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Microvascular Remodeling and Wound Healing: A Role for Pericytes

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Abstract

Physiologic wound healing is highly dependent on the coordinated functions of vascular and non-vascular cells. Resolution of tissue injury involves coagulation, inflammation, formation of granulation tissue, remodeling and scarring. Angiogenesis, the growth of microvessels the size of capillaries, is crucial for these processes, delivering blood-borne cells, nutrients and oxygen to actively remodeling areas. Central to angiogenic induction and regulation is microvascular remodeling, which is dependent upon capillary endothelial cell and pericyte interactions. Despite our growing knowledge of pericyte-endothelial cell crosstalk, it is unclear how the interplay among pericytes, inflammatory cells, glia and connective tissue elements shape microvascular injury response. Here, we consider the relationships that pericytes form with the cellular effectors of healing in normal and diabetic environments, including repair following injury and vascular complications of diabetes, such as diabetic macular edema and proliferative diabetic retinopathy. In addition, pericytes and stem cells possessing “pericyte-like” characteristics are gaining considerable attention in experimental and clinical efforts aimed at promoting healing or eradicating ocular vascular proliferative disorders. As the origin, identification and characterization of microvascular pericyte progenitor populations remains somewhat ambiguous, the molecular markers, structural and functional characteristics of pericytes will be briefly reviewed.

Keywords

Adipose-derived stem cell; Angiogenesis; Capillary; Diabetes; Extracellular matrix; Fibroblast; Glia; Inflammatory Cells; Injury; Mesenchymal stem cell

1. Introduction

Physiologic wound healing progresses through coagulation, inflammation, formation of granulation tissue, and remodeling events (Falanga, 2005), all of which rely upon the active involvement and contributions derived from the microvasculature and its associated cellular

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and metabolic components. As the epidermis and all epithelia are avascular, dermal angiogenesis is an essential response following injury. Importantly, post-injury capillary expansion is controlled by microvascular cell-cell interactions. Microvascular cellular responses are further shaped by cues from the extracellular matrix (ECM), local non-vascular resident and immigrant cells, and immune surveillance cells present within the wound microenvironment. As have been well studied, platelets, inflammatory cells, and connective tissue components foster neovascularization through regulated secretion of soluble factors and ECM reorganization (Schultz et al., 2011). How these cell-based and cell-extracellular signals provide meaningful wound healing-associated “crosstalk” to control microvascular remodeling in response to injury is of particular interest. Early studies identified a role for pericyte-endothelial cell (EC) interactions in wound healing, but its scope was limited to the vasculature (Crocker et al., 1970). In this review, we examine the molecular and cellular interactions governing wound healing responses, revealing what might be under-appreciated or emergent roles for pericytes in microvascular remodeling and wound repair.

Non-healing wounds, such as those seen in diabetic patients suffering from the ulceration of their extremity-associated wounds (plantar ulcers) or patients presenting with proliferative diabetic retinopathy (DR) remain a problem in the clinic. Diverse in nature, yet reflective of the important role that the local microenvironment plays in shaping the spectrum of pathologic microvascular wound healing responses, DR results in a marked formation of neovessels (Crawford et al., 2009); whereas, diabetic dermal wounds are deficient in angiogenic processes (Brem and Tomic-Canic, 2007). As changes to pericytes have been proposed to mediate DR (Hammes, 2005) and foot ulceration (Tilton et al., 1985), we will review the current literature on pericyte dysfunction in diabetic wound healing. Since it is hypothesized that DR embodies a wound healing response (Gariano and Gardner, 2005), we aim to highlight convergent, divergent and overlapping signaling pathways that may help to reveal the basic molecular and cellular mechanisms controlling pericyte-dependent microvascular remodeling observed during healing or proliferative DR.

Use of mesenchymal stem cells (MSC) to promote wound healing is an expanding field of therapeutic interest (Brower et al., 2011). Recent studies describe pericytes as a potential perivascular pool for MSCs, giving rise to skeletal myofibers, bone, cartilage, and adipocytes (Crisan et al., 2008a, 2008b). MSCs and adipose-derived stem cells (AdSC) are being deployed in experimental models of wound healing (Huang et al., 2012; Natesan 2011b), and MSCs (Sasaki et al., 2008) and AdSCs (Natesan et al., 2011b) have been described as possessing pericyte-specific properties; these attributes may be linked to enhanced healing. Herein, we propose a more uniform classification system for these stem cells or presumed pericyte progenitors, offering an overview of key molecular markers expressed, structural elements presented, and functional properties possessed by microvascular pericytes.

2. Pericytes, Microvascular Endothelial Cells and Non-vascular Cells Control Wound Repair

Microvascular pericytes act as a crucial cellular interface between blood-borne and connective tissue signals. In normal vascular physiology, pericytes are closely associated with a stable endothelium. However, in response to injury, pericytes are not only contacted with ECs, but also encounter infiltrating inflammatory and connective tissue cells (Figure 1). How pericytes interact with the microenvironment and cellular players of wound healing will be reviewed below.

2.1. Vascular Cells

2.1.1. Endothelial Cells—ECs serve as the fundamental unit of the vasculature.

Composing the inner lining of vessels, they act as a conduit for oxygen, nutrients and blood-borne cells. Endothelial tubes possess two surfaces: adluminal and abluminal. Adluminally, ECs are immunologically inactive, which prohibits spontaneous clot formation and immune cell activation. Low levels of adhesion receptors line inactive endothelial monolayers, allowing monocytes to roll along the vessel wall (Cook-Mills and Deem, 2005).

Abluminally, microvascular ECs are ensheathed by a shared basement membrane (BM) with pericytes; the abluminal surface is interspersed with EC-pericyte and EC-matrix contact points (Hirschi and D'Amore, 1996).

In injury, the endothelium acts to home infiltrating inflammatory cells, and provide nutrients to actively remodeling tissues. Chemokines and cytokines from the wound bed change the repertoire of luminal surface receptors on ECs. For example, P-selectin and E-selectin, glycoprotein receptors responsible for leukocyte rolling and adhesion, are virtually absent from normal EC surfaces. Yet, inflammatory factors induce translocation of P-selectin from Weibel-Palade bodies, and increase expression of E-selectin in the endothelium (Weller et al., 1992). P- and E-selectins appear to function concertedly as double deficient mice for P-/E-selectin display decreased leukocyte transmigration and wound closure (Subramaniam et al., 1997). Furthermore, tumor necrosis factor- α (TNF- α) and interleukin-1 (IL-1) upregulate vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1) in ECs, which cause arrest of leukocyte movement (Muller et al., 1993). Studies with membrane-bound ICAM-1 knockout mice and knockout mice for all isoforms of ICAM-1 demonstrate attenuated wound healing responses (Gay et al., 2011; Nagaoka et al., 2000). Since transmigration of leukocytes through the endothelial barrier is mediated by the binding of platelet endothelial cell adhesion molecule-1 (PECAM-1/CD31) on leukocytes and ECs (Muller et al., 1993), decreased leukocyte accumulation and angiogenesis is observed when endothelial PECAM-1 expression is disrupted. Results using this PECAM-deficient murine model suggest that wound healing may be negatively affected, as leukocyte extravasation into the connective tissue or wound bed is necessary to foster injury resolution (Solowiej et al., 2003). Taken together, leukocyte homing to injured tissue is highly dependent on EC expression of adhesion receptors, and these receptors are crucial for microvascular-immune cell interactions needed for the progression of the healing cascade.

As angiogenesis provides oxygen, nutrients and blood-borne cells to the site of tissue injury, microvascular expansion is a tightly regulated process influenced by local cell-matrix interactions and soluble mediators released or produced within the wound bed (Schultz et al., 2011). For example, formation of fibrin clots and release of platelet factors such as vascular endothelial growth factor (VEGF) (Möhle et al., 1997) stimulates EC migration and capillary morphogenesis. As healing progresses, matrix remodeling influences EC integrin-matrix interactions, which stimulates angiogenesis. During dermal wound healing, clot-derived fibronectin and vitronectin as well as EC production of laminins may foster increased angiogenesis. For example, fibronectin and vitronectin facilitate EC adhesion and migration (Tonnesen et al., 2000). Further, laminin 8 increases capillary tube formation *in vitro*, and laminin 10 is strongly expressed by microvessels in dermal granulation tissue (Li J et al., 2003). It is currently unknown how endothelial-pericyte interactions modulate laminin expression and whether this impacts matrix remodeling or the dynamic reciprocity driving healing (Shultz et al., 2011). Together, the microenvironment is an important factor in modulation of the endothelium, and during injury repair, induces a pro-angiogenic phenotype in ECs.

Pericyte crosstalk with the endothelium may regulate the extent of neovascularization in wound healing. Early wound healing studies observed gradual increases in pericyte coverage of ECs as the healing cascade progressed (Crocker et al., 1970), suggesting pericyte contact may mediate vessel stabilization in wound repair. Indeed, two independent pathways of pericyte regulation of endothelial growth state have been identified. Firstly, soluble transforming growth factor-beta (TGF- β) promotes endothelial quiescence. When pericytes and ECs are cocultured, latent TGF- β is converted to its active form, which inhibits EC growth (Antonelli-Orlidge et al., 1989). In addition, an alternative pathway of contact-dependent regulation of EC proliferative phenotype exists. Work by Lee et al. (2010) provides evidence that pericyte mechanical stiffness may control the tension of the underlying BM and endothelium. Furthermore, work from our group reveals adenoviral infection of pericytes with dominant negative or active Rho GTPase alters pericyte contractile state, which in turn, modulates EC proliferation (Kutcher et al., 2007). These results suggest that perturbations in pericyte contractility may serve as an angiogenic switch in wound healing or pathologic ocular angiogenesis seen in diabetes. Moreover, tissue inhibitor of metalloproteinase-2 (TIMP-2) and TIMP-3 expressed by ECs and pericytes respectively, enhance capillary stability (Saunders et al., 2006). Thus, pericyte regulation of EC proliferation is multiform, and modulation of these signaling pathways may play important roles in neovascularization, including the maturation of the capillary plexus associated with the wound bed.

2.1.2. Vascular Progenitor Cells—Unlike angiogenesis, which arises from a pre-existing microvascular plexus, vasculogenesis is driven by the *in situ* differentiation of endothelial (EPC) or vascular progenitor cells. The embryonic and postnatal vasculature is formed from multiple sources including mesodermal tissue, bone marrow and local stem cell reservoirs (Bautch, 2011). Mesoderm-derived angioblasts produce ECs of the major vessels, and angioblast migration is VEGF-dependent (Cleaver et al., 1997). Moreover, chimera studies demonstrate angioblasts also contribute to the vessels of the trunk and limbs, as well as perineural vessels (Ambler et al., 2001). Hemangioblasts, bipotential progenitors, are an additional source of endothelium in the developing vasculature. Fate map studies revealed that hemangioblasts give rise to erythrocytes and ECs (Vogeli et al., 2006). Newly formed vessels must be stabilized, which is fostered by mural cell associations. Mural progenitor cell recruitment and differentiation into smooth muscle cells (SMC)/pericytes are mediated by EC contact (Hirschi et al., 1998). Furthermore, during postnatal vasculogenic expansion, EPCs and native endothelium stimulate differentiation of vascular stem cells into pericytes by JAGGED-1 contact-dependent signals (Boscolo et al., 2011). Thus, the formation and maturation of nascent vascular networks relies on interactions among vascular progenitor cells, ECs and mural cells, and this process may be important at wound sites where areas of actively growing and remodeling microvessels are present.

EPCs produce functional vascular networks in cutaneous wounds and ischemic tissues (Asahara et al., 1999). Isolation and *in vitro* culture of putative EPCs, mononuclear blood cells expressing CD34, demonstrated that these cells possess EC lineage markers and form tube-like structures. Furthermore, *in vivo* hind limb ischemia studies reveal that CD34+ EPCs can be observed as they incorporate into the endothelium of neovessels (Asahara et al., 1997). These results highlight the blood-borne nature of this progenitor pool, which is capable of EC differentiation in wounded dermal compartments recovering from injury.

Peripheral vascular trauma places hypoxic stress on the surrounding tissue, as is the case in wound microenvironments (Knighton et al., 1983). Gill et al. (2001) examined the peripheral blood of burn patients for mobilization of EPCs, and observed significant increases in bone marrow derived EPCs concomitant with augmented VEGF plasma levels. Moreover, EPCs were shown to contribute to neovascularization in ischemic tissue, and this

EPC-driven neovascularization was enhanced by cytokine pre-treatment (Takahashi et al., 1999). Interestingly, during wound healing, chemokine signaling through the CCL5/CCR5 pathway appears to contribute to EPC homing since CCR5 null mice display decreased EPC accumulation and wound closure (Ishida et al., 2012). In addition, this study revealed that EPCs not only participate in wound neovascularization, but also secrete growth factors such as TGF- β and VEGF. Conversely, Bluff et al. (2007) demonstrated that dermal wound healing increases EPC accumulation 5–14 days after injury, but that EPCs do not significantly add to neovascularization as angiogenesis was implicated as the prevailing mechanism of neovascularization in the healing of surgical incisions. Together, these results suggest that soluble factors and low oxygen concentrations from the wound bed stimulate EPC mobilization and accumulation to foster increased vascularization. Furthermore, EPC-driven neovascularization may only function in wounds inflicted by significant trauma where the wound area is large and requires a more robust vascular response.

Pericytes may foster EPC differentiation while stabilizing neovessel formation during vasculogenesis. Yet, direct evidence revealing such a functional linkage is lacking. Interplay between pericytes and EPCs could be important in controlling microvascular morphogenesis, and results derived from *in vitro* experimentation support this notion. Matrigel-derived cocultures containing defined ratios of EPCs and pericytes reveal that EPCs can form capillary-like networks, and that pericytes become closely associated with these structures (Bagley et al., 2005). Further, pericyte extensions appeared to connect groups of EPCs; therefore, it was hypothesized that pericytes might guide EPC-derived vascular structures (Bagley et al., 2005). This observation could be important in wound healing, as pericytes may direct EPC migration and tube formation into the wound area. Moreover, shear stress or vascular smooth muscle cell (vSMC) contact have been shown to induce EPC differentiation into mature endothelium (Ye et al., 2008), suggesting that local mechanical or mural cell-based signals shape EPC differentiation *in situ*. However, whether pericyte-EPC contact acts as an initiator or merely helps to strengthen an already differentiating EPC remains equivocal. Clearly, more work will be needed to reveal whether pericyte and EPC interactions foster wound healing by promoting capillary EC differentiation and capillary network formation.

2.2. Inflammatory Cells

2.2.1. Platelets—Platelets, the primary cellular responders to injury, represent a plasma reservoir of pro-inflammatory, pro-coagulatory and pro-angiogenic factors that contribute to the healing cascade. During vessel injury, platelets adhere to exposed collagen fibers mediating the formation of fibrin clots (Brass, 2003); upon activation, platelets release a milieu of soluble factors including TGF- β , platelet derived growth factor (PDGF), basic-fibroblast growth factor (b-FGF), VEGF, ATP (Blair and Flaumenhaft, 2009) and sphingosine 1-phosphate (S1P) (English et al., 2000). And, with local control of coagulation critical for the initiation of the myriad of acute wound healing responses that ensue, platelets function to integrate healing effector pathways ranging from coagulation to angiogenesis and re-epithelialization.

At the onset of injury, cessation of hemorrhagic events requires formation of a fibrin clot. Interestingly, fibrin and fibrinogen, key players in the coagulation cascade, have been shown to bind VEGF and b-FGF. These growth factor-laden molecules induce angiogenesis, fibroblast migration and proliferation, and interact with infiltrating inflammatory cells (Laurens et al., 2006). Moreover, the use of platelet-rich fibrin matrices has been shown to induce wound neovascularization, and accelerate wound closure through the release of VEGF, TGF- β and PDGF-BB (Roy S et al., 2011). Because platelets and their associated soluble factors demonstrate pro-healing effects, it is not surprising that platelet-rich plasma

has been employed to foster enhanced healing in clinical settings. Indeed, application of platelet rich plasma to dermal lesions increases EC proliferation and migration, angiogenesis and injury resolution (Park HB et al., 2011; Yang et al., 2011). Furthermore, work in our laboratory and wound healing center indicate that synthetic peptides “mimicking” and augmenting the naturally occurring plasma- and/or platelet-derived healing activities can significantly promote wound healing *in vitro* and *in vivo* (Demidova-Rice et al., 2012). These bioactive wound healing peptides, which we engineered based on key platelet rich plasma fragments have a marked effect when added to capillary EC cultures, significantly augmenting cell proliferation and tube formation. Importantly, the wound healing peptides also markedly enhance epithelial cell migration *in vitro* and *in vivo*. Thus, platelet functionality ensures initiation of the healing responses to injury, while fostering wound closure through enhanced epithelialization

No direct interactions between platelets and pericytes have been identified, yet pericytes may cooperate with platelets to control vascular hemorrhage. Bouchard et al. (1997) demonstrated that brain pericytes possess the ability to modulate the extrinsic clotting cascade. Brain pericytes express tissue factor, and their surface permits the assembly of the prothrombinase complex. Further, dermal pericytes were also shown to express tissue factor (McDonald et al., 2008), although the clotting efficacy of dermal pericytes was not investigated. When pericyte-EC cocultures were embedded in fibrin clots, only EC sprouts were observed to emanate from the clot (Nehls et al., 1994), suggesting pericytes may remain within the fibrin matrix to mediate additional coagulation. Alternatively, fibrin may sequester pericytes in the clot to permit expansion of the capillary plexus into the wound bed. To test this, pericyte-EC cocultures on several matrix components such as collagen, laminin and fibrin could be assessed for endothelial S-phase entry and tube formation. Fibrin cocultures might exhibit significant angiogenic activity and capillary-like structures devoid of pericytes, indicating fibrin may switch pericyte physiology to a state that is permissive to endothelial activation, allowing revascularization of the wound bed.

2.2.2. Neutrophils—During the cellular response to injury, neutrophil transmigration is coordinated by pericytes. Feng et al. (1998) observed that neutrophil movement through the endothelium was followed by a transcellular migration path across cutaneous pericytes. Further, electron microscopy revealed pauses in neutrophil migration when contacted with pericytes. Indeed, studies on cremaster muscle venules have identified BM and possibly pericyte-produced matrix microdomains through which neutrophil extravasation is facilitated. Such matrix microdomains are heralded by decreased expression of laminin 10, collagen IV, and nidogen-2, i.e. low expression regions (LERs) (Wang et al., 2006). Strikingly, nearly 100% of LERs were associated with gaps between adjacent venular pericytes. Further, Voisin et al. (2010) demonstrated that LERs are not confined to venules of cremaster muscles, but are ubiquitous. Taken together, these results identify a critical role for pericytes in the construction of the BM as well as in shaping whether or to what extent immigrant cells can transit from the blood into the connective tissues of healing wounds. Furthermore, the close proximity of LERs to pericytes and preferential migration of neutrophils through these channels suggests pericytes and neutrophils may be in direct communication during inflammatory responses.

Through chemotactic signals neutrophils are able to home to sites of inflammation, transmigrate through the vasculature, and enter the wound bed (Woodfin et al., 2010). Neutrophils produce early inflammatory responses, as their entry into the wound area is strongest within 24 hours of injury. Investigation of neutrophil infiltration kinetics revealed that compared to saline treated wounds, wounds inoculated with *Staphylococcus aureus* exhibited higher numbers of neutrophils, but did not significantly alter wound closure (Kim MH et al., 2008). This result confirmed an early study by Simpson and Ross (1972), which

reported control and neutropenic wounds had comparable healing rates. However, a recent study implicated neutrophil derived-TNF- α as a promoter of neovascularization and re-epithelialization in *Pseudomonas aeruginosa* infected lesions (Kanno et al., 2011). Moreover, neutrophils were observed to secrete VEGF (Gaudry et al., 1997) and TIMP-free matrix metalloproteinase (MMP)-9 (Ardi et al., 2007), which stimulate angiogenesis *in vitro*. These results indicate neutrophil contribution to injury repair may be dictated by the presence or absence of infection, as well as the type of bacterial infection. While several studies indicate neutrophils possess pro-angiogenic molecules, they have yet to be tested *in vivo*. Therefore, further investigation is required to gain a fuller understanding of the extent of neutrophil participation in wound closure.

2.2.3. Monocytes/Macrophages—Pericytes and macrophages possess overlapping functions, and may coordinate wound neovascularization. Evidence for phagocytic properties of pericytes has been shown in brain-derived pericytes *in vivo* (Jeynes, 1985) and *in vitro* (Balabanov et al., 1996). Furthermore, injurious stimuli may convert brain pericytes to macrophages or upregulate their macrophage-like activities (Thomas, 1999). Therefore, pericytes could aid to clear wound debris in dermal lesions; although, the ability of dermal pericytes to phagocytose particles remains to be demonstrated.

Macrophage and pericyte interactions may foster vascular expansion and stability in the wound bed. *In vitro* and *in vivo* experiments revealed that macrophages and monocytes produce tube-like structures in Matrigel (Anghelina et al., 2004). These tubes were posited to influence neovessel distribution as erythrocytes and cells with EPC markers co-localized with these structures. Moreover, recruitment of bone marrow derived-pericytes and macrophages were shown to establish immature vascular networks in dermal Matrigel plugs via FGF-2 dependent mechanisms (Tigges et al., 2008). Macrophage migration may also influence neovascularization. Nucleoside triphosphate diphosphohydrolase-1 (CD39) mediates the breakdown of extracellular nucleoside di- and triphosphate molecules such as ATP and ADP. In nucleotide migration studies, CD39-null macrophage chemotaxis was altered (Goepfert et al., 2001). When CD39-null mice were used to investigate Matrigel plug capillary ingrowth, CD39-null mice exhibited a stratified cell infiltrate of macrophages, pericytes and ECs devoid of neovessel formation, suggesting macrophage migration is crucial to wound neovascularization (Goepfert et al., 2001). Further, macrophage-pericyte interactions may not be limited to early vessel formation as population and subsequent stabilization of the tumor vasculature by pericytes appears to be macrophage MMP-9 dependent (Chantrain et al., 2004).

Several subtypes of macrophages exist, including resident tissue, M1 and M2 macrophages; however, during wound healing, the distinction between M1 and M2 macrophages becomes blurred, and are commonly referred to as “wound associated macrophages” (Rodero and Khosrotehrani, 2010). Wound-associated macrophages function as phagocytic cells capable of interacting with vascular and non-vascular cells through an array of soluble factors. Indeed, through time-specific ablation of macrophages, Lucas et al. (2010) revealed that macrophages differentially regulate wound healing. For example, elimination of macrophages in the early stages of injury results in decreased neovascularization and re-epithelialization, whereas depletion of macrophages in the later stages of healing appears to have no significant effect on tissue repair (Lucas et al., 2010). Moreover, decreased macrophage infiltration mitigates angiogenesis and secretion of pro-angiogenic factors (Goren et al., 2009; Mirza et al., 2009). There is also evidence that macrophages may control the soluble milieu of the wound microenvironment through phenotypic switching. Wound macrophages produce high concentrations of cytokines such as TNF- α and IL-6 early, but secrete higher levels of TGF- β in the later stages of healing (Daley et al., 2010). As TGF- β mediates EC quiescence in pericyte-EC cocultures (Antonelli-Orlidge et al.,

1989), macrophage-derived TGF- β in concert with pericyte investment of nascent capillaries may further enhance vessel maturation. Thus, macrophages may be an additional regulator of angiogenic potential during wound repair, and contribute to vessel guidance and stabilization with pericytes.

2.2.4. Pericyte Regulation of Lymphocyte Function: A Role in Modulating Wound Healing

Pericytes may regulate T cell activation, and recruit T and B-lymphocytes to areas of tissue injury. Under inflammatory conditions, brain pericytes gain the ability to present antigens to T cells, which leads to lymphocyte activation (Balabanov et al., 1999). In contrast, retinal pericytes inhibit T lymphocyte activity (Tu et al., 2011). Hyperglycemia reduces pericyte inactivation of T cells, suggesting pericyte modulation of T lymphocytes is highly dependent on cues from the microenvironment. Therefore, pericytes may influence T cell activity during wound healing, and cues from the wound microenvironment may dictate whether these pericyte signals are stimulatory or inhibitory.

Pericyte recruitment of lymphocytes to inflamed tissue could be mediated by several cytokines. Immunohistochemical analysis of stromal-cell derived factor (SDF-1) and CXCR4 in the dermal microvasculature revealed a potential role for pericyte-derived SDF-1 in lymphocyte recruitment (Pablos et al., 1999). Moreover, secretion of CXCL9 and CXCL12 by tumor microvessel-associated pericytes enhances B and T cell tissue infiltration (Venetz et al., 2010). Further, dermal pericytes have been shown to express CXCL12 (Avniel et al., 2006), which signals through CXCR4 to mediate T cell chemotaxis (Prasad et al., 2007); therefore, the CXCL12/CXCR4 axis may represent an additional pathway for pericyte-lymphocyte communication.

Pericytes modulate adhesion receptor expression and lymphocyte infiltration. Stellate cells, pericyte-like cells of the liver, regulate adhesion molecule expression in response to injury and inflammation. *In vitro* application of inflammatory cytokines to stellate cells increased levels of ICAM-1 and VCAM-1 compared to control cultures. These results were recapitulated *in vivo* as acute liver injury resulted in augmented levels of ICAM-1 and VCAM-1 mRNA (Knittel et al., 1999). Further, brain pericyte exposure to TNF- α mediated T cell infiltration via very late antigen-4 (VLA-4)/VCAM-1 binding (Verbeek et al., 1995). Pericytes possess soluble and contact-dependent means of regulating lymphocyte infiltration to inflamed tissue; therefore, these pathways could importantly influence tissue repair.

2.3. Connective Tissue

2.3.1. Fibroblasts/Myofibroblasts—Fibroblasts are a major cellular component of connective tissue, and are crucial for maintenance of the ECM in normal and injured tissue. In the dermis, subpopulations of fibroblast exist, which exhibit differences in ECM deposition, and highlight fibroblast heterogeneity (Sorrell and Caplan, 2004). Characteristic of wounds, myofibroblasts represent specialized fibroblasts that express α -smooth muscle actin (α -SMA) (Darby et al., 1990). The concerted functions of these cells facilitate injury repair through matrix remodeling, growth factor secretion and contractile forces.

Release of PDGF from platelets initiates fibroblast migration and proliferation into the wound bed (Rajkumar et al., 2006). Fibroblasts secrete numerous growth factors including epidermal growth factor (EGF), FGF-2, TGF- β , PDGF and VEGF (Barrientos et al., 2008), which promote epithelialization, vascularization and granulation tissue formation. Activation of fibroblasts causes differentiation to contractile myofibroblasts (Li B and Wang, 2011). Fibroblast-myofibroblast transitions require both mechanical signals and TGF- β signaling (Serini et al., 1998), and myofibroblasts can liberate latent TGF- β to stimulate further fibroblast differentiation (Wipff et al., 2007). Collagen network stability is mediated by myofibroblast contractions. Tightening of previously secreted collagen fibers

allows adjacent myofibroblasts to secrete more collagen, thereby increasing the strength of wound granulation tissue (Tomasek et al., 2002). This cyclical process of contraction and ECM deposition may also facilitate wound closure in a TGF- β dependent manner (Grinnell and Ho, 2002). A recent study by Kilarski et al. (2009) illuminates an additional role for fibroblasts and myofibroblasts: non-angiogenic neovascularization. Myofibroblasts generate forces that are capable of translocating capillaries into the wound area, suggesting sprouting angiogenesis follows this contraction-mediated vascular growth. These studies illuminate critical roles for fibroblast and myofibroblast force generation; not only do these forces influence wound closure, but may also provide a blood supply to expanding granulation tissue.

The extent of fibroblast interactions with pericytes remains unclear. As fibroblasts are known to secrete numerous soluble factors including FGF-2, which induces phenotypic changes to pericytes (Papetti et al., 2003), it is conceivable that fibroblast secretions may increase pericyte numbers during the proliferative phase of wound healing. With the formation of immature capillary networks during repair, an enhanced pool of pericytes would be advantageous for stabilization and remodeling of these neovessels.

There is emerging evidence that pericytes may act as a source of myofibroblasts. *In vivo* dermal healing revealed overlapping immunologic markers between myofibroblasts and pericytes (Juniantito et al., 2012), and pericyte IL-33 induction caused differentiation to myofibroblasts (Sponheim et al., 2010). However, Sponheim et al. (2010) suggested these IL-33 positive myofibroblasts could have originated from pre-existing dermal fibroblasts as well. Furthermore, pericyte to myofibroblast differentiation appears to not be skin specific, but can also occur in kidney fibrosis through PDGF-dependent mechanisms (Chen et al., 2011). These studies offer an intriguing novel role for pericytes as a potential source of myofibroblasts in wound healing. However, whether these pericyte-derived myofibroblasts are bona fide myofibroblasts remains to be determined. An alternative hypothesis is that these myofibroblasts within the wound bed could be contractile, ECM depositing pericytes that have become dissociated from microvessels (Figure 2). Fate map studies of microvascular pericytes during wound healing could elucidate the origin of myofibroblast-like cells found in wound tissue. Thus, pericyte to myofibroblast differentiation may reflect a migration and phenotypic switching event, whereby pericytes derived from a vascular niche are activated to migrate into the wound ECM and possess the functional roles of a myofibroblast.

2.4. Neuronal Support Cells

2.4.1. Müller Cells/Astrocytes—Association of mural cells with non-vascular, resident parenchymal cells has long been thought to influence microvascular dynamics during development and disease. For example, Müller cell (MC) and astrocytes are glial support cells that act as a critical interface between the vasculature and nervous system in what has been described as “the neurovascular unit” (Fisher, 2009). Confined to the retina, MCs function to provide lactate and pyruvate to neurons, and maintain the blood-retina barrier (Bringmann et al., 2006; Tout et al., 1993). Also, MCs secrete growth factors that promote retinal cell survival (Saint-Geneiz et al., 2008). Similarly, astrocytes are glia of the spinal cord and brain. Central nervous system (CNS) astrocytes interact with the vasculature and neurons, regulating blood flow dynamics, ion transport, and metabolism (Ransom and Ransom, 2012).

During injury, glial cells maintain neurovascular homeostasis through gliosis (Streit et al., 1999), which is characterized by an upregulation of glial fibrillary acidic protein (GFAP) expression (Pekny and Nilsson, 2005). Gliosis represents a spectrum of astrocyte responses aimed at maintaining neuronal homeostasis (Sofroniew, 2009). When reactive gliosis is

prolonged in MCs and astrocytes, the result is the formation of a glial scar. Glial scars impede axon regeneration (Silver and Miller, 2004), and in the retina, produce abnormal neuron growth and neovascularization (Bringmann et al., 2006). A recent study by Göritz et al. (2011) illuminates a subpopulation of CNS pericytes that participates in scar formation during spinal cord injury, suggesting glial scar formation may no longer be a glial-specific phenomenon, but include pericytes as well. Therefore, development of future therapeutics may benefit from examining glial and mural cell interactions in CNS lesions.

MCs and astrocytes are in close contact with capillaries of the CNS and retina, where astrocytic end-feet are observed to make contacts with pericytes (Mathiisen et al., 2010). Functionally, glia and pericyte interactions have been shown to maintain the blood-retina and blood-brain barriers (Al Ahmad et al., 2011; Kim JH et al., 2009). This linkage may also be important in response to injury in DR. Rescue of MC apoptosis in the diabetic retina appears to prevent capillary cell death, including pericyte loss. These results suggest that pathology to neuronal and glial cells might directly affect the health of vascular cells (Hammes et al., 1995). When pericytes and astrocytes are cultured with ECs *in vitro* both cell types migrate to capillary-like structures; furthermore, pericytes and astrocytes become invested in these endothelial structures as well (Itoh et al., 2011; Minakawa et al., 1991). Astrocyte-EC cocultures reveal that astrocytes inhibit EC proliferation through contact-dependent means (Garcia et al., 2004). Therefore, pericyte and astrocyte investment of microvessels may cooperate to maintain a quiescent endothelium, and regulate the angiogenic potential of the CNS microvasculature in response to injury.

3. Wound Healing in Diabetes: A Focus on Pericytes

Diabetes presents a significant burden on society, affecting 25.8 million Americans and costing \$174 billion dollars annually (American Diabetes Association, 2011), with a projected 64% increase in patients and \$514 billion in health care delivery costs by 2025 (Rowley and Bezold, 2012). Diabetic complications affect multiple organ systems, increasing the risk for myocardial infarction, stroke and atherosclerosis (Beckman et al., 2002). In addition, diabetic vascular complications plague macro- and microvascular structures causing stroke, atherosclerosis, impaired healing, retinopathy, neuropathy and nephropathy (Reddy and Natarajan, 2011). The cellular and molecular changes associated with DR encompass a misregulated healing response (Gariano and Gardner, 2005); therefore, we will frame the progression of DR within the events of a normal wound healing cascade (Figure 3). Here, we will also examine the pathogenesis of impaired injury repair in the context of pericyte dysfunction.

3.1. Diabetic Retinopathy

Recent successes with anti-angiogenic therapies in age-related macular degeneration (AMD) have turned attention to seeking advances in combating other ocular diseases such as DR (Durham and Herman, 2011). Development of innovative treatments is imperative, as diabetes-induced retinopathy causes the majority of new cases of adult blindness, and from 2005–2008 it was estimated that 28.5% of diabetics were afflicted with DR (American Diabetes Association, 2011). While the initial stimuli driving retinal neovascularization remain unknown, hyperglycemia produces a chaotic biochemical and cellular environment, a combined result of oxidative stresses and advanced glycation end products. Through altered signaling pathways and cellular interactions, an aberrant wound healing response is initiated, and if left unchecked, results in pathologic angiogenesis and retinal detachment.

Similar to coagulation events in physiologic wound healing, stasis of retinal blood flow (Frank, 2004) and abnormal platelet function produce microthrombi, which release vasoactive substances that may alter capillary integrity, permitting egress of platelet

fragments and hyperglycemic plasma into perivascular spaces (Dobbie et al., 1974). While platelets do not adhere to uninjured endothelium in normal physiology, hyperglycemic plasma induces platelet hyper-reactivity through increased adhesion receptor and platelet activation molecule expression (Ferreiro et al., 2010). Moreover, hypoxia downstream of microthrombosis and concomitant leukostasis may also contribute to retinal microvascular leakage and angiogenic induction. Indeed, fibrinogen and fibrin, potent inflammatory cell and EC activators, were found in diabetic retinal exudates (Murata et al., 1992). Thus, platelets may play an important role in the early progression of DR by facilitating augmented clot formation and vascular leakage.

Prolonged exposure to hyperglycemia perturbs vascular cell physiology, and creates a scenario reminiscent of the inflammatory and proliferative phases of tissue repair. *In vitro* culture of ECs and pericytes provide evidence that the diabetic microenvironment may upregulate inflammatory gene expression (Kowluru et al., 2010; Perrone et al., 2009) and matrix deposition (Roy S et al., 1996). *In vivo* mouse studies reveal a critical role for inflammation in DR progression. Diabetic CD18 knockout and ICAM knockout mice both exhibited decreased leukostasis, vessel leakage and vascular lesions compared to wild type diabetic mice. Moreover, pericyte loss was attenuated in diabetic knockout mice (Joussen et al., 2004). When cultured in hyperglycemic conditions, retinal pericytes exhibit attenuated inhibition of T lymphocyte activity (Tu et al., 2011). Taken together, retinal pericytes are sensitive to inflammation, and their inactivation of lymphocytes is essential to the stability of the microcirculation.

Retinal endothelial dysfunction and inflammatory responses produce vascular permeability that permits increased amounts of glucose to invade the mural cell microenvironment. Elevated levels of glucose have a deleterious effect on pericyte physiology, and have been shown to induce pericyte apoptosis (Geraldes et al., 2009), a histopathologic hallmark of DR (Hammes, 2005). Loss of pericytes may facilitate a permissive environment for neovascularization, as a 50% reduction in pericyte density was shown to lead to the re-initiation of EC proliferation (Enge et al., 2002). Ultimately, pathologic angiogenesis ensues, producing retinal capillaries that become acellular entities devoid of pericytes and ECs, enclosed by a thickened BM (Lorenzi and Gerhardinger, 2001).

3.2. Chronic Wounds in Diabetes

As retinal pericyte dysfunction and degeneration promotes pathologic angiogenesis, perturbations in dermal pericyte physiology result in decreased neovascularization and healing responses. These observations highlight the heterogeneity of microvascular function, and reflect differences between pericytes of the retina and dermis. Impaired wound healing is a significant complication in diabetes, which often results in foot ulcers. Diabetic foot ulcers have a lifetime incidence upwards of 25%, and are found in 70–84% of lower limb amputees (Evans et al., 2011). The exact mechanisms of chronic wound healing are unclear, but evidence suggests pathology to the microvasculature is essential to the pathogenesis of diabetic wound healing (Pham et al., 1998). Because pericytes mediate interactions with numerous cellular effectors of wound healing, their loss may be crucial to the pathogenesis of non-healing wounds in diabetes.

Pericyte loss from dermal and muscle capillary networks of diabetics has been documented (Tilton 1981, 1985); yet, little connection has been made linking pericyte absence with alterations to inflammatory responses, microvascular stability, wound closure and matrix deposition. Chronic wounds possess impaired inflammatory cell infiltration and function (Fahey et al., 1991; Nolan et al., 1978). Pericyte dysfunction may affect neutrophil and macrophage accumulation in chronic wounds, as pericyte-derived matrix components and localization to LERs is crucial for inflammatory cell infiltration. In addition, pericytes

modulate lymphocyte activity, suggesting alterations to pericyte physiology could prolong or shorten the inflammatory response.

The microenvironment of chronic wounds is hostile, characterized by markedly elevated levels of proteases and glycated matrix components. Punch biopsies of diabetic foot ulcers reveal increased concentrations of MMP-1, MMP-2, MMP-8, and MMP-9, but decreased concentrations of TIMP-2 (Lobmann et al., 2002). Imbalances in proteases and their inhibitors would begin to cannibalize growth factors and growth factor receptors, prohibiting capillary expansion. Moreover, glycated matrices possess altered elasticity and rigidity. When fibroblasts were challenged to proliferate and contract on glycated matrix, they were prohibited (Liao et al., 2009). These results could have implications for pericyte-EC signaling, as changes to the mechanical properties of the BM may impair chemo-mechanical regulation of endothelial growth state by pericytes. Thus, abnormal protease activity and glycation of ECM components render the wound bed to a state of angiogenic incompetence, thereby inhibiting neovascularization of granulation tissue.

Impaired wound healing illuminates the complexity of molecular and cellular organization required for proper healing. Wound healing events are interconnected, and as pericytes coordinate with multiple cell types, understanding pericyte pathology in diabetic ulcers may drive the discovery of future wound healing therapeutics.

4. Experimental Use of Pericyte-like Stem Cells: Implications for Cell-Based Wound Healing Therapeutics

4.1. Adipose-derived Stem Cells

AdSCs represent a source of multipotent stem cells that are typically isolated from the stromal vascular fraction derived from adipose (Lin et al., 2010). Traktuev et al. (2008) first identified CD34+ AdSCs as pericyte-like cells. These AdSCs express “pericyte” or mural cell markers, NG2 and α -SMA, as well as the mesenchymal marker, CD90. On Matrigel, AdSCs make abluminal associations with EC tube networks (Traktuev et al., 2008). In addition, a recent *in vitro* study confirmed the pericyte-like properties of AdSCs, and identified soluble and contact-dependent interactions are responsible for the production of EC tube morphogenesis and increased vessel stability (Merfeld-Clauss et al., 2010). However, AdSC differentiation appears to not be limited to pericytes, as a study using CD90+/CD34– AdSCs observed that AdSCs not only differentiate into pericytes, but also into EC lineages (Natesan et al., 2011b). Thus, while there is increasing evidence that AdSCs are capable of pericyte differentiation, questions still remain as to what microenvironmental cues direct AdSCs to mural cell lineages, and what subpopulations are capable of this transition.

While the endothelium and mural cells appear to be unsuitable for the toxic microenvironment of diabetic ulcers, AdSCs may represent a more resilient cell type. Because neovascularization is absent in diabetic lesions, the wound bed becomes extremely hypoxic. Evidence suggests that CD34+/NG2+/ α -SMA+ AdSCs, cells with pericyte-like properties, increase secretion of pro-angiogenic molecules, and maintain vascularization in inflamed tissue (Amos 2008, 2011). Further, application of conditioned media derived from CD90+/CD34– AdSCs cultured in hypoxia augmented wound area closure (Lee EY et al., 2009). Moreover, AdSCs have been shown to enhance wound healing in diabetic mice through secretion of growth factors and neovascularization (Kim EK et al., 2011; Nambu et al., 2009). However, one study using diabetic rats observed increased healing, but no significant difference in wound vascularization when treated with AdSCs (Maharlooeei et al., 2011). Thus, the mechanisms underlying AdSC mediated healing requires further

characterization, and more studies are needed to determine if AdSC to pericyte transitions are necessary to foster increased healing.

Taken together, AdSCs promote wound healing in diabetic and hypoxic conditions. Because AdSCs are an abundant and easily isolated population of adult stem cells in both normal (Yoshimura et al., 2006) and injured tissues (Natesan et al., 2011a), they may be an excellent tool for future pericyte replacement and chronic wound therapies.

4.2. Mesenchymal Stem Cells

Controversy seems to surround MSCs and pericytes. Both cell types have been noted to share several mesenchymal markers including CD44, CD90, CD105 and CD73 (Corselli et al., 2010), and evidence suggests that perivascular MSCs express the pericyte markers NG2 and PDGFR- β (Crisan et al., 2008b). Furthermore, pericytes have been purported to be stem cells themselves, capable of differentiation into osteogenic, adipogenic and chondrogenic lineages (Dar et al., 2012). This multipotency has been observed in CNS pericytes as well (Dore-Duffy et al., 2006). Early *in vivo* studies add more complexity, as MSCs were unable to differentiate into pericytes, although, these MSCs expressed the pericyte marker α -SMA; furthermore, these α -SMA⁺ cells were not found in perivascular locations (Anjos-Afonso et al., 2004; Silva et al., 2005). As therapeutic use of MSCs increases, and the delineation between MSCs and pericytes becomes further blurred, we suggest that pericytes and MSCs are two separate cell types. Pericytes may undergo phenotypic switching upon leaving the vascular niche, but do not undergo differentiation. In contrast, MSCs may reside in perivascular locations, but may not possess functional similarities to pericytes. Additional experimentation is required to determine whether MSCs regulate vascular stability and tone as well as endothelial quiescence.

MSCs may represent a potential source of pericytes in dermal wound healing. Sasaki et al. (2008) identified capillary associated α -SMA⁺/CD31⁺ pericytes in dermal lesions using GFP labeled MSCs (Sasaki et al., 2008); it should be noted that these GFP⁺ cells composed only 33% of wound bed α -SMA⁺ cells. This study suggests bone marrow MSCs may participate in wound healing by differentiating into a variety of cells including pericytes; although, it appears that a majority of pericytes arise from local vascular sources.

Innovative delivery systems are being deployed to enhance MSC-dependent healing. For example, MSC-containing hydrogels are “pro-angiogenic,” augment cytokine secretion, and accelerate wound closure *in vivo* (Rustad et al., 2012). Further, approximately 39% of implanted MSCs were NG2 positive. Another study provides evidence that microspheres loaded with MSCs could increase healing, as wounds treated with microspheres had significantly greater wound closure compared to control tissues (Huang et al., 2012). In addition to experimental models of healing, MSCs are being used clinically to alleviate limb ischemia. A recent Phase II trial suggests administration of EPCs and MSCs may elevate limb perfusion through increased angiogenesis or vascular remodeling (Lasala et al., 2011).

These studies illuminate a potential role for MSCs as progenitors of wound bed pericytes. Furthermore, animal studies demonstrate MSCs possess potent wound healing effects. These healing properties potentially hold therapeutic value in human wounds as MSCs were shown to be effective in treating limb ischemia. To enhance the pericytic properties of MSCs, more accurate sorting and pre-conditioning of MSCs could be used. Moreover, treating MSCs with molecules such as TGF- β , which induce pericyte-like phenotypes (Hirschi et al., 1998), before implantation might better ensure that stem cells produce a pericyte outcome in diseased tissues.

5. Pericyte Markers: A Perivascular Identity Crisis?

Pericytes are an extremely heterogeneous population of mural cells that accompany microvascular structures of the body. Enveloped by BM in the abluminal perivascular space of microvessels (Sims, 1986), proper identification is dependent on a mixture of molecular markers and location cues. While a growing list of pericyte markers exists, a bona fide marker remains to be discovered. As reviewed by Armulik et al. (2011), molecular markers of pericytes are promiscuous. For example, the glycoprotein 3G5 (Nayak et al., 1988), proteoglycan NG2 (Ozerdem et al., 2001) and PDGFR- β (Hellstrom et al., 1999) react with other cell types including renal medullary cells, vSMC and myofibroblasts, respectively. Moreover, several pericyte markers may be useful in areas of actively remodeling vessels only. These markers include an intermediate filament protein desmin (Nehls et al., 1992), high molecular weight melanoma-associated antigen (Schlingemann et al., 1991) and aminopeptidase A (Schlingemann et al., 1996). Therefore, with such ambiguity concerning the characterization of pericytes, it warrants the following line of questioning: “What defines a pericyte based on the current molecular markers, and can we develop methods to better identify pericytes?”

5.1. The Pericyte Actin Network: An Old Story with a New Twist

Original studies by Herman and D’Amore revealed actin networks as potential pericyte markers. Immunofluorescence analyses of actin networks could be used to distinguish pericytes from vSMCs and ECs *in vitro* and *in vivo* (Herman and D’Amore, 1985). Pericyte stress fibers were shown to strongly react with antibodies to both muscle and non-muscle actin, whereas stress fibers of vSMCs and ECs reacted only with muscle or non-muscle actin isoform-specific antibodies, respectively. Moreover, anti-muscle actin antibodies illuminated qualitative differences between pericyte and vSMC cytoskeletons. Pericytes and vSMCs possess robust muscle actin enriched stress fibers, yet only pericytes exhibit a granular meshwork of muscle actin in motile cytoplasmic segments (Figure 4). Non-vascular cells such as dermal fibroblasts also express α -SMA as do myofibroblasts, stellate cells and mesangial cells (Johnson et al., 1991; Rubbia-Brandt et al., 1997; Skalli et al., 1986). Recent work from our lab suggests that the actin cytoskeleton not only acts as a biomarker, but could be used as a functional marker for pericytes as well. Kolyada et al. (2003) revealed alteration to Rho GTPase signaling affects pericyte contractile phenotype through actin cytoskeletal rearrangements. Building on these results, Kutcher et al. (2007) demonstrated that perturbations to pericyte contraction influences pericyte regulation of endothelial proliferation. Therefore, comparing EC growth inhibition in AdSC/MS-EC cocultures with pericyte-EC cocultures could determine whether these stem cells are functionally equivalent to pericytes. Further, *in vitro* contractility assays paired with investigation of the non-muscle and muscle actin networks could provide evidence that MSCs and AdSCs are capable of mechanical regulation of capillary physiology. Taken together, pericytes are dynamic regulators of capillary stability and flow (Kutcher and Herman, 2009); therefore, functional assays may add another level of resolution for distinguishing pericytes from other pericyte-like stem cells.

6. Conclusion

Orchestration of cellular and molecular events is crucial for wound repair in normal physiology. Pericyte interactions with inflammatory cells, glia and connective tissue highlight the diverse roles pericytes play in mediating injury repair. These interactions may extend to other pathologies such as tumor progression, where pericyte investment may not only stabilize the tumor microvasculature, but also aid the tumor in recruiting stromal and immune components. Moreover, induction of wound healing pathways may not be limited to tissue injuries, as events in DR exhibit many similarities with the phases of physiologic

wound repair. DR and chronic wounds represent reciprocal wound healing responses; DR possesses enhanced angiogenic, inflammatory and proliferative processes, while diabetic ulcers are lacking, yet both exhibit pericyte loss. Therefore, elucidating the signaling pathways of either disease, and targeting pericytes may be beneficial to treatment of the other. Further, it is advantageous that a more uniform classification system be developed, as pericyte identification remains unclear and pericyte-like stem cells are emerging as therapeutic options in wound healing. Molecular markers and an association with the perivascular space may no longer be sufficient to identify pericytes. Pericytes are not static cells, but dynamic regulators of microvascular physiology. Thus, while “pericytic stem cells” are enticing candidates for pro-wound healing treatments, they must be rigorously validated before they can be truly called pericytes.

Abbreviations

AdSC	adipose-derived stem cell
BM	basement membrane
DR	diabetic retinopathy
EC	endothelial cell
ECM	extracellular matrix
EPC	endothelial progenitor cell
LER	low expression region
MC	Müller cell
MSC	mesenchymal stem cell

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Figure 1. Schematic Diagram of Pericyte Interactions with Cellular Effectors of Wound Healing
 Pericytes form relationships with vascular, immune, connective tissue and glial cells during injury repair. This diagram demonstrates that pericytes may regulate multiple points of the wound healing cascade. Pericyte functions are not confined to the microvasculature, and range from modulation of immune cell infiltration and activation to glial scar formation. Hypothesized interactions are indicated by (?).

Figure 2. Myofibroblast Emergence: A Perivascular Origin?

In normal tissue, the microvasculature is stable and covered by pericytes, and inactivated fibroblasts occupy the surrounding connective tissue. During wound healing, activated fibroblasts may differentiate into myofibroblasts or alternatively, pericytes may differentiate into myofibroblasts. However, pericytes may migrate from the vasculature, and enter the wound bed. Signals and matrix from the wound microenvironment may cause pericytes to assume a more contractile and matrix depositing phenotype, similar to that of a myofibroblast. Further validation will reveal the essence of the different contractile cell types that occupy the wound bed. Hypothesized interactions are indicated by (?).

Figure 3. Microvascular Dynamics During Normal Wound Healing, Diabetic Retinopathic and Chronic Wound-Associated Healing

(A) Normal tissue repair progresses through coagulation, inflammation, proliferation and remodeling phases without complication resulting in wound closure. (B) DR exhibits abnormal coagulation, inflammation and proliferation phases. Abnormalities in these phases of wound healing result in pathologic neovascularization and vascular leakage. (C) Chronic wounds possess normal coagulation, but display perturbed inflammation and proliferation phases. Without proper immune and vascular cell responses, wound closure is impaired or prevented.

Figure 4. The Pericyte Actin Network Regulates Cell Shape, Contractility and Endothelial Cell Quiescence

Immunofluorescence imaging of the smooth muscle and cytoskeletal containing compartments within a Bovine Retinal Pericyte. Phalloidin staining (green) and anti- α -SMA antibody (red) reveals F-actin and muscle actin networks in pericytes. α -SMA may be an important molecular and functional marker of pericytes and pericyte-like stem cells.