HHS Public Access

Author manuscript

Wound Repair Regen. Author manuscript; available in PMC 2017 March 10.

Published in final edited form as:

Wound Repair Regen. 2016 March ; 24(2): 204–214. doi:10.1111/wrr.12415.

Pericytes: A Newly Recognized Player in Wound Healing

Richard J Bodnar[1,4,5,‡], **Latha Satish**[2,4,‡], **Cecelia C Yates**[1,3,4,5], and **Alan Wells**[1,4,5]

[1]Department of Pathology, University of Pittsburgh, Pittsburgh, PA 15261

[2]Department of Plastic Surgery, University of Pittsburgh, Pittsburgh, PA 15261

[3]Department of Health Promotions and Development, University of Pittsburgh, Pittsburgh, PA 15261

[4]McGowan Institute for Regenerative Medicine, Pittsburgh, PA 15213

[5]Veterans Affairs Medical Center, Pittsburgh, PA 15240

Abstract

Pericytes have generally been considered in the context of stabilizing vessels, ensuring the blood barriers, and regulating the flow through capillaries. However, new reports suggest that pericytes may function at critical times to either drive healing with minimal scarring or, perversely, contribute to fibrosis and ongoing scar formation. Beneficially, pericytes likely drive much of the vascular involution that occurs during the transition from the regenerative to the resolution phases of healing. Pathologically, pericytes can assume a fibrotic phenotype and promote scarring. This perspective will discuss pericyte involvement in wound repair and the relationship pericytes form with the parenchymal cells of the skin. We will further evaluate the role pericytes may have in disease progression in relation to chronic wounds and fibrosis.

Introduction

Physiologic wound healing is a multi-focal, highly orchestrated process that involves four distinct but overlapping phases; coagulation, inflammation, tissue replacement, and resolution. Each one of these phases is dependent on the active involvement and contributions of various cells. In the coagulation phase, clotting of serum and degranulation of platelets release matrix components and a host of growth factors and chemokines. These growth factors and chemokine promotes the immigration and activation of hematopoietically-derived inflammatory cells followed by fibroblasts, endothelial cells, and keratinocytes. During the tissue replacement phase, these signals promote the dedifferentiation of the formed cells to enable rapid motility and production of the granulation tissue. The neo-vasculature forms at this stage via angiogenesis to supply oxygen and nutrients to the highly metabolic environment. During the resolution phase, the fibroblasts and myofibroblast contract the matrix and promote collagen deposition and collagen fibril organization. Throughout the first three phases of wound healing; platelets,

Address Correspondence to: rbodnar2131@gmail.com or las238@pitt.edu.

The authors have no conflicts of interest to declare.

inflammatory cells and the provisional matrix foster angiogenesis through the secretion of growth factors, cytokines and extracellular matrix (ECM) components. However, during the last phase of wound resolution, up to 90% of the neo-vasculature involutes, in response to not only clearance of trophic factors but active signaling from late-stage chemokines via the CXCR3 G protein coupled receptor (1–3). It is during this last phase that the matrix matures and the skin reverts to a pauci-cellular state.

The vascular system plays a critical role in promoting the progression of the wound healing process. However, its dysfunction, either too much or too little early angiogenesis or conversely late regression, leads to a host of problems including dehiscence, ecchymoses and ulcer formation. As the vasculature is regulated by pericytes, attention has recently turned to these ill-defined but ubiquitous cells in controlling wound repair. These cells have been found to contribute to more than regulation of the neo-vasculature during repair, and are the focus of this perspective.

During wound repair, pericytes interact with platelets, inflammatory cells and connective tissue cells in addition to endothelial cells. Although no direct interaction between platelets and pericytes has been identified, pericytes have been implicated in modulating the extrinsic clotting cascade (4). Pericytes have been observed to possess phagocytic properties and may function to assist macrophages during the inflammatory phase of wound healing (5). Under inflammatory condition, pericytes have been observed to act as antigen presenting cells and promote the activation of T-cells (6). Pericytes secrete several cytokines including stromal-cell derived factor (SDF-1), CXCL9 and CXCL12, which was found to enhance lymphocyte infiltration (7, 8). There is emerging evidence-indicating pericytes as a source of myofibroblast and transition could occur through a PDGF-dependent mechanism (9). And lastly, the role for which pericytes are most known, communication with endothelial cells controls neo-vascularization during wound healing by regulating vessel formation and stabilization (1, 10, 11). These finding highlight the diverse role of pericytes in mediating cellular response during wound healing. Herein we will discuss the new evidence demonstrating that pericytes are dynamic regulators of the wound healing cascade.

Pericytes

Pericytes are considered to act as the vascular smooth muscle cells of the microvasculature providing tone and integrity to the capillary bed. This is in part based on their location. They are found surrounding the arterioles, venules and capillaries. Pericytes may not be restricted to the microvasculature as pericyte-like cells having characteristics of both pericytes and smooth muscle cells have been found in small blood vessels (12). Their coverage, consisting of a single layer, of the microvasculature is variable with density being dependent on the organ. The ratios of pericytes to endothelial can be as high as 1:1 in the retina and central nervous system (CNS) cells to as low as 1:100 in capillary bed of skeletal muscle of the upper extremities (10, 13, 14). Pericyte density appears to correlate with blood pressure. Vessels with higher blood pressure gradients such as the retina and lower extremities have higher pericytes coverage (15), supporting this view of pericytes as microvascular support structure.

Morphologically, pericytes tend to have a large cell body with an elongated membrane having numerous secondary branching processes, which allows a single pericyte to interact with and regulate many endothelial cells. Pericytes are typically embedded in the basal membrane of the microvessel allowing for direct interaction with endothelial cells. There are three principal connections made between endothelial cells and pericytes; gap and adherence junctions, and adhesion plaques, which contribute to transmission of biological and mechanical signals between the cells (10, 16, 17). Gap junctions allow for the exchange of ions and small molecules allowing direct pericyte-endothelial cell communication. The adherence junctions and adhesion plaques support the transmission of contractile forces allowing for the regulation of vessel diameter and blood flow. Because of the heterogeneity of pericytes (15, 18), their identity is typically determined through morphological features along with distinct molecular markers.

There are a number of cell surface markers that define pericytes but these are not unique to pericytes and can be dynamically expressed on pericytes and other cells (10, 19)(Table 1). Smooth muscle cells, endothelial cells and fibroblasts also express a number of these markers complicating identification of these cells. The most common markers used to identify pericytes include PDGFRβ, chondroitin sulfate proteoglycan 4 (NG2), alpha smooth muscle actin (αSMA), desmin, CD146/MUC18, and desmin regulator of GTPase signaling 5 (RGS5). Although these markers are expressed on subsets of pericytes, expression is not ubiquitous across all pericytes. Expression of these markers on pericytes can vary depending on the type of vessel they surround and pericyte phenotype. Studies have shown pericytes surrounding arterioles are generally $NG2^{+}/\alpha SMA^{+}$, venules $NG2^{-}/\alpha SMA^{+}$, and capillaries $NG2^{+}/\alpha SMA^{-}$ (20). These markers may provide a method to identify pericyte location within the vasculature. Levels of αSMA expression have been found to correlate with increased and decreased vasoconstriction of vessels and could be used to determine an activation state (21). Desmin expression may be a marker for maturity as immature pericytes express a high level (22). Typically CD146 and PDGFRβ are ubiquitously expressed. However, it must be appreciated that these molecules are present in numerous non-pericyte cells. In addition, pericytes also express a number of mesenchymal stem cell (MSC) markers, which include CD105, CD73 and CD90 (20). The use of pericyte morphology combined with cellular marker expression can be used to identify both vessel and pericyte phenotype. Still, the lack of even relatively specific or selective markers limits the ability to determine the source of pericytes prior to the interaction with the microvasculature. This hampers investigations into pericyte function in the earlier stages of wound healing when the microvasculature is poorly organized.

Pericyte Origin

A further question relates to the origin of pericytes. The embryonic linage, for the most part, is thought to be of a mesodermal origin. These may be part and parcel due to pericytes having a mesenchymal stem cells/multipotent stromal cells (MSC) phenotype (23). Pericytes and MSC reside in the same perivascular niche (24–26). Both MSC and pericytes share common markers (CD44, CD73, CD90, CD105) and exhibit multi-linage potential leading to pericytes having been viewed as a subset of the MSC population (25). Pericytes as a MSC phenotype is under much debate due to insufficient understanding of pericyte origin. It has

been observed that a subpopulation of pericytes lack expression of αSMA, a protein necessary for cell contraction, suggesting that two distinct populations of pericytes exist in the vasculature (27) a smooth muscle (αSMA^+) and MSC (αSMA^-) phenotype. In support of pericyte variability, two distinct pericyte populations have been identified as $nestin^-/NG2^+$ (type-1) and $nestin^+/NG2^+$ (type-2) (28, 29). The type-1 pericyte are αSMA^+ pericytes and may play a role in regulating vessel tone, and thus are mainly found around arterioles and venules. The type-2 pericytes participate in angiogenesis and have a MSC-like behavior in being able to differentiate into a neuronal linage (30, 31). These findings posit that pericyte phenotype and function may be regulated by its association with the vasculature; pericytes function as smooth muscle cells when associated with the vasculature and dissociation promotes dedifferentiation to a MSC phenotype. Thus, pericytes isolated and grown in culture revert to an MSC phenotype, having a regenerative or reparative capacity. Additionally, their location within all tissues make them ideal candidates for local recruitment for tissue repair. Additionally, there is some evidence that pericytes may come from bone marrow-derived cells in the circulation (32), though this may relate more to vasculogenesis with angiogenic support originating in neighboring tissue.

Pericyte Function

The well-described function of pericytes is to regulate vessel tone and permeability of mature vessels as well as modulating angiogenesis. By this alone, pericytes would impact wound healing. But even more so, in these functions we see hints of how pericytes may alter repair beyond the vasculature. Pericytes promote endothelial migration and cell survival (33, 34) in part by responding to hypoxia with upregulation of matrix metalloproteinases (MMP) 2, 3 and 9 (35). These proteinases help clear the tissue debris during early stages of wound healing but prolonged expression leads to non-healing ulcers.

In addition to promoting vessel maturation and stabilization, pericytes may also regulate vessel guidance and promote regression of non-essential nascent vessels. Upon initiation of angiogenesis pericytes detach from the angiogenic vessel and migrate into the parenchyma forming a guidance tunnel for the angiogenic sprout (36, 37). During development individual pericytes have been found associated with tip cells of the angiogenic sprout (38). These cells were observed to guide the vascular sprout and thus may play a role in determining the formation and location of a new vessel (39). We have shown that pericytes have the ability to negatively regulate angiogenesis through activation of CXCR3 on endothelial cells (1). Activated pericytes secrete the CXCR3 ligands IP-9 (CXCL11) and IP-10 (CXCL10). This signaling limits endothelial cell migration in response to growth factors by inhibiting m-calpain-mediated rear release (40) and triggers anoikis of endothelial cells via calpain-mediated cleavage of the β3 integrin (41) (Figure 1). The involution of such vessels is in large part responsible for the loss of the majority of microvasculature during the resolution phase of wound healing (42).

It needs to be noted that along with CXCR3 activation on endothelial cells, vessel regression can also be mediated by additional factors, which include angiopoietins and withdrawal of trophic factors. The decrease in VEGF and other trophic factors later in wound healing would act as if ‘taking the foot off the gas’ and limiting the driving force for angiogenesis.

Angiopoietins are complex having both vessel stabilizing properties as well as anti-angiogenic signaling (43). This is further complicated by angiopoietins impacting pericytes in addition to acting on endothelial cells directly (44, 45).

How the pericytes can play a dual role of both stabilizing vessels while pruning excess microvasculature may be explained by the maturation-dependent nature of CXCR3 expression. CXCR3 expression is nearly absent on mature vessels but expression is regained during wound-induced angiogenesis (41, 46). During wound healing, initial angiogenesis in the tissue replacement phase occurs in the absence of pericytes (1). The endothelial cells that venture into the provisional matrix without pericytes express CXCR3. As the vessel begins to mature, the endothelial cells adhere to each other and quiesce; concomitantly CXCR3 expression on endothelial cells diminishes. This would allow for the association of pericytes with the microvasculature without involution. Consistent with this, pericyte-stabilized vessels in mature wounds failed to express CXCR3 (1, 41, 47).

Pericytes in Wound Healing

From the foregoing, pericytes could play numerous roles in wound healing – angiogenic modulation, paracrine effectors (factors and enzymes), and stem cell contribution to tissue. The main function by which pericytes were recognized, vascular development and stability, is central to wound healing and pathologies thereof (Figure 2). The process of wound healing is a complex and dynamic process involving various players for secretion of soluble mediators and deposition of extracellular matrix along with migration of various cell types, including fibroblasts, keratinocytes, macrophages, leukocytes, endothelial cells and pericytes.

Pericytes interaction during various stages of healing

Inflammation followed by the proliferation phase is characterized by accumulation of fibroblasts and macrophages, formation of new blood vessel with a healthy granulation tissue. Activated macrophages release growth factors, such as vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF) to initiate angiogenesis. During this stage, PDGF receptor beta β (PDGFRβ) signaling is essential not only for angiogenesis but, also for recruitment and proliferation and normal function of fibroblasts and pericytes. Studies by Rajkumar et al. (48) show that PDGFR-β inhibition *in vivo* was accompanied by abnormal microvascular morphogenesis reminiscent of that observed in PDGFR-β $^{-/-}$ mice with significantly reduced immunostaining of the pericyte marker NG2 implying the importance of PDGFR-β signaling during the early phases of wound healing (48). Pericytes are typically thought to regulate the microvasculature but are now being viewed as have a broad range of functions. When associated with the microvasculature, pericytes act as smooth muscle to maintain vascular integraty and regulate blood flow.

Pericytes involvement in angiogenesis

Upon injury of the skin, initiation of the coagulation pathway occurs to establish homeostasis. Platelets and fibrinogen are key mediators in the formation of the fibrin clot and initiation of the inflammatory response. Platelets release various factors that can

stimulate pericyte activity. Platelets release PDGF and TGF-β, which promote pericyte detachment from endothelial cells and migration into the parenchyma. Activated pericytes can express tissue factor to promote activation of the extrinsic coagulation pathway (4, 49). Platelet activation of pericytes may facilitate or regulate neovascularization. Pericytes can facilitate angiogenesis through secretion of MMPs (driven in part by the hypoxic environment (35)) to degrade the basement membrane allowing endothelial cells to migrate into the provisional matrix. The dissociation of pericytes from the vasculature allows for destabilization of the endothelial tube, which can promote endothelial migration and proliferation. Vasculogenesis also may be influenced by pericytes. Endothelial progenitor cell (EPC) differentiation may be regulated by pericyte and may contribute EPC recruitment into the neovasculature (50). Upon formation of the nascent vasculature pericytes can promote stabilization through cell-cell interactions and paracrine signaling (TGF-β and Ang-1) (10, 39, 51), with TGF-β also promoting collagen I deposition in the wound bed. Dissolution of non-functional or excessive nascent vessels can be regulated by pericytes (1). We have found that pericytes can promote involution of nascent vessels through the activation of CXCR3 on endothelial cells driving anoikis (1). Thus, the pericytes may contribute to the angio-response in wound healing distinctly at different stages of repair. At the earlier point, dissociation from existing small vessels allows for endothelial proliferation and invasion into the provisional matrix, later pruning the excess immature vessels that characterize the tissue replacement phase, and finally stabilizing the remaining structures during and after wound resolution.

Pericytes interaction with leukocytes and neutrophils

Pericytes are not just important players in vascular development (10), but also have significant paracrine functions. They play a role in regulating immune responses, inflammation and may be interpreters of hazard/damage signals (52, 53, 54). In particular, pericytes can inducibly express key adhesion molecules (e.g., ICAM-1, VCAM-1), chemokines (egg., human and murine CXCL1, CXCL8, MIF), and receptors for proinflammatory molecules (TNFR1, TNFRII, IL-1R, TLRS, Nod like receptors) (53–55). A large variation in the profile of pericyte-leukocyte communications has been reported, and this may be due to the heterogeneity of pericytic cells from different blood vessels, tissues, species, isolation procedures, and inflammatory settings. The relevance of such interactions to leukocyte trafficking *in vivo* is only just beginning to emerge (54). Pericytes have shown to provide an adhesive substrate for neutrophils crawling within the vascular wall and seeking portals to the extravascular tissue (56). This response was mediated through the interaction of pericyte-expressed ICAM-1 with neutrophil Mac-1 (macrophage-1 antigen) and LFA-1 (leukocyte function associated antigen-1). Evidence points out that the use of an endothelial cell-pericyte *in vitro* co-culture model shows that the trans endothelial cell migration process itself can prime neutrophils for enhanced interactions with pericytes (57). Adhesion of fully extravasated myeloid cells with the abluminal aspect of pericytes has also been proposed as a mechanism through which various leukocyte effector functions are enhanced by specific instructing signals presented by subsets of inflamed pericytes (55). It should also be noted that the generation of pericyte-derived chemokines and/or chemokine depots is likely to play a key role in facilitating continued migration of leukocytes through vascular walls after the breaching of the endothelium.

Role of pericytes in-vivo in murine wounds

In addition to modulating immune function via paracrine effectors, pericytes secrete a number of chemokine, growth factors and matrix proteins that can regulate the function of parenchymal cells of the skin (58). An elegant study by Paquet-Fifield et al. (59) demonstrated the mesenchymal stem cell property of pericytes by investigating the pericytes located adjacent to the proliferative basal layer of the skin's epidermis. In essence, their findings showed that pericytes represent a potent cell population in the skin and are important microenvironmental regulators of skin regeneration as pericytes could promote epithelial proliferation, differentiation, and tissue regeneration in the absence of angiogenesis. Recent pilot studies from the same group suggested that treatment of murine excisional wounds with human-derived pericytes delayed the rate of dermal wound resolution but, did not significantly affect wound re-epithelialization and wound closure. The authors attribute this phenomenon to the failure to down-regulate the inflammatory response which warrants further experimentation (60) but may relate to the above-described pro-inflammatory effects of much of pericyte paracrine signaling.

Stemness and pericytes

Lastly, the stem cell-like properties of pericytes must be considered. Recent evidence has shown that pericyte dissociation from the vasculature induces a dedifferentiation from a smooth muscle cell phenotype to a mesenchymal phenotype. In vitro studies have shown the ability of pericytes to differentiate into various cell types (24, 61–65). Pericytes have the potential to convert into macrophages (66). The pericytes as a pool of macrophage-like cells have been widely studied in brain vasculature (5) and conversion of pericytes into macrophages, including microglia, have also been pointed out (67, 68). These cells have been described to have the capacity for pinocytosis and phagocytosis and shown to express the macrophage markers CD4, Fc receptor and MHC I and II (5, 69, 70) thus may function to support the inflammatory response. Pericytes can act as antigen-presenting cells for primed T-lymphocytes via the expression of MHC class II (71) and are the first line of immunologic defense in the brain. Recruitment of lymphocytes may also occur through pericyte secretion of SDF-1 and CXCL12 (8, 72). The fraction of pericyte-like cells that behave as macrophages (pericytal macrophages), contributing along with lymphocytes to an immune response (5), may originate from the bone marrow mesenchymal progenitors, named fibrocytes (70). In fact, BM-derived precursor cells have been shown to give rise to a subset of pericytes (32, 34, 73) that in turn may contribute to the inflammatory cell population in the wound bed.

Pericytes in tissue regeneration

Pericytes have been used in a various animal models for wound repair, muscle, central nervous system (CNS), cartilage, cardiac and bone therapy (24, 61–65). The use of pericytes for skeletal muscle repair has been well demonstrated *in vivo*. Pericytes have been shown to colonize host muscle upon transplantation and can spontaneously fuse into myotubes promoting muscle regeneration (62). Injection of muscle-derived pericytes into injured skeletal muscle improved function better than injected myoblasts (19). When used in a mouse model of muscular dystrophy, pericyte transplantation shows an enhancement in

skeletal muscle function, with the use of genetically-corrected pericytes ameliorating the disease phenotype (62, 74). Given the myotube-intrinsic nature of the dystrophy phenotype, it is assumed that the pericytes directly contributed to the myotube as a stem cell. However, secreted factors, soluble and matrix, may also contribute to the survival of the existing myotubes and limiting the inflammatory damage.

Another current area investigating the use of pericytes is myocardium repair. Initial cell therapy used MSC that were directly injected into the heart (75–77). These studies provided modest benefit but significant improvement in cardiac function was still lacking. A comparative study between bone marrow MSCs and saphenous vein pericytes for cardiac repair after myocardial infarct MI, showed that the pericytes were superior in efficacy of cardiac function with no myocardial calcification in MI (78). This was attributed to the paracrine signaling from the pericytes improving revascularization of the tissue. Pericytes also were found to home back to perivascular sites and this homing was suggested to support the long-term survival of the transplanted pericytes (61). These studies suggest that pericyte treatment attributes multiple cardioprotective mechanisms for cardiac repair rather than acting as stem cells directly contributing the repair (79). While not directly applicable to skin wound healing, these findings suggest similarities in supporting the neo-angiogenic functioning.

Role of Pericytes in Skin Fibrosis

The excessive accumulation of extracellular matrix (ECM) components, including collagen I, fibronectin, and hyaluronan, in injured tissue results in fibrosis. Unchecked fibrosis can cause permanent damage to the tissues and may result in morbidity. Despite their heterogeneous origins, different fibrotic disorders, including those of the liver, heart, lungs, skin and kidney all involve the activation of fibroblasts and myofibroblasts (80–84). Multiple origins have been hypothesized for myofibroblasts (81); the principal sources postulated being pre-existing fibroblasts, bone marrow-derived circulating mesenchymal progenitors (bone marrow stromal cells and fibrocytes) and pericytes (32, 85). It has been shown that myofibroblasts are capable of synthesizing essential components of the ECM such as collagen types I, III, IV and V (81). It has been described in kidney fibrosis that pericytes, detach from the vessel walls, migrate, acquire a fibroblast-like phenotype where they differentiate into myofibroblasts (activated fibroblasts) and act as collagen-producing cells (86).

Functional changes in endothelial cells and pericytes have been associated with excessive skin fibrosis resulting in hypertrophic scars and keloids. Kischer et al. (87) noted that microvessels in hypertrophic scar and keloid are occluded or partially occluded, apparently owing to an excess of endothelial cells (87, 88). Microvessels of hypertrophic scars and keloids appear to be surrounded by multiple satellite cells (pericytes), and the innermost satellite cells closely resemble myofibroblasts. These pericapillary myofibroblasts can migrate from their perivascular position to the interstitial area. The authors also stated that the observed extent of microvascular occlusion supports a previously published theory that hypoxia is involved in hypertrophic scarring and keloids (88). Hypertension has also been cited as a risk factor for keloids and hypertrophic scars (Reviewed extensively by (89)). The

response to hypertension by pericytes/myofibroblasts in the fibrotic state is mediated by hypoxia. Pericytes seem to be sensitive to hypoxia, and their contraction during hypoxia may promote blood flow impairment (90). The pericyte contraction as well as pericapillary myofibroblast contractions can aggravate the occlusion of the blood vessel lumen, leading to a vicious cycle resulting in ever-increasing hypoxia and results in fibroproliferation. As the pericytes produce numerous MMPs that would lead to matrix degradation products that are chemotactic for cells of the innate immune system that in turn drives more myofibroblast-mediated fibrosis. A recent study by Dulauroy et al. postulated the role for pericytes as myofibroblast precursors of dermal fibrosis (91). These studies showed evidence that the majority of collagen-producing, α-SMA+ myofibroblasts formed following acute dermal or muscle injury are generated from tissue-resident ADAM12^{+} (ADAM12^{+} is a matrix metalloprotease-12 and is expression is restricted to embryogenesis and fibrosis) cells and furthermore from a PDGFRβ^{+} NG2^{+} perivascular subpopulation (92). Pericytes isolated from skeletal muscle that were nestin^{-}/NG2^{+} (type-1) were found to express αSMA; when these were exposed to transforming growth factor-β (TGF-β) they became fibrogenic (93). In addition, these type-1 pericytes were also observed to express collagen I (93). Taken together expression of both nestin and ADAM12 may be key regulators that determine whether pericytes can be transformed to a myofibroblast phenotype.

Systemic sclerosis (SSc), a complex autoimmune disease is characterized by fibrosis of the skin and visceral organs. SSc is a vascular disease that is marked by chronic and prolonged tissue injury and inflammation (reviewed by (94)). Endothelial and pericyte activation has been noted as one of the contributing factors for initiation of vasculopathy and pathological accumulation of excessive ECM proteins in SSc (95, 96). Microvascular endothelium activation and damage is shown to be always present followed by dysregulation in vascular remodeling in samples from patients affected with SSc ((97, 98)). Among the many factors that result in SSc fibrotic lesions, abnormal characteristics of microvascular pericytes are cited as a major source as pericytes have important functional roles in the regulation of vascular development, maturation and remodeling. Studies suggest that endothelial cells and pericytes both undergo changes in the early stages of SSc (99). In SSc fibrotic lesions, pericytes showed markers of activation such as platelet-derived growth factor receptor beta and high-molecular-weight melanoma associated antigen (100). Strong evidence shows convergence of microvascular pericytes and resident fibroblasts to a myofibroblast lineage and thereby contributing to SSc by synthesizing excessive ECM components (101).

Interestingly, it has been reported that there is a difference in the properties of bone marrow mesenchymal stem cells (BM-MSCs) derived from healthy controls and SSc patients. SSc-derived MSCs behave as pericytes and display a more mature and myofibroblast-like phenotype probably due to changing microenvironmental cues during disease progression (102). Considering the impactful role played by pericytes in SSc pathology, molecular targets against PDGF signaling using pharmacological agents such as Imatinib mesylate, sunitinib, and sorafenib are currently under investigation.

Pericytes in Non-Healing (Chronic) Skin Wounds

In chronic metabolic diseases, diabetes in particular, the healing process is defective in not going to completion, leading to what is termed a “chronic wound” (103). Diabetic complications impair both macro-and micro-vascular structures causing stroke, atherosclerosis, impaired wound healing, retinopathy, neuropathy and nephropathy (104). It has been estimated that between 3–6 million people in the United States suffer from one of the three main types of chronic wound -- diabetic, venous or pressure ulcers (103). Microvascular pericytes act as a crucial interface between blood-borne and connective tissue signals. In normal vascular physiology, pericytes are closely associated with a stable endothelium and upon injury, are encountered by other cell types and environmental signals.

In chronic wounds, the microenvironment is hostile with an imbalance in protease activity and chemokine release. We have seen that pericytes produce many of the enzymes whose persistent presence not only mark chronic wounds but also contribute to the stalled healing process. Thus, in this situation the feed-forward cycle of matrix remodeling leading to a chronic inflammation is coupled with paracrine signals such as TGFβ and CXCR3 ligands that impair neovascularization. This provides for a model in which pericyte presence would prolong the non-healing state.

An early investigation by Laaff et al. (105) on patients with chronic venous insufficiency (CVI) that lead to leg ulcers demonstrated that most of the patients (31 out of 42) with CVI showed pericyte changes. The investigators attributed these changes to the destruction of the pericyte envelope, which might lead to microcirculatory dysfunction. However, how the pericyte dysfunction relates to failure to heal will require new explorations.

Pericytes as Therapeutic Agents

Patients suffering from diabetes, vasculitis and ischemic diseases are prone to develop chronic ulcers that are difficult to treat due to many of the patients having co-morbidities, which further hinders the healing process. Chronic wounds fail to heal at a rate up to 33% with implementation of current wound management strategies (106). Current strategies have focused on the use of cell therapy to enhance the healing of chronic wounds. The focus has been on tissue engineering due to the potential of returning tissue to it original state. Classical cell based therapies use fibroblasts or keratinocytes sheets from autologous and allogenic tissue. The use of cell sheets has improved wound healing but fail to fully incorporate the tissue graft into the new tissue. Furthermore, the tissue grafts are difficult to maintain and are expensive.

Experimental therapies have used mesenchymal stem cells (MSCs) to enhance wound healing in preclinical models (107–110). MSC use has been found to have limitations for their ability to enhance wound healing (110). They require expansion prior to use risking mutational events, and exhibit low levels of long-term survival. Thus, the noted therapeutic benefit mainly from the release of tropic factors rather than a direct contribution (110, 111). These limitations make MSC less than ideal for therapeutic use.

Recent studies have identified pericytes as a cell with mesenchymal differentiation potential (25). These cells are currently being tested in experimental models of tissue regeneration because of their plasticity, regenerative capacity and availability (24, 61–65). The data from these studies indicate that pericytes may be a more viable option then MSC for tissue regeneration.

Pericytes are easier to obtain, since they can be harvested from more accessible tissue including, subcutaneous fat, umbilical cord and placenta. Sufficiently large numbers of pericytes can be isolated from subcutaneous fat enabling transplantation without expansion. The use of pericytes for tissue regeneration has gained substantial momentum in the last 10 years. Pericytes isolated from human umbilical cord were found to improve re-epithelialization and reconstitute the dermis (24). In this study, pericytes were administered in a fibrin gel on full thickness wounds. The pericyte-treated full thickness wounds had a higher tensile strength, denser and organized collagen fibers, enhanced angiogenic response and a more organized dermis (24). In an excisional wound model, pericytes isolated from adipose tissue were found to increase vascularization, enhanced remodeling and promote earlier collagen deposition (112). Using an organotypic skin culture, pericytes were found to enhance epidermal regeneration through secretion of laminin α5 (59). These studies show that pericytes display mesenchymal stem cell properties, making them a potential cell for therapy in treating non-healing wounds.

Although these initial studies are promising, new data suggest the possibility of two subtypes of pericytes. Type 1 ($Nestin^{-}/NG2^{+}$) and type 2 ($Nestin^{+}/NG2^{+}$) pericytes were found to play diverse roles in tissue repair (28, 29, 93). The type 2 pericytes were observed to promote angiogenesis, found to be associated with vessels and possessed myogenic potential (28, 113). On the other hand, type 1 pericytes were found to promote fibrosis in injured skeletal muscle through the deposition of collagen (93). Since pericytes have been implicated in contributing to fibrosis or even chronic wounds, these finding may help in understanding the possible role pericytes may play (114). Understanding to what extent type 1 pericytes may contribute to fibrosis and to what extent type 2 pericytes may contribute to tissue regeneration will be necessary to design effective therapeutic approaches to limit undesirable side effects. Taken together, pericytes have the potential to be used as a cell therapy for non-healing wound but further understanding the function of these cells is necessary to reduce unwanted outcomes.

Conclusions

Pericytes perform multiple functions that are critical during wound healing. Their regulation of the angiogenic response, including both growing and pruning nascent vessels, and then stabilizing the surviving ones, alone would constitute a major role in the repair process. However, beyond this well-documented function, their ability to act akin to MSC as both a source of paracrine/matricrine signals and potential stem cell opens up new avenues that require exploration. The reports to-date offer tantalizing glimpses of a world of various pericyte functions that can be exploited to control healing, to promote closure or reducing scarring. However, our ability to redirect these processes are limited by the diversity of

pericytes that posses these different functions and thus lack the ability to exquisitely target the interventions.

Acknowledgments

This work was supported by grants from the National Institute of General Medical Sciences (NIGMS) at NIH (USA).

We thank the members of the Wells and Yates laboratories for helpful discussions and suggestions.

References

1. Bodnar RJ, Rodgers ME, Chen WC, Wells A. Pericyte regulation of vascular remodeling through the CXC receptor 3. Arterioscler Thromb Vasc Biol. 2013; 33:2818–2829. [PubMed: 24135023]
2. Bodnar RJ, Wells A. Differential Regulation of Pericyte Function by the CXC Receptor 3. Wound Repair Regen. 2015
3. Balesterieri ML, Balestrieri A, Mancini FP, Napoli C. Understanding the immunoangiostatic CXC chemokine network. Cardiovascular Research. 2008; 78:250–256. [PubMed: 18252760]
4. Bouchard BA, Shatos MA, Tracy PB. Human brain pericytes differentially regulate expression of procoagulant enzyme complexes comprising the extrinsic pathway of blood coagulation. Arterioscler Thromb Vasc Biol. 1997; 17:1–9. [PubMed: 9012630]
5. Thomas WE. Brain macrophages: on the role of pericytes and perivascular cells. Brain Res Brain Res Rev. 1999; 31:42–57. [PubMed: 10611494]
6. Balabanov R, Beaumont T, Dore-Duffy P. Role of central nervous system microvascular pericytes in activation of antigen-primed splenic T-lymphocytes. J Neurosci Res. 1999; 55:578–587. [PubMed: 10082080]
7. Venetz D, Ponzoni M, Schiraldi M, Ferreri AJ, Bertoni F, Doglioni C, et al. Perivascular expression of CXCL9 and CXCL12 in primary central nervous system lymphoma: T-cell infiltration and positioning of malignant B cells. Int J Cancer. 2010; 127:2300–2312. [PubMed: 20872671]
8. Avniel S, Arik Z, Maly A, Sagie A, Basst HB, Yahana MD, et al. Involvement of the CXCL12/CXCR4 pathway in the recovery of skin following burns. J Invest Dermatol. 2006; 126:468–476. [PubMed: 16385346]
9. Chen YT, Chang FC, Wu CF, Chou YH, Hsu HL, Chiang WC, et al. Platelet-derived growth factor receptor signaling activates pericyte-myofibroblast transition in obstructive and post-ischemic kidney fibrosis. Kidney Int. 2011; 80:1170–1181. [PubMed: 21716259]
10. Armulik A, Abramsson A, Betsholtz C. Endothelial/pericyte interactions. Circulation Research. 2005; 97:512–523. [PubMed: 16166562]
11. Edelman DA, Jiang Y, Tyburski J, Wilson RF, Steffes C. Pericytes and their role in microvasculature homeostasis. Journal of Surgical Research. 2006; 135:305–311. [PubMed: 16930620]
12. Andreeva ER, Pugach IM, Gordon D, Orekhov AN. Continuous subendothelial network formed by pericyte-like cells in human vascular bed. Tissue Cell. 1998; 30:127–135. [PubMed: 9569686]
13. von Tell D, Armulik A, Betsholtz C. Pericytes and vascular stability. Experimental Cell Research. 2006; 312:623–629. [PubMed: 16303125]
14. Shepro D, Morel NM. Pericyte physiology. FASEB Journal. 1993; 7:1031–1038. [PubMed: 8370472]
15. Sims DE. Diversity within pericytes. Clin Exp Pharmacol Physiol. 2000; 27:842–846. [PubMed: 11022980]
16. Fernandez-Klett F, Priller J. Diverse functions of pericytes in cerebral blood flow regulation and ischemia. J Cereb Blood Flow Metab. 2015; 35:883–887. [PubMed: 25853910]
17. van Dijk CG, Nieuweboer FE, Pei JY, Xu YJ, Burgisser P, van Mulligen E, et al. The complex mural cell: pericyte function in health and disease. Int J Cardiol. 2015; 190:75–89. [PubMed: 25918055]

18. Armulik A, Genove G, Betsholtz C. Pericytes: developmental, physiological, and pathological perspectives, problems, and promises. Dev Cell. 2011; 21:193–215. [PubMed: 21839917]
19. Crisan M, Yap S, Casteilla L, Chen CW, Corselli M, Park TS, et al. A perivascular origin for mesenchymal stem cells in multiple human organs. Cell Stem Cell. 2008; 3:301–313. [PubMed: 18786417]
20. Crisan M, Corselli M, Chen WC, Peault B. Perivascular cells for regenerative medicine. J Cell Mol Med. 2012; 16:2851–2860. [PubMed: 22882758]
21. Dore-Duffy P, Wang S, Mehedi A, Katyshev V, Cleary K, Tapper A, et al. Pericyte-mediated vasoconstriction underlies TBI-induced hypoperfusion. Neurol Res. 2011; 33:176–186. [PubMed: 21801592]
22. Hughes S, Gardiner T, Hu P, Baxter L, Rosinova E, Chan-Ling T. Altered pericyte-endothelial relations in the rat retina during aging: implications for vessel stability. Neurobiol Aging. 2006; 27:1838–1847. [PubMed: 16387390]
23. Pierantozzi E, Badin M, Vezzani B, Curina C, Randazzo D, Petraglia F, et al. Human pericytes isolated from adipose tissue have better differentiation abilities than their mesenchymal stem cell counterparts. Cell and Tissue Research. 2015; 361:769–778. [PubMed: 25820673]
24. Zebardast N, Lickorish D, Davies JE. Human umbilical cord perivascular cells (HUCPVC): A mesenchymal cell source for dermal wound healing. Organogenesis. 2010; 6:197–203. [PubMed: 21220956]
25. Corselli M, Chen CW, Crisan M, Lazzari L, Peault B. Perivascular ancestors of adult multipotent stem cells. Arterioscler Thromb Vasc Biol. 2010; 30:1104–1109. [PubMed: 20453168]
26. Minteer DM, Marra KG, Rubin JP. Adipose stem cells: biology, safety, regulation, and regenerative potential. Clin Plast Surg. 2015; 42:169–179. [PubMed: 25827561]
27. Nehls V, Drenckhahn D. Heterogeneity of microvascular pericytes for smooth muscle type alpha-actin. J Cell Biol. 1991; 113:147–154. [PubMed: 2007619]
28. Birbrair A, Zhang T, Wang ZM, Messi ML, Mintz A, Delbono O. Pericytes at the intersection between tissue regeneration and pathology. Clin Sci (Lond). 2014; 128:81–93. [PubMed: 25236972]
29. Birbrair A, Zhang T, Wang ZM, Messi ML, Enikolopov GN, Mintz A, et al. Skeletal muscle pericyte subtypes differ in their differentiation potential. Stem Cell Res. 2013; 10:67–84. [PubMed: 23128780]
30. Birbrair A, Zhang T, Files DC, Mannava S, Smith T, Wang Z-M, et al. Type-1 pericytes accumulate after tissue injury and produce collagen in an organ-dependent manner. Stem Cell Research and Therapy. 2014; 5:e122.
31. Birbrair AZT, Wang ZM, Messi ML, Olson JD, Mintz A. Type-2 pericytes participate in normal and tumoral angiogenesis. American Journal of Physiology - Cell Physiology. 2014; 307:C25–C38. [PubMed: 24788248]
32. Rodrigues M, Rennert RC, Januszyk M, Li AY, Kooreman N, Fernhoff NB, et al. Identification and characterization of a circulating pericyte that homes to ischemia. submitted. 2016
33. Virgintino D, Girolamo F, Errede M, Capobianco C, Robertson D, Stallcup WB, et al. An intimate interplay between precocious, migrating pericytes and endothelial cells governs human fetal brain angiogenesis. Angiogenesis. 2007; 10:35–45. [PubMed: 17225955]
34. Ozerdem U, Stallcup WB. Early contribution of pericytes to angiogenic sprouting and tube formation. Angiogenesis. 2003; 6:241–249. [PubMed: 15041800]
35. Davis GE, Saunders WB. Molecular balance of capillary tube formation versus regression in wound repair: role of matrix metalloproteinases and their inhibitors. Journal of Investigative Dermatology. Symposium Proceedings. 2006; 11:44–56.
36. Valarmathi MT, Davis JM, Yost MJ, Goodwin RL, Potts JD. A three-dimensional model of vasculogenesis. Biomaterials. 2009; 30:1098–1112. [PubMed: 19027154]
37. Stratman AN, Malotte KM, Mahan RD, Davis MJ, Davis GE. Pericyte recruitment during vasculogenic tube assembly stimulates endothelial basement membrane matrix formation. Blood. 2009; 114:5091–5101. [PubMed: 19822899]
38. Gerhardt H, Betsholtz C. Endothelial-pericyte interactions in angiogenesis. Cell Tissue Res. 2003; 314:15–23. [PubMed: 12883993]

39. Betsholtz C, Lindblom P, Gerhardt H. Role of pericytes in vascular morphogenesis. EXS. 2005:115–125. [PubMed: 15617474]
40. Bodnar RJ, Yates CC, Wells A. IP-10 blocks vascular endothelial growth factor-induced endothelial cell motility and tube formation via inhibition of calpain. Circ Res. 2006; 98:617–625. [PubMed: 16484616]
41. Bodnar RJ, Yates CC, Rodgers ME, Du X, Wells A. IP-10 induces dissociation of newly formed blood vessels. Journal of Cell Science. 2009; 122:2064–2077. [PubMed: 19470579]
42. Yates CC, Krishna P, Whaley D, Bodnar R, Turner T, Wells A. Lack of CXC Chemokine Receptor 3 Signaling Leads to Hypertrophic and Hypercellular Scarring. The American Journal of Pathology. 2010; 176:1743–1755. [PubMed: 20203286]
43. Augustin HG, Koh GY, Thurston G, Alitalo K. Control of vascular morphogenesis and homeostasis through the angiopoietin-Tie system. Nature Reviews - Molecular Cell Biology. 2009; 10:165–177. [PubMed: 19234476]
44. Aguilera KY, Brekken RA. Recruitment and retention: factors that affect pericyte migration. Cellular and Molecular Life Sciences. 2014; 71:299–309. [PubMed: 23912898]
45. Park SW, Yun J-H, Kim JH, Kim K-W, Cho C-H, Kim JH. Angiopoietin 2 induces pericyte apoptosis via alpha3beta1 integrin signaling in diabetic retinopathy. Diabetes. 2014; 63:3057–3068. [PubMed: 24722242]
46. Romagnani P, Annunziato F, Lasagni L, Lazzeri E, Beltrame C, Francalanci M, et al. Cell cycle-dependent expression of CXC chemokine receptor 3 by endothelial cells mediates angiostatic activity. J Clin Invest. 2001; 107:53–63. [PubMed: 11134180]
47. Yates CC, Whaley D, Kulasekeran P, Hancock WW, Lu B, Bodnar R, et al. Delayed and deficient dermal maturation in mice lacking the CXCR3 ELR-negative CXC chemokine receptor. American Journal of Pathology. 2007; 171:484–495. [PubMed: 17600132]
48. Rajkumar VS, Shiwen X, Bostrom M, Leoni P, Muddle J, Ivarsson M, et al. Platelet-derived growth factor-beta receptor activation is essential for fibroblast and pericyte recruitment during cutaneous wound healing. American Journal of Pathology. 2006; 169:2254–2265. [PubMed: 17148686]
49. McDonald AG, Yang K, Roberts HR, Monroe DM, Hoffman M. Perivascular tissue factor is down-regulated following cutaneous wounding: implications for bleeding in hemophilia. Blood. 2008; 111:2046–2048. [PubMed: 18055866]
50. Bagley RG, Weber W, Rouleau C, Teicher BA. Pericytes and endothelial precursor cells: cellular interactions and contributions to malignancy. Cancer Research. 2005; 65:9741–9750. [PubMed: 16266995]
51. Lee S, Zeiger A, Maloney JM, Kotecki M, Van Vliet KJ, Herman IM. Pericyte actomyosin-mediated contraction at the cell-material interface can modulate the microvascular niche. J Phys Condens Matter. 2010; 22:194115. [PubMed: 21386441]
52. Nourshargh S, Hordijk PL, Sixt M. Breaching multiple barriers: leukocyte motility through venular walls and the interstitium. Nature Reviews Molecular Cell Biology. 2010; 11:366–378. [PubMed: 20414258]
53. Pober JS, Tellides G. Participation of blood vessel cells in human adaptive immune responses. Trends in Immunology. 2012; 33:49–57. [PubMed: 22030237]
54. Voisin MB, Nourshargh S. Neutrophil transmigration: emergence of an adhesive cascade within venular walls. Journal of Innate Immunity. 2013; 5:336–347. [PubMed: 23466407]
55. Stark K, Eckart A, Haidari S, Tirniceriu A, Lorenz M, vonBruhl ML, et al. Capillary and arteriolar pericytes attract innate leukocytes exiting through venules and 'instruct' them with pattern-recognition and motility programs. Nature Immunology. 2013; 14:41–51. [PubMed: 23179077]
56. Proebstl D, Voisin MB, Woodfin A, Whiteford J, D'Acquisto F, Jones GE, et al. Pericytes support neutrophil subendothelial cell crawling and breaching of venular walls in vivo. J Exp Med. 2012; 209:1219–1234. [PubMed: 22615129]
57. Ayres-Sander CE, Lauridsen H, Maier CL, Sava P, Pober JS, Gonzalez AL. Transendothelial migration enables subsequent transmigration of neutrophils through underlying pericytes. PLoS One. 2013; 8:e60025. [PubMed: 23555870]

58. Dulmovits BM, Herman IM. Microvascular remodeling and wound healing: a role for pericytes. Int J Biochem Cell Biol. 2012; 44:1800–1812. [PubMed: 22750474]
59. Paquet-Fifield S, Schluter H, Li A, Aitken T, Gangatirkar P, Blashki D, et al. A role for pericytes as microenvironmental regulators of human skin tissue regeneration. J Clin Invest. 2009; 119:2795–2806. [PubMed: 19652362]
60. Mills SJ, Zhuang L, Arandjelovic P, Cowin AJ, Kaur P. Effects of human pericytes in a murine excision model of wound healing. Experimental Dermatology. 2015; 24:881–882. [PubMed: 25959561]
61. Chen CW, Okada M, Proto JD, Gao X, Sekiya N, Beckman SA, et al. Human pericytes for ischemic heart repair. Stem Cells. 2013; 31:305–316. [PubMed: 23165704]
62. Peault B, Rudnicki M, Torrente Y, Cossu G, Tremblay JP, Partridge T, et al. Stem and progenitor cells in skeletal muscle development, maintenance, and therapy. Molecular Therapy. 2007; 15:867–877. [PubMed: 17387336]
63. Askarinam A, James AW, Zara JN, Goyal R, Corselli M, Pan A, et al. Human perivascular stem cells show enhanced osteogenesis and vasculogenesis with Nel-like molecule I protein. Tissue Eng Part A. 2013; 19:1386–1397. [PubMed: 23406369]
64. Cohen JA. Mesenchymal stem cell transplantation in multiple sclerosis. J Neurol Sci. 2013; 333:43–49. [PubMed: 23294498]
65. Osawa A, Harner CD, Gharaibeh B, Matsumoto T, Mifune Y, Kopf S, et al. The use of blood vessel-derived stem cells for meniscal regeneration and repair. Med Sci Sports Exerc. 2013; 45:813–823. [PubMed: 23247715]
66. Baron M, Gallego A. The relation of the microglia with the pericytes in the cat cerebral cortex. Zeitschrift fur Zellforschung und mikroskopische Anatomie. 1972; 128:42–57. [PubMed: 4336601]
67. Jordan FL, Thomas WE. Brain macrophages: questions of origin and interrelationship. Brain Research. 1988; 472:165–178. [PubMed: 3289689]
68. Monteiro RA, Rocha E, Marini-Abreu MM. Do microglia arise from pericytes? An ultrastructural and distribution study in the rat cerebellar cortex. Journal of Submicroscopic Cytology and Pathology. 1996; 28:457–469. [PubMed: 8933732]
69. Bergers G, Song S. The role of pericytes in blood-vessel formation and maintenance. Neuro Oncol. 2005; 7:452–464. [PubMed: 16212810]
70. Diaz-Flores L, Gutierrez R, Madrid JF, Varela H, Valladares F, Acosta E, et al. Pericytes. Morphofunction, interactions and pathology in a quiescent and activated mesenchymal cell niche. Histol Histopathol. 2009; 24:909–969. [PubMed: 19475537]
71. Guillemin GJ, Brew BJ. Microglia, macrophages, perivascular macrophages, and pericytes: a review of function and identification. Journal of Leukocyte Biology. 2004; 75:388–397. [PubMed: 14612429]
72. Pablos JL, Amara A, Bouloc A, Santiago B, Caruz A, Galindo M, et al. Stromal-cell derived factor is expressed by dendritic cells and endothelium in human skin. Am J Pathol. 1999; 155:1577–1586. [PubMed: 10550315]
73. Rajantie I, Ilmonen M, Alminaite A, Ozerderm U, Alitalo K, Salven P. Adult bone marrow-derived cells recruited during angiogenesis comprise precursors for periendothelial vascular mural cells. Blood. 2004; 104:2084–2086. [PubMed: 15191949]
74. Tedesco FS, Gerli MF, Perani L, Benedetti S, Ungaro F, Cassano M, et al. Transplantation of genetically corrected human iPSC-derived progenitors in mice with limb-girdle muscular dystrophy. Sci Transl Med. 2012; 4:140ra89.
75. Behfar A, Faustino RS, Arrell DK, Dzeja PP, Perez-Terzic C, Terzic A. Guided stem cell cardiopoiesis: discovery and translation. J Mol Cell Cardiol. 2008; 45:523–529. [PubMed: 18835562]
76. Gnecchi M, Zhang Z, Ni A, Dzau VJ. Paracrine mechanisms in adult stem cell signaling and therapy. Circ Res. 2008; 103:1204–1219. [PubMed: 19028920]
77. Loffredo FS, Steinhauser ML, Gannon J, Lee RT. Bone marrow-derived cell therapy stimulates endogenous cardiomyocyte progenitors and promotes cardiac repair. Cell Stem Cell. 2011; 8:389–398. [PubMed: 21474103]

78. Katare R, Riu F, Mitchell K, Gubernator M, Campagnolo P, Cui Y, et al. Transplantation of human pericyte progenitor cells improves the repair of infarcted heart through activation of an angiogenic program involving micro-RNA-132. Circ Res. 2011; 109:894–906. [PubMed: 21868695]
79. Kennedy-Lyon T, Rosenthal N. Cardiac regeneration: epicardial mediated repair. Proceedings of the Royal Society B. 2015; 282:e1821.
80. Gabbiani G. The myofibroblast in wound healing and fibroconnective diseases. Journal of Pathology. 2003; 200:500–503. [PubMed: 12845617]
81. Hinz B. Formation and function of the myofibroblast during tissue repair. Journal of Investigative Dermatology. 2007; 127:526–537. [PubMed: 17299435]
82. Desmouliere A, Darby IA, Gabbiani G. Normal and pathologic soft tissue remodeling: role of the myofibroblast, with special emphasis on liver and kidney fibrosis. Laboratory Investigation. 2003; 83:1689–1707. [PubMed: 14691287]
83. Virag JI, Murry CE. Myofibroblast and endothelial cell proliferation during murine myocardial infarct repair. American Journal of Pathology. 2003; 163:2433–2440. [PubMed: 14633615]
84. Phan SH. The myofibroblast in pulmonary fibrosis. Chest. 2002; 122:286–29S.
85. Diaz-Flores L, Gutierrez R, Madrid JF, Varela H, Vallardares F, Acosta E, et al. Pericytes. Morphofunction, interactions and pathology in a quiescent and activated mesenchymal cell niche. Histology and Histopathology. 2009; 24:909–969. [PubMed: 19475537]
86. Humphries BD, Lin SL, Kobayashi A, Hudson TE, Nowlin BT, Bonventre JV, et al. Fate tracing reveals the pericyte and not epithelial origin of myofibroblasts in kidney fibrosis. American Journal of Pathology. 2010; 176:85–97. [PubMed: 20008127]
87. Kischer CW, Thies AC, Chvapil M. Perivascular myofibroblasts and microvascular occlusion in hypertrophic scars and keloids. Human Pathology. 1982; 13:819–824. [PubMed: 7106747]
88. Kischer CW, Thies AC, Chvapil M. Hypertrophic scars and keloids: a review and new concept concerning their origin. Scanning Electron Microscopy. 1982; Pt 4:1699–1713. [PubMed: 7184146]
89. Huang C, Ogawa R. The link between hypertension and pathological scarring: does hypertension cause or promote keloid and hypertrophic scar pathogenesis? Wound Repair and Regeneration. 2014; 22:462–466. [PubMed: 24899409]
90. Yemisci M, Ozdemir YG, Vural A, Can A, Topalkara K, Turgay D. Pericyte contraction induced by oxidative-nitrative stress impairs capillary reflow despite successful opening of an occluded cerebral artery. Nature Medicine. 2009; 15:1031–1037.
91. Dulauroy S, DiCarlo SE, Langa F, Eberl G, Peduto L. Lineage tracing and genetic ablation of ADAM12(+) perivascular cells identify a major source of profibrotic cells during acute tissue injury. Nature Medicine. 2012; 18:1262–1270.
92. Duffield JS. The elusive source of the myofibroblasts: problem solved? Nature Medicine. 2012; 18:1178–1180.
93. Birbrair A, Zhang T, Wang ZM, Messi ML, Mintz A, Delbono O. Type-1 pericytes participate in fibrous tissue deposition in aged skeletal muscle. Am J Physiol Cell Physiol. 2013; 305:C1098–C1113. [PubMed: 24067916]
94. Ho YY, Lagares D, Tager AM, Kapoor M. Fibrosis--a lethal component of systemic sclerosis. Nat Rev Rheumatol. 2014; 10:390–402. [PubMed: 24752182]
95. Koch AE, Kronfeld-Harrington LB, Szekanecz Z, Cho MM, Haines GK, Harlow LA, et al. In situ expression of cytokines and cellular adhesion molecules in the skin of patients with systemic sclerosis. Their role in early and late disease. Pathobiology. 1993; 61:239–246. [PubMed: 7507681]
96. Gruschwitz MS, Vieth G. Up-regulation of class II major histocompatibility complex and intercellular adhesion molecule 1 expression on scleroderma fibroblasts and endothelial cells by interferon-gamma and tumor necrosis factor alpha in the early disease stage. Arthritis Rheum. 1997; 40:540–550. [PubMed: 9082943]
97. Guiducci S, Giacomelli R, Cerinic MM. Vascular complications of scleroderma. Autoimmun Rev. 2007; 6:520–523. [PubMed: 17854742]
98. Kuwana M, Okazaki Y, Yasuoka H, Kawakami Y, Ikeda Y. Defective vasculogenesis in systemic sclerosis. Lancet. 2004; 364:603–610. [PubMed: 15313361]

99. Trojanowska M. Cellular and molecular aspects of vascular dysfunction in systemic sclerosis. Nat Rev Rheumatol. 2010; 6:453–460. [PubMed: 20585340]
100. Rajkumar VS, Sundberg C, Abraham DJ, Rubin K, Black CM. Activation of microvascular pericytes in autoimmune Raynaud's phenomenon and systemic sclerosis. Arthritis Rheum. 1999; 42:930–941. [PubMed: 10323448]
101. Rajkumar VS, Howell K, Csiszar K, Denton CP, Black CM, Abraham DJ. Shared expression of phenotypic markers in systemic sclerosis indicates a convergence of pericytes and fibroblasts to a myofibroblast lineage in fibrosis. Arthritis Res Ther. 2005; 7:R1113–R1123. [PubMed: 16207328]
102. Cipriani P, Marrelli A, Benedetto PD, Liakouli V, Carubbi F, Ruscitti P, et al. Scleroderma Mesenchymal Stem Cells display a different phenotype from healthy controls; implications for regenerative medicine. Angiogenesis. 2013; 16:595–607. [PubMed: 23413114]
103. Menke NB, Ward KR, Witten TM, Bonchev DG, Diegelmann RF. Impaired wound healing. Clin Dermatol. 2007; 25:19–25. [PubMed: 17276197]
104. Reddy MA, Natarajan R. Epigenetic mechanisms in diabetic vascular complications. Cardiovasc Res. 2011; 90:421–429. [PubMed: 21266525]
105. Laaff H, Vandscheidt W, Weiss JM, Schaefer HE, Schoepf E. Immunohistochemical investigation of pericytes in chronic venous insufficiency. Vasa. 1991; 20:323–328. [PubMed: 1776342]
106. Fife CE, Carter MJ. Wound Care Outcomes and Associated Cost Among Patients Treated in US Outpatient Wound Centers: Data From the US Wound Registry. Wounds. 2012; 24:10–17. [PubMed: 25875947]
107. Peng LH, Wei W, Shan YH, Zhang TY, Zhang CZ, Wu JH, et al. beta-Cyclodextrin-Linked Polyethylenimine Nanoparticles Facilitate Gene Transfer and Enhance the Angiogenic Capacity of Mesenchymal Stem Cells for Wound Repair and Regeneration. J Biomed Nanotechnol. 2015; 11:680–690. [PubMed: 26310074]
108. Pelizzo G, Avanzini MA, Icaro Cornaglia A, Osti M, Romano P, Avolio L, et al. Mesenchymal stromal cells for cutaneous wound healing in a rabbit model: pre-clinical study applicable in the pediatric surgical setting. J Transl Med. 2015; 13:219. [PubMed: 26152232]
109. Huang S, Wu Y, Gao D, Fu X. Paracrine action of mesenchymal stromal cells delivered by microspheres contributes to cutaneous wound healing and prevents scar formation in mice. Cytotherapy. 2015; 17:922–931. [PubMed: 25939802]
110. Isakson M, de Blacam C, Whelan D, McArdle A, Clover AJ. Mesenchymal Stem Cells and Cutaneous Wound Healing: Current Evidence and Future Potential. Stem Cells Int. 2015; 2015:831095. [PubMed: 26106431]
111. Duscher D, Barrera J, Wong VW, Maan ZN, Whittam AJ, Januszyk M, et al. Stem Cells in Wound Healing: The Future of Regenerative Medicine? A Mini-Review. Gerontology. 2015 epub ahead of print.
112. Zamora DO, Natesan S, Becerra S, Wrice N, Chung E, Suggs LJ, et al. Enhanced wound vascularization using a dsASCs seeded FPEG scaffold. Angiogenesis. 2013; 16:745–757. [PubMed: 23709171]
113. Birbrair A, Zhang T, Wang ZM, Messi ML, Enikolopov GN, Mintz A, et al. Role of pericytes in skeletal muscle regeneration and fat accumulation. Stem Cells Dev. 2013; 22:2298–2314. [PubMed: 23517218]
114. Greenhalgh SN, Conroy KP, Henderson NC. Healing scars: targeting pericytes to treat fibrosis. QJM. 2014; 108:3–7. [PubMed: 24659747]

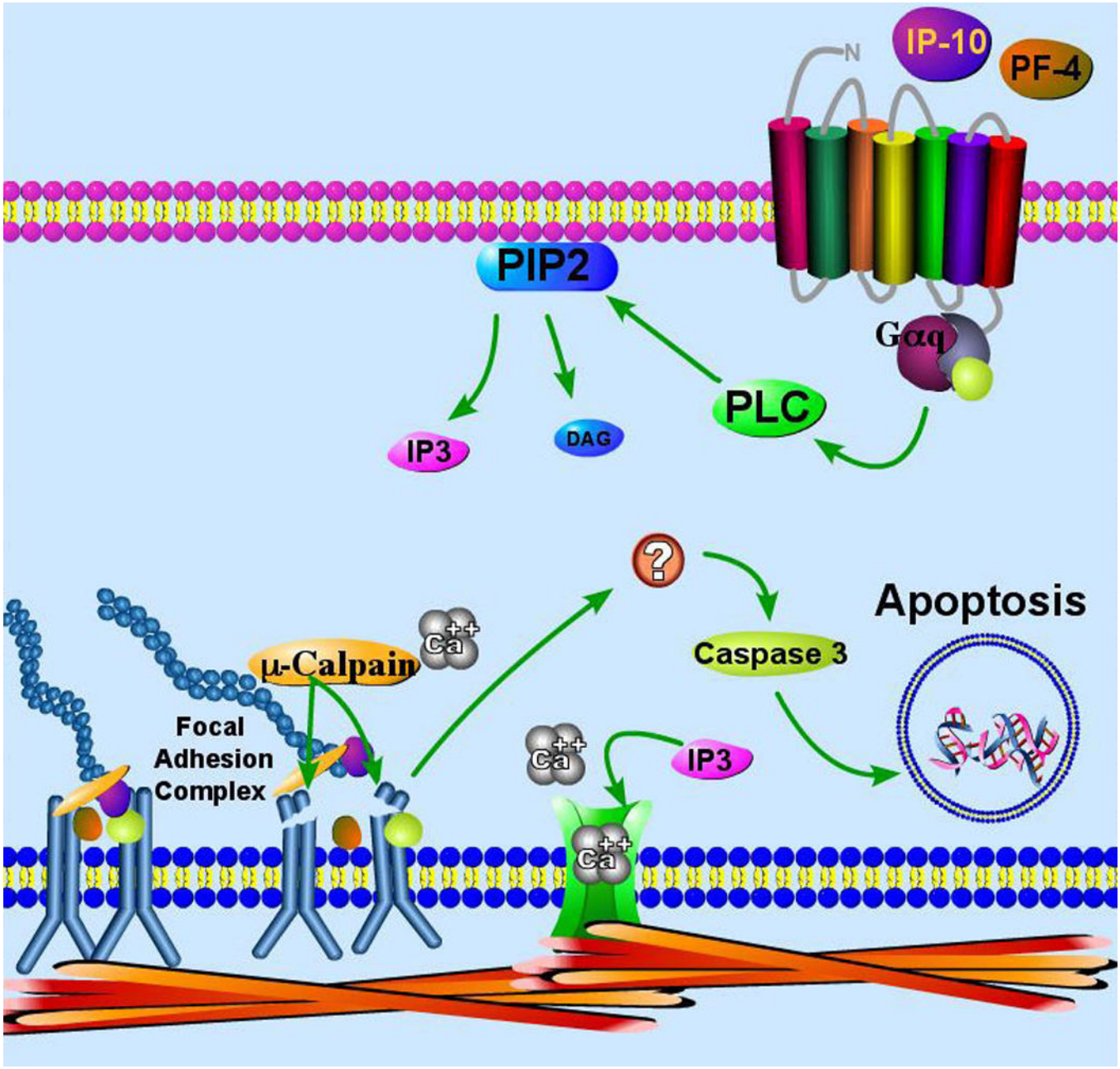

Figure 1.

CXCR3 signaling leading to anoikis in endothelial cells. The ligands for CXCR3 (CXCL4/PF4, CXCL9/Mig, CXCL10/IP-10, and CXCL11/IP-9/I-TAC), derived from pericytes and other cells, lead to calcium influx. In the calcium sparks at the membrane, the calcium concentration can be sufficient to activate μ-calpain which then cleaves the β3 integrin. As β3 integrin is the main isoform in endothelial cells, this leads to anoikis and endothelial cell death.

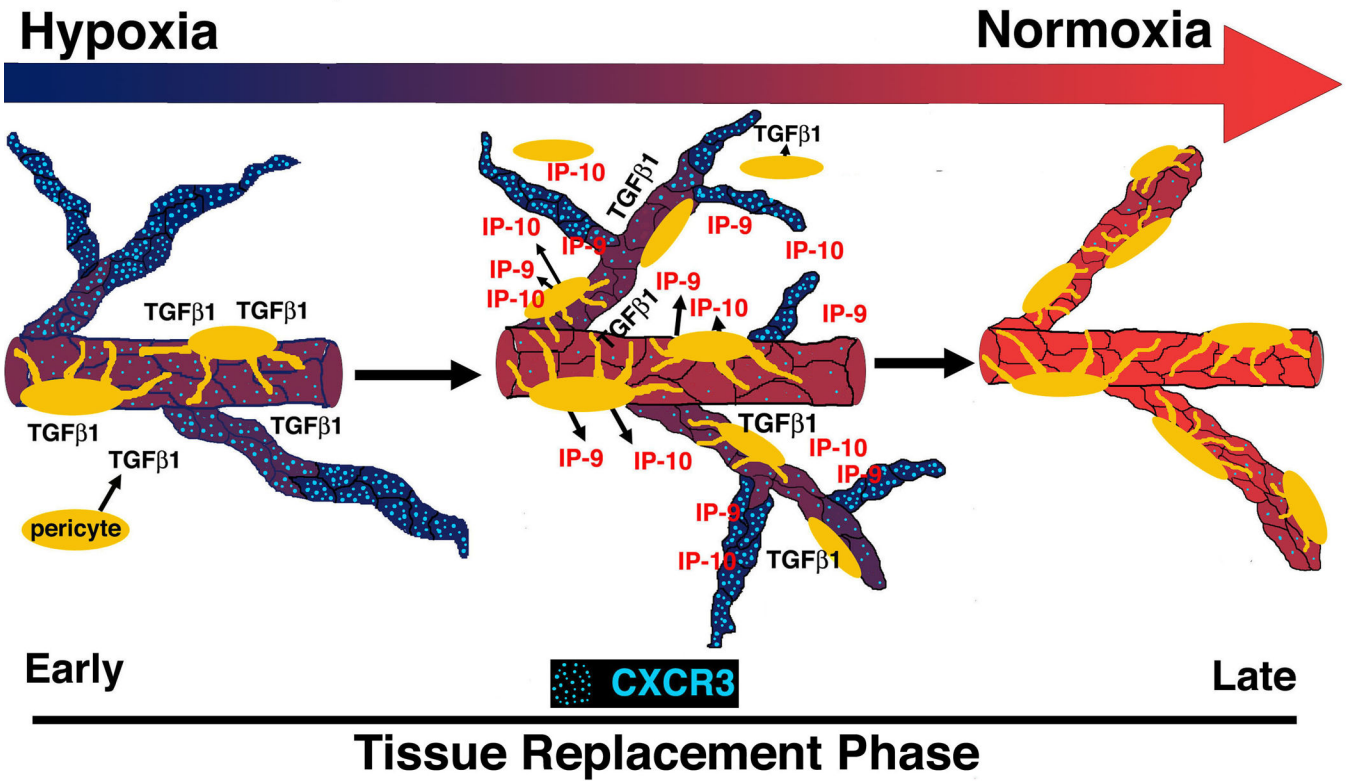

Figure 2.

Pericytes regulate the angiogenic response during wound healing. As the tissue replacement phase initiates, the wound bed is largely anoxic, with the VEGF response driving angiogenic sprouting; the pericytes are driven to express TGFβ1 being both angiogenic and pro-fibrotic. These immature endothelial cells express high levels of CXCR3. Upon establishing functional vascular conduits, the endothelial cells mature, concomitantly downregulating CXCR3 expression but producing CXCR3 ligands that attract pericytes. The pericytes also express CXCR3 ligands, with these ligands driving anoikis of nearby immature endothelial cells resulting in vascular involution. Late in wound healing the pericytes coat and stabilize the remaining, CXCR3-negative endothelial cell tubes.

Table 1

Characteristic Markers for Identifying Pericytes

Pericyte Markers	Target/Localization	Main Functions	Physiological Role
CD-146 (melanoma cell-adhesion molecule)	Cell surface protein	Angiogenesis, signal transduction, mesenchymal stem cells differentiation, cell migration	Pericyte differentiation
Chondroitin sulfate proteoglycan marker NG-2 (or high molecular weight melanoma antigen)	Cell surface protein. Expressed only in arteriolar and capillary perivascular cells (vSMC ad pericytes) and is absent on venular pericytes.	Cell proliferation, migration, differentiation	Vasculogenesis or angiogenesis
Desmin	Intermediate filament protein	Cellular contraction	Regulation of blood flow
Alpha- SMA	Cytoskeletal protein	Cellular contraction	Contraction, regulation of blood flow
Platelet-derived growth factor receptorβ (PDGFR-β)	Cell surface protein	Tyrosine-protein kinase; kinase receptor	Proliferation, migration, development
Aminopeptidases A and N and RGS5 (regulator of G-protein signaling)	Intracellular protein	G-protein couple receptor signaling	Active vessel remodeling
Angiopoietin-1 (Ang-1)	Interstitial stem cells destined to become microvascular pericytes	Vessel maturation, adhesion, migration, and survival	Vessel stabilization