Stem cells* **25**:69-77.

He W, Nieponice A, Soletti L, Hong Y, Gharaibeh B, Crisan M, Usas A, Peault B, Huard J, Wagner WR and Vorp DA (2010) Pericyte-based human tissue engineered vascular grafts. *Biomaterials* **31**:8235-8244.

Hellstrom M, Kalen M, Lindahl P, Abramsson A and Betsholtz C (1999) Role of PDGF-B and PDGFR-beta in recruitment of vascular smooth muscle cells and pericytes during embryonic blood vessel formation in the mouse. *Development* **126**:3047-3055.

Hong HS, Lee J, Lee E, Kwon YS, Lee E, Ahn W, Jiang MH, Kim JC and Son Y (2009) A new role of substance P as an injury-inducible messenger for mobilization of CD29(+) stromal-like cells. *Nature medicine* **15**:425-435.

Hoogduijn MJ, Verstegen MM, Engela AU, Korevaar SS, Roemeling-van Rhijn M, Merino A, Franquesa M, de Jonge J, Ijzermans JN, Weimar W, Betjes MG, Baan CC and van der Laan LJ (2014) No evidence for circulating mesenchymal stem cells in patients with organ injury. *Stem cells and development* **23**:2328-2335.

Hu C, Yong X, Li C, Lü M, Liu D, Chen L, Hu J, Teng M, Zhang D, Fan Y and Liang G (2013) CXCL12/CXCR4 axis promotes mesenchymal stem cell mobilization to burn wounds and contributes to wound repair. *Journal of Surgical Research* **183**:427-434.

Hu J, Srivastava K, Wieland M, Runge A, Mogler C, Besemfelder E, Terhardt D, Vogel MJ, Cao L, Korn C, Bartels S, Thomas M and Augustin HG (2014) Endothelial cell-derived angiopoietin-2 controls liver regeneration as a spatiotemporal rheostat. *Science* **343**:416-419.

Humphreys BD, Lin SL, Kobayashi A, Hudson TE, Nowlin BT, Bonventre JV, Valerius MT, McMahon AP and Duffield JS (2010) Fate tracing reveals the pericyte and not epithelial origin of myofibroblasts in kidney fibrosis. *The American journal of pathology* **176**:85-97.

Hung C, Linn G, Chow YH, Kobayashi A, Mittelsteadt K, Altemeier WA, Gharib SA, Schnapp LM and Duffield JS (2013) Role of lung pericytes and resident fibroblasts in the pathogenesis of pulmonary fibrosis. *American journal of respiratory and critical care medicine* **188**:820-830.

Hyldahl RD, Xin L, Hubal MJ, Moeckel-Cole S, Chipkin S and Clarkson PM (2011) Activation of nuclear factor-kappaB following muscle eccentric contractions in humans is localized primarily to skeletal muscle-residing pericytes. *FASEB journal : official publication of the Federation of American Societies for Experimental Biology* **25**:2956-2966.

Iida M, Tanabe K, Matsushima-Nishiwaki R, Kozawa O and Iida H (2013) Adenosine monophosphate-activated protein kinase regulates platelet-derived growth factor-BB-induced vascular smooth muscle cell migration. *Archives of biochemistry and biophysics* **530**:83-92.

Jain RK and Booth MF (2003) What brings pericytes to tumor vessels? *The Journal of clinical investigation* **112**:1134-1136.

James AW, Zara JN, Corselli M, Askarinam A, Zhou AM, Hourfar A, Nguyen A, Megerdichian S, Asatrian G, Pang S, Stoker D, Zhang X, Wu B, Ting K, Peault B and Soo C (2012a) An abundant perivascular source of stem cells for bone tissue engineering. *Stem cells translational medicine* **1**:673-684.

James AW, Zara JN, Corselli M, Chiang M, Yuan W, Nguyen V, Askarinam A, Goyal R, Siu RK, Scott V, Lee M, Ting K, Peault B and Soo C (2012b) Use of human perivascular stem cells for bone regeneration. *Journal of visualized experiments : JoVE*:e2952.

James AW, Zara JN, Zhang X, Askarinam A, Goyal R, Chiang M, Yuan W, Chang L, Corselli M, Shen J, Pang S, Stoker D, Wu B, Ting K, Peault B and Soo C (2012c) Perivascular stem cells: a prospectively purified mesenchymal stem cell population for bone tissue engineering. *Stem cells translational medicine* **1**:510-519.

Jo DH, Kim JH, Heo JI, Kim JH and Cho CH (2013) Interaction between pericytes and endothelial cells leads to formation of tight junction in hyaloid vessels. *Molecules and cells* **36**:465-471.

Johnson JR, Folestad E, Rowley JE, Noll EM, Walker SA, Lloyd CM, Rankin SM, Pietras K, Eriksson UP and Fuxe J (2015) Pericytes contribute to airway remodeling in a mouse model of chronic allergic asthma. *American journal of physiology Lung cellular and molecular physiology*:ajplung 00286 02014.

Karp JM and Leng Teo GS (2009) Mesenchymal Stem Cell Homing: The Devil Is in the Details. *Cell Stem Cell* **4**:206-216.

Katare R, Riu F, Mitchell K, Gubernator M, Campagnolo P, Cui Y, Fortunato O, Avolio E, Cesselli D, Beltrami AP, Angelini G, Emanueli C and Madeddu P (2011) Transplantation of human pericyte progenitor cells improves the repair of infarcted heart through activation of an angiogenic program involving micro-RNA-132. *Circulation research* **109**:894-906.

Kitaori T, Ito H, Schwarz EM, Tsutsumi R, Yoshitomi H, Oishi S, Nakano M, Fujii N, Nagasawa T and Nakamura T (2009) Stromal cell-derived factor 1/CXCR4 signaling is critical for the recruitment of mesenchymal stem cells to the fracture site during skeletal repair in a mouse model. *Arthritis and rheumatism* **60**:813-823.

Kong Y, Wang H, Lin T and Wang S (2014) Sphingosine-1-phosphate/S1P receptors signaling modulates cell migration in human bone marrow-derived mesenchymal stem cells. *Mediators of inflammation* **2014**:565369.

Kunisaki Y, Bruns I, Scheiermann C, Ahmed J, Pinho S, Zhang D, Mizoguchi T, Wei Q, Lucas D, Ito K, Mar JC, Bergman A and Frenette PS (2013) Arteriolar niches maintain haematopoietic stem cell quiescence. *Nature* **502**:637-643.

Li C, Kong Y, Wang H, Wang S, Yu H, Liu X, Yang L, Jiang X, Li L and Li L (2009) Homing of bone marrow mesenchymal stem cells mediated by sphingosine 1-phosphate contributes to liver fibrosis. *Journal of hepatology* **50**:1174-1183.

Li M and Ikehara S (2013) Bone-marrow-derived mesenchymal stem cells for organ repair. *Stem cells international* **2013**:132642.

Li Y and Thompson WJ (2011) Nerve terminal growth remodels neuromuscular synapses in mice following regeneration of the postsynaptic muscle fiber. *The Journal of neuroscience : the official journal of the Society for Neuroscience* **31**:13191-13203.

Lin CS and Lue TF (2013) Defining vascular stem cells. *Stem cells and development* **22**:1018-1026.

Lin SL, Chang FC, Schrimpf C, Chen YT, Wu CF, Wu VC, Chiang WC, Kuhnert F, Kuo CJ, Chen YM, Wu KD, Tsai TJ and Duffield JS (2011) Targeting endothelium-pericyte cross talk by inhibiting VEGF receptor signaling attenuates kidney microvascular rarefaction and fibrosis. *The American journal of pathology* **178**:911-923.

Lin SL, Kisseleva T, Brenner DA and Duffield JS (2008) Pericytes and perivascular fibroblasts are the primary source of collagen-producing cells in obstructive fibrosis of the kidney. *The American journal of pathology* **173**:1617-1627.

Liu L, Yu Q, Lin J, Lai X, Cao W, Du K, Wang Y, Wu K, Hu Y, Zhang L, Xiao H, Duan Y and Huang H (2011) Hypoxia-inducible factor-1alpha is essential for hypoxia-induced mesenchymal stem cell mobilization into the peripheral blood. *Stem cells and development* **20**:1961-1971.

Liu N, Tian J, Cheng J and Zhang J (2013) Migration of CXCR4 gene-modified bone marrow-derived mesenchymal stem cells to the acute injured kidney. *Journal of cellular biochemistry* **114**:2677-2689.

Liu S, Taghavi R and Leask A (2010) Connective tissue growth factor is induced in bleomycin-induced skin scleroderma. *Journal of cell communication and signaling* **4**:25-30.

Loibl M, Binder A, Herrmann M, Duttenhoefer F, Richards RG, Nerlich M, Alini M and Verrier S (2014) Direct cell-cell contact between mesenchymal stem cells and endothelial progenitor cells induces a pericyte-like phenotype in vitro. *BioMed research international* **2014**:395781.

Lozito TP, Kuo CK, Taboas JM and Tuan RS (2009a) Human mesenchymal stem cells express vascular cell phenotypes upon interaction with endothelial cell matrix. *Journal of cellular biochemistry* **107**:714-722.

Lozito TP, Taboas JM, Kuo CK and Tuan RS (2009b) Mesenchymal stem cell modification of endothelial matrix regulates their vascular differentiation. *Journal of cellular biochemistry* **107**:706-713.

Main H, Munsie M and O'Connor MD (2014) Managing the potential and pitfalls during clinical translation of emerging stem cell therapies. *Clinical and translational medicine* **3**:10.

Mansilla E, Marin GH, Drago H, Sturla F, Salas E, Gardiner C, Bossi S, Lamonega R, Guzman A, Nunez A, Gil MA, Piccinelli G, Ibar R and Soratti C (2006) Bloodstream cells phenotypically identical to human mesenchymal bone marrow stem cells circulate in large amounts under the influence of acute large skin damage: new evidence for their use in regenerative medicine. *Transplantation proceedings* **38**:967-969.

Mederacke I, Hsu CC, Troeger JS, Huebener P, Mu X, Dapito DH, Pradere JP and Schwabe RF (2013) Fate tracing reveals hepatic stellate cells as dominant contributors to liver fibrosis independent of its aetiology. *Nature communications* **4**:2823.

Montiel-Eulefi E, Nery AA, Rodrigues LC, Sanchez R, Romero F and Ulrich H (2012) Neural differentiation of rat aorta pericyte cells. *Cytometry Part A : the journal of the International Society for Analytical Cytology* **81**:65-71.

Morikawa S, Baluk P, Kaidoh T, Haskell A, Jain RK and McDonald DM (2002) Abnormalities in pericytes on blood vessels and endothelial sprouts in tumors. *The American journal of pathology* **160**:985-1000.

Mosna F, Sensebe L and Krampera M (2010) Human bone marrow and adipose tissue mesenchymal stem cells: a user's guide. *Stem cells and development* **19**:1449-1470.

Murray IR, West CC, Hardy WR, James AW, Park TS, Nguyen A, Tawonsawatruk T, Lazzari L, Soo C and Peault B (2014) Natural history of mesenchymal stem cells, from vessel walls to culture vessels. *Cellular and molecular life sciences : CMLS* **71**:1353-1374.

Nakagomi T, Molnar Z, Nakano-Doi A, Taguchi A, Saino O, Kubo S, Clausen M, Yoshikawa H, Nakagomi N and Matsuyama T (2011) Ischemia-induced neural stem/progenitor cells in the pia mater following cortical infarction. *Stem cells and development* **20**:2037-2051.

Onoyama M, Kitadai Y, Tanaka Y, Yuge R, Shinagawa K, Tanaka S, Yasui W and Chayama K (2013) Combining molecular targeted drugs to inhibit both cancer cells and activated stromal cells in gastric cancer. *Neoplasia* **15**:1391-1399.

Orlic D, Kajstura J, Chimenti S, Jakoniuk I, Anderson SM, Li B, Pickel J, McKay R, Nadal-Ginard B, Bodine DM, Leri A and Anversa P (2001) Bone marrow cells regenerate infarcted myocardium. *Nature* **410**:701-705.

Orlova VV, Drabsch Y, Freund C, Petrus-Reurer S, van den Hil FE, Muenthaisong S, Dijke PT and Mummery CL (2014a) Functionality of endothelial cells and pericytes from human pluripotent stem cells demonstrated in cultured vascular plexus and zebrafish xenografts. *Arteriosclerosis, thrombosis, and vascular biology* **34**:177-186.

Orlova VV, van den Hil FE, Petrus-Reurer S, Drabsch Y, Ten Dijke P and Mummery CL (2014b) Generation, expansion and functional analysis of endothelial cells and pericytes derived from human pluripotent stem cells. *Nature protocols* **9**:1514-1531.

Osawa A, Harner CD, Gharaibeh B, Matsumoto T, Mifune Y, Kopf S, Ingham SJM, Schreiber V, Usas A and Huard J (2013) The use of blood vessel-derived stem cells for meniscal regeneration and repair. *Med Sci Sport Exer* **45**:813-823.

Owen M and Friedenstein AJ (1988) Stromal stem cells: marrow-derived osteogenic precursors. *Ciba Foundation symposium* **136**:42-60.

Ozen I, Deierborg T, Miharada K, Padel T, Englund E, Genove G and Paul G (2014) Brain pericytes acquire a microglial phenotype after stroke. *Acta neuropathologica* **128**:381-396.

Ozerdem U and Stallcup WB (2003) Early contribution of pericytes to angiogenic sprouting and tube formation. *Angiogenesis* **6**:241-249.

Pi L, Xia H, Liu J, Shenoy AK, Hauswirth WW and Scott EW (2011) Role of connective tissue growth factor in the retinal vasculature during development and ischemia. *Investigative ophthalmology & visual science* **52**:8701-8710.

Pitchford SC, Furze RC, Jones CP, Wengner AM and Rankin SM (2009) Differential mobilization of subsets of progenitor cells from the bone marrow. *Cell Stem Cell* **4**:62-72.

Pontikoglou C, Deschaseaux F, Sensebe L and Papadaki HA (2011) Bone marrow mesenchymal stem cells: biological properties and their role in hematopoiesis and hematopoietic stem cell transplantation. *Stem cell reviews* **7**:569-589.

Proebstl D, Voisin MB, Woodfin A, Whiteford J, D'Acquisto F, Jones GE, Rowe D and Nourshargh S (2012) Pericytes support neutrophil subendothelial cell crawling and breaching of venular walls in vivo. *The Journal of experimental medicine* **209**:1219-1234.

Pukac L, Huangpu J and Karnovsky MJ (1998) Platelet-derived growth factor-BB, insulin-like growth factor-I, and phorbol ester activate different signaling pathways for stimulation of vascular smooth muscle cell migration. *Experimental cell research* **242**:548-560.

Rajkumar VS, Howell K, Csiszar K, Denton CP, Black CM and Abraham DJ (2005) Shared expression of phenotypic markers in systemic sclerosis indicates a convergence of pericytes and fibroblasts to a myofibroblast lineage in fibrosis. *Arthritis research & therapy* **7**:R1113-1123.

Rankin SM (2012) Chemokines and adult bone marrow stem cells. *Immunology letters* **145**:47-54.

Ray S, Kulkarni SS, Chakraborty K, Pessu R, Hauer-Jensen M, Kumar KS and Ghosh SP (2013) Mobilization of progenitor cells into peripheral blood by gamma-tocotrienol: a promising radiation countermeasure. *International immunopharmacology* **15**:557-564.

Raza A, Franklin MJ and Dudek AZ (2010) Pericytes and vessel maturation during tumor angiogenesis and metastasis. *American journal of hematology* **85**:593-598.

Ren G, Zhang L, Zhao X, Xu G, Zhang Y, Roberts AI, Zhao RC and Shi Y (2008) Mesenchymal stem cell-mediated immunosuppression occurs via concerted action of chemokines and nitric oxide. *Cell Stem Cell* **2**:141-150.

Ren S, Johnson BG, Kida Y, Ip C, Davidson KC, Lin SL, Kobayashi A, Lang RA, Hadjantonakis AK, Moon RT and Duffield JS (2013) LRP-6 is a coreceptor for multiple fibrogenic signaling pathways in pericytes and myofibroblasts that are inhibited by DKK-1. *Proceedings of the National Academy of Sciences of the United States of America* **110**:1440-1445.

Rochefort GY, Delorme B, Lopez A, Herault O, Bonnet P, Charbord P, Eder V and Domenech J (2006) Multipotential mesenchymal stem cells are mobilized into peripheral blood by hypoxia. *Stem cells* **24**:2202-2208.

Rock JR, Barkauskas CE, Cronce MJ, Xue Y, Harris JR, Liang J, Noble PW and Hogan BL (2011) Multiple stromal populations contribute to pulmonary fibrosis without evidence for epithelial to mesenchymal transition. *Proceedings of the National Academy of Sciences of the United States of America* **108**:E1475-1483.

Rojas M, Xu J, Woods CR, Mora AL, Spears W, Roman J and Brigham KL (2005) Bone marrow-derived mesenchymal stem cells in repair of the injured lung. *American journal of respiratory cell and molecular biology* **33**:145-152.

Ruan J, Luo M, Wang C, Fan L, Yang SN, Cardenas M, Geng H, Leonard JP, Melnick A, Cerchietti L and Hajjar KA (2013) Imatinib disrupts lymphoma angiogenesis by targeting vascular pericytes. *Blood* **121**:5192-5202.

Sacchetti B, Funari A, Michienzi S, Di Cesare S, Piersanti S, Saggio I, Tagliafico E, Ferrari S, Robey PG, Riminucci M and Bianco P (2007) Self-renewing osteoprogenitors in bone

marrow sinusoids can organize a hematopoietic microenvironment. *Cell* **131**:324-336.

Sava P, Cook IO, Mahal RS and Gonzalez AL (2015) Human microvascular pericyte basement membrane remodeling regulates neutrophil recruitment. *Microcirculation* **22**:54-67.

Scharpfenecker M, Floot B, Russell NS, Coppes RP and Stewart FA (2013) Endoglin haploinsufficiency attenuates radiation-induced deterioration of kidney function in mice. *Radiotherapy and oncology : journal of the European Society for Therapeutic Radiology and Oncology* **108**:464-468.

Scharpfenecker M, Floot B, Russell NS, Coppes RP and Stewart FA (2014) Thalidomide ameliorates inflammation and vascular injury but aggravates tubular damage in the irradiated mouse kidney. *International journal of radiation oncology, biology, physics* **89**:599-606.

Schrimpf C, Xin C, Campanholle G, Gill SE, Stallcup W, Lin SL, Davis GE, Gharib SA, Humphreys BD and Duffield JS (2012) Pericyte TIMP3 and ADAMTS1 modulate vascular stability after kidney injury. *J Am Soc Nephrol* **23**:868-883.

Sendtner M, Carroll P, Holtmann B, Hughes RA and Thoenen H (1994) Ciliary neurotrophic factor. *Journal of neurobiology* **25**:1436-1453.

Shu HK, Yoon Y, Hong S, Xu K, Gao H, Hao C, Torres-Gonzalez E, Nayra C, Rojas M and Shim H (2013) Inhibition of the CXCL12/CXCR4-axis as preventive therapy for radiation-induced pulmonary fibrosis. *PloS one* **8**:e79768.

Simonavicius N, Ashenden M, van Weverwijk A, Lax S, Huso DL, Buckley CD, Huijbers IJ, Yarwood H and Isacke CM (2012) Pericytes promote selective vessel regression to regulate vascular patterning. *Blood* **120**:1516-1527.

Smith AN, Willis E, Chan VT, Muffley LA, Isik FF, Gibran NS and Hocking AM (2010) Mesenchymal stem cells induce dermal fibroblast responses to injury. *Experimental cell research* **316**:48-54.

Song JS, Kang CM, Kang HH, Yoon HK, Kim YK, Kim KH, Moon HS and Park SH (2010) Inhibitory effect of CXC chemokine receptor 4 antagonist AMD3100 on bleomycin induced murine pulmonary fibrosis. *Experimental & molecular medicine* **42**:465-472.

Soriano P (1994) Abnormal kidney development and hematological disorders in PDGF beta-receptor mutant mice. *Genes & development* **8**:1888-1896.

Stark K, Eckart A, Haidari S, Tirniceriu A, Lorenz M, von Bruhl ML, Gartner F, Khandoga AG, Legate KR, Pless R, Hepper I, Lauber K, Walzog B and Massberg S (2013) Capillary and arteriolar pericytes attract innate leukocytes exiting through venules and 'instruct' them with pattern-recognition and motility programs. *Nature immunology* **14**:41-51.

Sundberg C, Kowanetz M, Brown LF, Detmar M and Dvorak HF (2002) Stable expression of angiopoietin-1 and other markers by cultured pericytes: phenotypic similarities to a subpopulation of cells in maturing vessels during later stages of angiogenesis in vivo. *Laboratory investigation; a journal of technical methods and pathology* **82**:387-401.

Takahashi T, Kalka C, Masuda H, Chen D, Silver M, Kearney M, Magner M, Isner JM and Asahara T (1999) Ischemia- and cytokine-induced mobilization of bone marrow-derived endothelial progenitor cells for neovascularization. *Nature medicine* **5**:434-438.

Tang W, Zeve D, Suh JM, Bosnakovski D, Kyba M, Hammer RE, Tallquist MD and Graff JM (2008) White fat progenitor cells reside in the adipose vasculature. *Science* **322**:583-586.

Tatsumi K, Otani H, Sato D, Enoki C, Iwasaka T, Imamura H, Taniuchi S, Kaneko K, Adachi Y and Ikehara S (2008) Granulocyte-colony stimulating factor increases donor mesenchymal stem cells in bone marrow and their mobilization into peripheral circulation but does not repair dystrophic heart after bone marrow transplantation. *Circulation journal : official journal of the Japanese Circulation Society* **72**:1351-1358.

Tourovskaia A, Fauver M, Kramer G, Simonson S and Neumann T (2014) Tissue-engineered microenvironment systems for modeling human vasculature. *Experimental biology and medicine*.

Uezumi A, Fukada S, Yamamoto N, Takeda S and Tsuchida K (2010) Mesenchymal progenitors distinct from satellite cells contribute to ectopic fat cell formation in skeletal muscle. *Nature cell biology* **12**:143-152.

Wan M, Li C, Zhen G, Jiao K, He W, Jia X, Wang W, Shi C, Xing Q, Chen YF, Jan De Beur S, Yu B and Cao X (2012) Injury-activated transforming growth factor beta controls mobilization of mesenchymal stem cells for tissue remodeling. *Stem cells* **30**:2498-2511.

Wang CH, Cherng WJ, Yang NI, Kuo LT, Hsu CM, Yeh HI, Lan YJ, Yeh CH and Stanford WL (2008a) Late-outgrowth endothelial cells attenuate intimal hyperplasia contributed by mesenchymal stem cells after vascular injury. *Arteriosclerosis, thrombosis, and vascular biology* **28**:54-60.

Wang J, Liao L and Tan J (2011) Mesenchymal-stem-cell-based experimental and clinical trials: current status and open questions. *Expert Opinion on Biological Therapy* **11**:893-909.

Wang Y, Deng Y and Zhou GQ (2008b) SDF-1alpha/CXCR4-mediated migration of systemically transplanted bone marrow stromal cells towards ischemic brain lesion in a rat model. *Brain research* **1195**:104-112.

Wang Y, Johnsen HE, Mortensen S, Bindslev L, Ripa RS, Haack-Sorensen M, Jorgensen E, Fang W and Kastrup J (2006) Changes in circulating mesenchymal stem cells, stem cell homing factor, and vascular growth factors in patients with acute ST elevation myocardial infarction treated with primary percutaneous coronary intervention. *Heart* **92**:768-774.

Welti JC, Powles T, Foo S, Gourlaouen M, Preece N, Foster J, Frentzas S, Bird D, Sharpe K, van Weverwijk A, Robertson D, Soffe J, Erler JT, Pili R, Springer CJ, Mather SJ and Reynolds AR (2012) Contrasting effects of sunitinib within in vivo models of metastasis. *Angiogenesis* **15**:623-641.

Wilkinson-Berka JL, Babic S, De Gooyer T, Stitt AW, Jaworski K, Ong LG, Kelly DJ and Gilbert RE (2004) Inhibition of platelet-derived growth factor promotes pericyte loss and angiogenesis in ischemic retinopathy. *The American journal of pathology* **164**:1263-1273.

Xie J, Wang W, Si JW, Miao XY, Li JC, Wang YC, Wang ZR, Ma J, Zhao XC, Li Z, Yi H and Han H (2013) Notch signaling regulates CXCR4 expression and the migration of mesenchymal stem cells. *Cellular immunology* **281**:68-75.

Xu X, Zhu F, Zhang M, Zeng D, Luo D, Liu G, Cui W, Wang S, Guo W, Xing W, Liang H, Li L, Fu X, Jiang J and Huang H (2013) Stromal cell-derived factor-1 enhances wound healing through recruiting bone marrow-derived mesenchymal stem cells to the wound area and promoting neovascularization. *Cells, tissues, organs* **197**:103-113.

Yamanishi S, Katsumura K, Kobayashi T and Puro DG (2006) Extracellular lactate as a dynamic vasoactive signal in the rat retinal microvasculature. *American journal of physiology Heart and circulatory physiology* **290**:H925-934.

Yang D, Sun S, Wang Z, Zhu P, Yang Z and Zhang B (2013) Stromal cell-derived factor-1 receptor CXCR4-overexpressing bone marrow mesenchymal stem cells accelerate wound healing by migrating into skin injury areas. *Cellular reprogramming* **15**:206-215.

Zehendner CM, Wedler HE and Luhmann HJ (2013) A novel in vitro model to study pericytes in the neurovascular unit of the developing cortex. *PloS one* **8**:e81637.

Zhan Y, Kim S, Izumi Y, Izumiya Y, Nakao T, Miyazaki H and Iwao H (2003) Role of JNK, p38, and ERK in platelet-derived growth factor-induced vascular proliferation, migration, and gene expression. *Arteriosclerosis, thrombosis, and vascular biology* **23**:795-801.

Zhang F, Zhang Z, Chen L, Kong D, Zhang X, Lu C, Lu Y and Zheng S (2014) Curcumin attenuates angiogenesis in liver fibrosis and inhibits angiogenic properties of hepatic stellate cells. *Journal of cellular and molecular medicine* **18**:1392-1406.

Ziegler T, Horstkotte J, Schwab C, Pfetsch V, Weinmann K, Dietzel S, Rohwedder I, Hinkel R, Gross L, Lee S, Hu J, Soehnlein O, Franz WM, Sperandio M, Pohl U, Thomas M, Weber C, Augustin HG, Fassler R, Deutsch U and Kupatt C (2013) Angiopoietin 2 mediates microvascular and hemodynamic alterations in sepsis. *The Journal of clinical investigation* **123**:3436-3445.
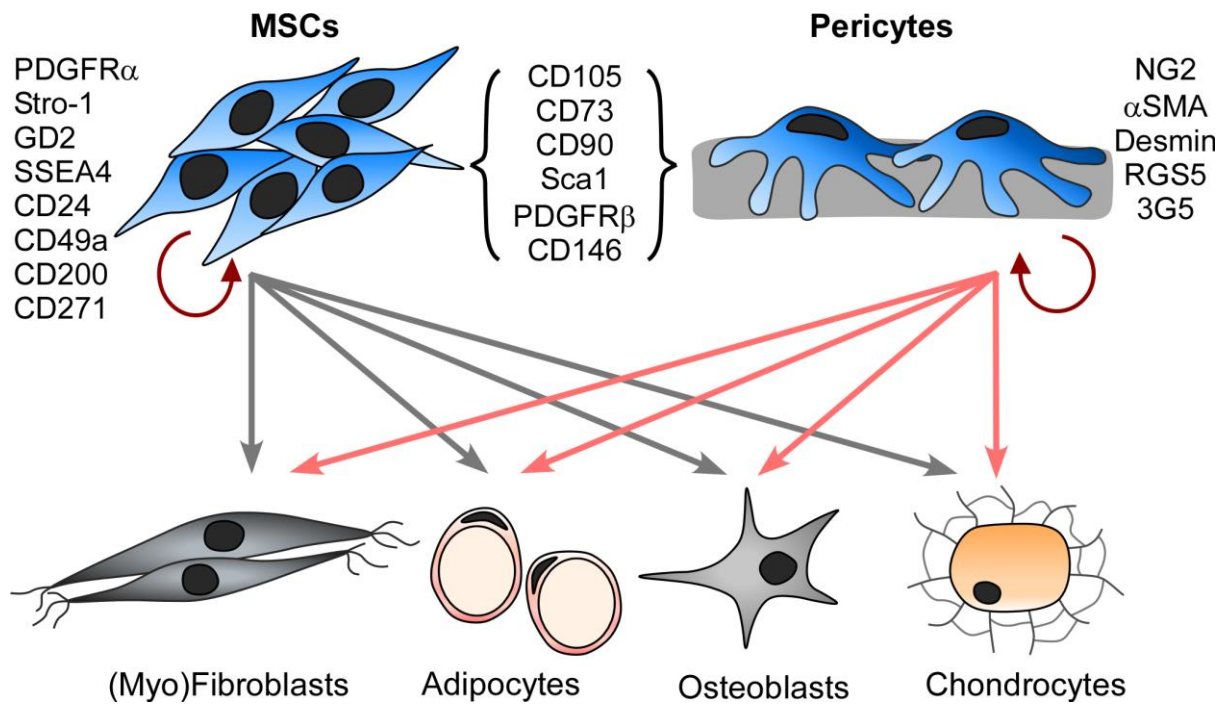
Figure 1. The schematic diagram illustrates the capacity for MSC and pericyte differentiation into separate lineages (grey and pink arrows) and for self-renewal (red arrows). The unique and shared markers of both cell types are shown.
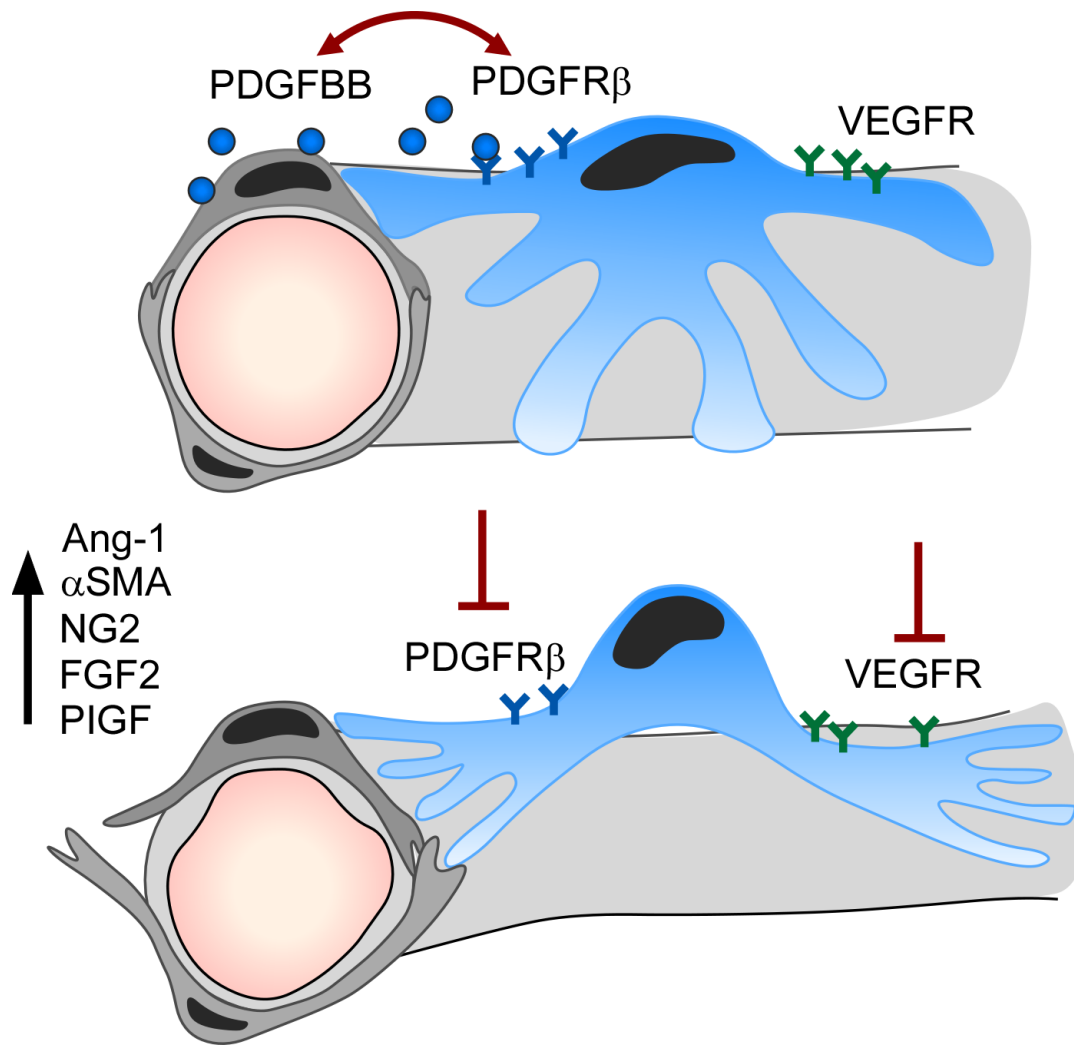
Figure 2. In a quiescent state (top), pericytes have a stellate morphology and a complex system of extensions which protect ECs and reinforce the vasculature. The balance between ECs and pericytes are tightly controlled by signalling pathways operating in a paracrine manner, such as PDGF-BB. In injury, disruption of PDGF-BB expression dissociates pericyte-EC connections, which leads to detachment of ECs and corresponding vessel collapse (bottom). Pericytes are then activated, such that they develop a bulky cytoplasm with shortened processes which allow migration through the basement membrane to invade newly vascularised tissue.
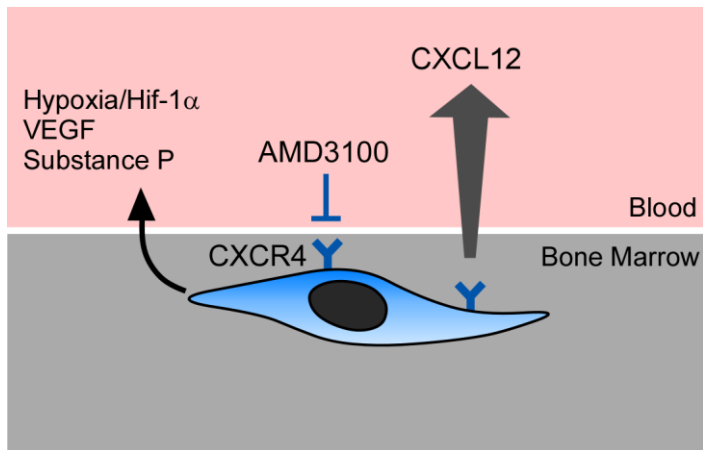
Figure 3. Therapeutic interventions to increase MSC mobilisation from the bone marrow niche have primarily focused on inhibiting CXCR4 signalling on bone marrow MSCs, thereby releasing them from their niche. This strategy can be combined with other systemic stimuli, such as hypoxia, VEGF and substance P. The arrow indicates the gradient of CXCL12 expression.
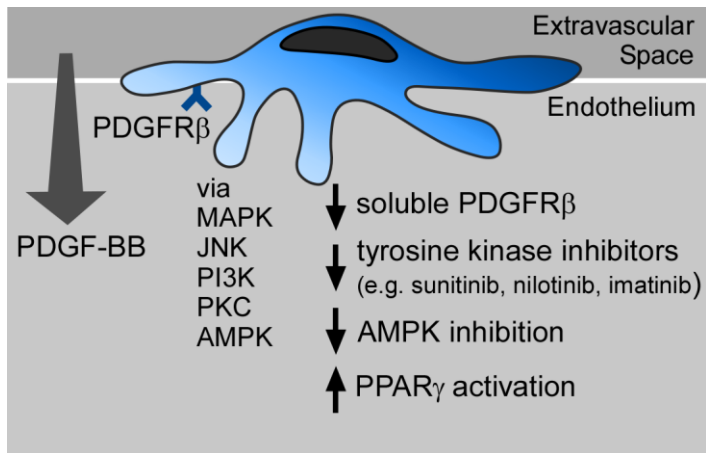
Figure 4. Therapeutic interventions to modulate pericyte mobilisation have attempted to inhibit the migration of these cells to the vasculature by impairing PDGFRβ signalling through the use of soluble receptors, tyrosine kinase inhibitors (sunitinib, nilotinib, imatinib, etc.), PPAR-γ activation and AMPK inhibition. The arrow indicates the gradient of PDGF-BB expression.