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From Pathobiology to the Targeting of Pericytes for the Treatment of Diabetic Retinopathy

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Abstract Pericytes, the mural cells that constitute the capillaries along with endothelial cells, have been associated with the pathobiology of diabetic retinopathy; however, therapeutic implications of this association remain largely unexplored. Pericytes appear to be highly susceptible to the metabolic challenges associated with a diabetic environment, and there is substantial evidence that their loss may contribute to microvascular instability leading to the formation of microaneurysms, microhemorrhages, acellular capillaries, and capillary nonperfusion. Since pericytes are strategically located at the interface between the vascular and neural components of the retina, they offer extraordinary opportunities for therapeutic interventions in diabetic retinopathy. Moreover, the availability of novel imaging methodologies now allows for the in vivo visualization of pericytes, enabling a new generation of clinical trials that use pericyte tracking as clinical endpoints. The recognition of multiple signaling mechanisms involved in pericyte development and survival should allow for a renewed interest in pericytes as a therapeutic target for diabetic retinopathy.

Keywords Pericytes · Diabetic retinopathy · Imaging · Cell signaling

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Introduction

Pericytes, the vascular cells that together with the endothelial cells make up the capillaries, have long been associated with the pathobiology of diabetic retinopathy (DR). However, translational applications of this link remain largely unexplored. Here, we review cell signaling mechanisms of pericytes in models of diabetes as well as new imaging technologies that open the door for novel therapeutic interventions and the possibility of assessing pericytes as clinical end points in clinical trials.

Microvascular Degeneration and Neurovascular Unit Dysfunction in Diabetic Retinopathy

The retinal requirements for oxygen and nutrients rank as the highest among all the other tissues in the human body, exceeding even that of the brain [1–4]. Thus, it is not surprising that two independent blood supplies operate to meet the demand. The superficial layers of the retina, including the ganglion cell layers and the inner nuclear layer, are nourished through a network of capillaries deriving from the central retinal artery. This vascular network has been the focus of intense research in the field of DR, as it is the target of overt degenerative changes that are easily detected by clinicians using standard fundoscopy examination or retinal photographs [5]. In fact, DR is classified according to the nature of changes observed in the retinal microvasculature. Early-stage DR, also known as nonproliferative DR, is characterized by the presence of microaneurysms, microhemorrhages, cotton-wool spots (an ischemic lesion of the nerve fiber layer), venous caliber changes, and intraretinal microvascular abnormalities; late-stage DR, also known as proliferative DR, is defined by the presence of aberrant neovascular outgrowths originating from the retinal vessels [6–9]. The outer retina, including the photoreceptors, receives a majority of its oxygen

and nutrients through diffusion from a rich plexus of fenestrated capillaries located in the choroid. This vascular network, called the choriocapillaris, is separated from the retina by Bruch's membrane and a monolayer of retinal pigment epithelium (RPE).

Multiple lines of evidence coming from studies in cell culture, animal models, and human studies strongly indicate that all the components of the retina, not only its vessels but also the neural elements, the choroid, and the RPE, are impacted by the metabolic and signaling challenges imposed by diabetes [10–14]. Neuronal apoptosis, astrocyte dysfunction, microglial activation, Müller cell dysfunction, and loss of RPE barrier function have all been observed in diabetic retinopathy [5, 15–18]. Moreover, studies of human specimens and animal models of diabetes have revealed a key role for inflammatory cells and cytokines (reviewed in [19] and [20]; also see [21–23]).

Recognizing the complexity of DR, however, should not detract from efforts to pursue therapeutic approaches that focus on specific cellular targets and signaling pathways; such targeted approaches may be clinically effective and involve fewer side effects. Based on this premise, this review focuses on the critical role of pericytes, a cell that, along with endothelial cells, comprises the retinal capillaries and is an early target of diabetes in both humans and experimental animals.

Cogan and colleagues first reported the loss of pericytes in DR in 1961 [24]. Termed as pericyte dropout, a loss of retinal pericytes was the earliest morphological change observed in diabetic retinopathy, with the endothelial cell-to-pericyte ratio dropping from 1:1 in normal retinal tissue to 4:1 in diabetic retinas [25]. Studies of the time course of diabetic complications in humans revealed that pericyte dropout in DR is linked to the development of microangiopathies such as microaneurysms, acellular capillaries, vessel tortuosity, hyperpermeability, and capillary nonperfusion [26, 24, 27–31]. This correlation was corroborated by evidence from animal models of diabetes consistently showing that pericyte loss preceded the development of microangiopathies (STZ, db/db, and galactosemia models) (summarized in [32]). Pericytes, however, are not the only cell type damaged in the course of diabetes. Rather, there is overwhelming evidence that pericytes are an integral component of what is known as the neurovascular unit, a functional and architectural arrangement of cells comprising vascular and neural components that support visual perception by maintaining an appropriate blood supply to retinal tissues [33, 34].

Pericytes and endothelial cells both synthesize and share a common basement membrane [35]. Discontinuities in the basement membrane allow intercellular contact and, thus, communication between pericytes and endothelial cells [36, 37]. These junctions consist of membrane invaginations that are rich in adherence and gap junctions [36–39]. Heterotypic cell-cell interactions also take place on the other side of the neurovascular unit with glial cells extending end-feet processes that surround vessels and regulate their function through diffusible molecules. In fact, there is evidence that the formation and maintenance of a functional blood-barrier is highly dependent on factors derived from glial cells [40]. In the superficial vascular plexus, astrocytes play a major role in regulating vascular integrity whereas Müller cells contribute to vascular integrity in the inner nuclear layer [41]. Accordingly, ablation of Müller cells has been shown to trigger photoreceptor apoptosis, vascular telangiectasias, and breakdown of the blood-retina barrier [41]. Similarly, genetic manipulations affecting astrocyte development have been shown to be associated with vascular abnormalities [42–44]. In consideration of the strong associations among the different cellular components of the neurovascular unit, pericyte loss should be understood both as cause and consequence of the dysfunction associated with diabetes.

Heterotypic Cell Signaling in the Neurovascular Unit

As described in detail above, pericytes and endothelial cells communicate through junctions that extend through discontinuities in a shared basement membrane. These direct cell-cell interactions, as well as the close proximity of the two cell types in vivo, are thought to facilitate cell signaling through key signaling pathways including PDGF-B/platelet-derived growth factor receptor beta (PDGFRβ), TGFβ, and angiopoietin-1/Tie2. Cell loss as a result of high glucose, inflammation, and/or the thickening and rarefaction of the basement membrane, all hallmarks of DR, may disrupt or abrogate cell signaling [45, 46]. In fact, not only a significant thickening of the capillary basement membrane in DR but also a change in the composition of diabetic basement membranes, with increased production of both collagen type IV and laminin, is found [47, 48]. The summary of cell signaling pathways linked to DR, the model system in which these observations were made, and the conclusions of the study are shown in Table 1. PDGF-B/PDGFRβ, TGFβ, and angiopoietin-1/Tie2 are discussed in detail because they are potentially amenable to therapeutic intervention.

PDGF-B/PDGFRβ Pericyte recruitment is coordinated by the interplay of the PDGF-B acting through PDGFRβ. Proliferating endothelial cells secrete PDGF-B, whereas pericytes and their precursors express PDGFRβ, the receptor for PDGF-B. In development, PDGF-B is secreted from the endothelium of angiogenic sprouts and newly formed vessels where it serves as an attractant for PDGFRβ-expressing comigrating pericytes or pericyte precursors [49–51]. Consistent with this function, transgenic mouse models clearly indicate a role for PDGF-B and PDGFRβ in the development of a mature neurovascular unit. *Pdgfb* and *Pdgfrb*-deficient mice

Table 1 Summary of pathways that may contribute to induced pericyte loss in diabetic retinopathy

Pathway	Model	Conclusions	Reference
CD38	Cultured primary human pericytes	• Complement activation contributes to functional loss and death of retinal pericytes in diabetes	[118]
Glyceraldehyde 3-phosphate dehydrogenase (GAPDH)	Bovine retinal endothelial cells and pericytes	• High glucose decreases GAPDH activity, expression, and nuclear translocation • Overexpression of GAPDH prevents glucose-induced inhibition of its activity, nuclear translocation, apoptosis, and activation of protein kinase C and hexosamine pathways	[119]
Forkhead box protein O1 (FOXO1)	Bovine retinal pericytes	• Tumor necrosis factor alpha and carboxymethyllysine (CML)-collagen can induce pericyte apoptosis through activation of the transcription factor FOXO1	[120]
Transforming growth factor beta (TGFβ)	Bovine retinal endothelial cells and pericytes	• TGFβ signaling in retinal endothelial cells and pericytes plays a role in basal lamina thickening in preclinical diabetic retinopathy	[76]
Platelet-derived growth factor B (PDGF-B)	Mouse/Cre-loxP system to target an inactivating mutation to the mouse PDGF-B gene selectively in endothelial cells	• Endothelium-restricted ablation of PDGF-B generates viable mice with extensive inter- and intraindividual variation in the density of pericytes throughout the central nervous system • Proliferative retinopathy invariably develops when pericyte density is <50 % of the normal level • A reduction of pericyte density during development is sufficient to cause retinopathy in mice, implying that pericyte loss may also be a causal pathogenic event in human diabetic retinopathy	[55]
PDGF-B	Mouse heterozygous *Pdgf-B*$^{+/-}$	• Pericyte numbers in nondiabetic *Pdgf-B*$^{+/-}$ mice are reduced by 30 %, as compared with wild-type mice; a small but significant increase in acellular capillaries is also observed • Pericyte numbers are decreased by 50 % in diabetic *Pdgf-B*$^{+/-}$ mice compared with nondiabetic wild-type littermates • Incidence of acellular capillaries is increased by 3.5-fold compared to nondiabetic *Pdgf-B*$^{+/-}$ mice • Retinal capillary coverage with pericytes is crucial for the survival of endothelial cells, particularly under stress conditions like diabetes	[30]
PDGF-B	Bovine retinal pericytes and *Prkcd*$^{-/-}$ mice	• Hyperglycemia persistently activates protein kinase C and p38 mitogen-activated protein kinase (MAPK) to increase the expression of a novel target, Src homology region 2 domain-containing phosphatase-1 (SHP-1), leading to PDGF receptor beta dephosphorylation, and increases pericyte apoptosis, independent of nuclear factor kappa B (NF-κB) • Unlike diabetic controls, diabetic *Prkcd*$^{-/-}$ mice did not exhibit p38 MAPK/SHP-1 activation, PDGF resistance, or acellular capillaries	[57]
Platelet-derived growth factor receptor B (PDGFR-B)	Mouse mutant *Pdgfrb*$^{redeye/redeye}$	• Mutation in the *Pdgfrb* gene at position +2 of intron causes a partial loss of normal splicing resulting in a frame shift and premature termination (termed redeye) • Mice exhibit features of nonproliferative diabetic retinopathy, including retinal neurodegeneration and defective pericyte recruitment restricted to the central nervous system	[121]
Angiopoietin (Ang)/tyrosine kinase with immunoglobulin-like and EGF-like domain (Tie) 1	Streptozotocin (STZ) diabetic rats/STZ diabetic Ang-2 LacZ$^{+/-}$ knock-in mice	• Expression of Ang-1 is upregulated by 2.5-fold, and expression of Ang-2 is upregulated by more than 30-fold in the retina of diabetic rats • Heterozygous Ang-2 deficiency (diabetic Ang-2 LacZ$^{+/-}$ mice) completely prevents diabetes-induced pericyte loss and reduces the number of acellular capillary segments • Upregulation of angiopoietin-2 plays a critical role in the loss of pericytes in the diabetic retina	[86]

Table 1 (continued)

Pathway	Model	Conclusions	Reference
Ang/Tie	$Ins2^{Akita-/-}XLacZ^{-/-}$	• Diabetic pericyte loss is the result of pericyte migration, a process modulated by the Ang/Tie	[89]
Ang/Tie	Bovine retinal pericytes	• Pericytes expressing a functionally active Tie2 receptor upregulate by both Ang-1 and Ang-2 • Ang-1 increases the survival of pericytes and enhances CD13 expression following apoptosis induced by TNF-α or high glucose • Tie2 may play an important role in the progression of diabetic retinopathy, by regulating pericyte loss and influencing the activation state and recruitment of pericytes	[93]
Polyol/sorbitol glucose metabolism	STZ diabetic rats/human retinal organ culture	• Aldose reductase immunoreactivity is observed in retinal pericytes and endothelial cells of rats and in retinal endothelial cells of humans • The human retinas exposed to high glucose in organ culture increase sorbitol production by a degree similar to that observed in the rat • Inhibition of aldose reductase prevents vascular processes culminating in acellular capillaries	[122]
Poly (ADP-ribose) polymerase (PARP)/NF-κB	STZ diabetic rats	• Activity of PARP is increased in the whole retina and in endothelial cells and pericytes • Administration of a PARP inhibitor significantly inhibits the diabetes-induced death of retinal microvascular cells and the development of early lesions of diabetic retinopathy, including acellular capillaries and pericyte ghosts • PARP activation plays an important role in the diabetes-induced death of retinal capillary cells, at least in part via its regulation of NF-κB	[123]
Thiamine/benfotiamine	Bovine and human retinal pericytes	• High glucose increases pericyte apoptosis and Bcl-2/Bax consistent with DNA fragmentation, whereas p53 is unchanged • Treatment with the vitamins thiamine and benfotiamine rescues high glucose-induced apoptosis in human pericytes	[124]

display vascular abnormalities, including microaneurysms and increased microvascular permeability, associated with the absence of pericytes and/or abnormal endothelial cell ultrastructure [49, 52–54]. As a systemic knockout of the *Pdgfb* and/or *Pdgfrb* genes is embryonically lethal, mice heterozygous for $PDGF\text{-}B^{+/-}$ and mice with an endothelial cell-specific conditional knockout of PDGF-B were created to study the loss of PDGF-B on pericytes [30, 51, 55, 56]. Although pericyte loss occurs in these models, the extent of impact is variable. The pericyte population in $PDGF\text{-}B^{+/-}$ mice is reportedly reduced by approximately 30 %, and a slight increase in acellular capillaries is observed [30]. Mice with an endothelial cell-specific conditional knockout of PDGF-B begin to display microaneurysms and increased microvascular regression when pericyte coverage is less than 50 % [55, 56]. Additionally, studies have shown that hyperglycemia leads to persistent activation of protein kinase C delta (PKCδ) and p38α MAPK, which results in increased expression of Src homology region 2 domain-containing phosphatase 1 (SHP-1) and PDGFβ dephosphorylation [57]. Unlike diabetic control mice, diabetic null for the *PKCδ* gene showed a reduced number of acellular capillaries compared to controls. Thus, these studies indicate that pericytes and PDGF-B play an important role in the development of the neurovascular unit and have generally been interpreted as further evidence of the key role played by pericytes in vascular integrity in diabetes.

However, whether PDGF signaling plays a role in pericyte survival and maintenance in adult tissues requires further analyses involving conditional knockouts. This question is highly relevant now that PDGF signaling inhibitors (Fovista™ (E10030), Ophthotech) are entering clinical trials for neovascular conditions in the eye including neovascular age-related macular degeneration (AMD) [58].

In wet AMD, neovascular outgrowths, which originate from the choriocapillaris and grow into the subretinal space, are treated with anti-VEGF therapies [59–64]. This therapy is generally effective at reducing vascular permeability and

inducing vascular regression. However, it has been observed that pericyte investment of vascular outgrowths correlates with poor response to anti-VEGF therapy. This is not surprising as the association of the pericyte with the new vessels leads to increased vessel stability. The addition of anti-PDGF therapy [65–67] to anti-VEGF is based on the premise that pericyte association with nascent vessels makes those vessels relatively (but not totally) insensitive to vascular regression by VEGF neutralization; however, considering the precedent of pericyte dropout in DR, it is essential to evaluate long-term safety of this treatment in both diabetic and nondiabetic eyes.

TGFβ/TGFβ Receptors Both pericytes and endothelial cells express TGFβ as well as TGFβ receptors, and the interactions between these two cell types are important in TGFβ signaling. Studies using co-culture of pericytes and endothelial cells demonstrated that physical contact between the cells is necessary for activation of latent TGFβ1 [68, 69]. Furthermore, in vivo studies targeting endothelial cell-specific deletion of activin receptor-like kinase 5 (Alk-5), a TGFβ type I receptor, showed a reduction in endothelial cell secretion of TGFβ1 [70]. This, in turn, resulted in reduced signaling of TGFβ/Alk-5 in pericytes [70]. Taken together, these data suggest an important role for TGFβ signaling between pericytes and endothelial cells.

Both TGFβ and angiopoietin-1 (Ang-1)/Tie2 (discussed in detail below) signaling between pericytes and endothelial cells appears to play a significant role in vessel stability. The majority of TGFβ/TGFβ receptor knockout models (*Tgfb1*, *Alk5*, *Eng*, and *Smad5*) are embryonically lethal due to major abnormalities in the vasculature [71–75]. Furthermore, mice deficient of either Alk-5, a TGFβ type I receptor, or endoglin, a TGFβ binding protein, lack smooth muscle cell investment and formation, respectively [72, 75]. Consistent with this biology, misregulation of TGFβ signaling has been associated with DR in humans and animal models, including thickening of the basal lamina [76] and cicatricial contraction of proliferative fibrous membranes [77]. Moreover, there is evidence indicating that TGFβ is also an indirect target of drugs that prevents experimental DR. Concordant treatment of diabetic rats with the aldose reductase inhibitor sorbinil and aspirin reduced the diabetes-induced upregulation of genes in the TGFβ pathway [78], and inhibition of ROCK, a key downstream mediator of TGFβ, dramatically suppressed PDR/PVR-induced collagen gel contraction [77], albeit clinical trials of sorbinil in humans did not show clinical efficacy [79]. Notwithstanding this set back, a recent systemic meta-analysis of vitreous biomarkers associated with DR identified blockade of TGFβ using cell therapy as a viable therapeutic candidate for DR therapy [80].

Ang-1/Tie2 Many studies have indicated the importance of pericyte and endothelial cell interactions in angiopoietin-1/Tie2 signaling, a system known to participate in vascular development. Ang-1 is produced by the pericytes, whereas endothelial cells express Tie2 [39, 81–83]. Studies have shown that Ang-1 signaling via Tie2 is important for capillary sprouting, endothelial cell survival, and vascular remodeling [81, 83–85]. Ang-1 has also been implicated in the stabilization of vessels by pericytes and smooth muscle cells to the vessel wall [86]. Ang-2, another member of the angiopoietin family, can act as a competitive inhibitor of Ang-1 signaling through Tie2 [87, 88]. Overexpression of Ang-2 in transgenic mice disrupts blood vessel formation during embryo development [87] and, over the course of several months, causes the pericyte dropout and the formation of acellular capillaries, similar to that observed in early-stage DR [86, 89, 90]. Similar to *TGFβ/TGFβ* receptor knockout models, *Ang-1* and *Tie2*-deficient mice lack pericytes and are embryonically lethal due cardiovascular failure at mid-gestation [84, 91]. In addition, intravitreal injection of Ang-1 has been shown to rescue high-order architecture of the developing vasculature in an anti-PDGFRβ antibody model of pericyte deficiency [92]. The Ang-1/Tie2 signaling system has also been shown to play a major role in the pericyte loss observed in DR by modulating pericyte migration [89] and influencing the activation state and recruitment of pericytes [93]. Additionally, Ang-1 may be useful for reducing microvascular leakage, as vessels in transgenic mice overexpressing Ang-1 were not only nonleaky but also resistant to leaks caused by inflammatory agents [94]. Targeting of the Ang-1/Tie2 signaling system for treatment of diabetic macular edema (DME) in humans has shown to be of promise, as a phase 1 trial using a subcutaneously administered agonist of this signaling system has been completed and a phase 2 trial is currently ongoing in patients with DME (ClinicalTrials.gov identifier: NCT02050828).

While extensive research has shown that the PDGF-B/PDGFRβ, TGFβ, and Ang-1/Tie2 signaling systems all play an important role in the pathogenesis of DR, a number of other pathways also have been identified to be associated with pericyte loss. These include the complement system, glyceraldehyde 3-phosphate dehydrogenase (GAPDH), forkhead box protein O1 (FOXO1), glucose metabolism (polyol/sorbitol), poly (ADP-ribose) polymerase (PARP), NF-κB, and vitamins thiamine and benfotiamine (see Table 1).

Tracking Pericytes in the Neurovascular Unit

Measurements of pericyte loss have been used extensively as an indication of diabetic retinopathy in animal models [57, 95–97] and in postmortem studies in human tissues [79, 98]. Pericytes can be identified in whole-mount retinal preparations using a combination of their location and one or more of a number of markers of differentiation, such as alpha-smooth

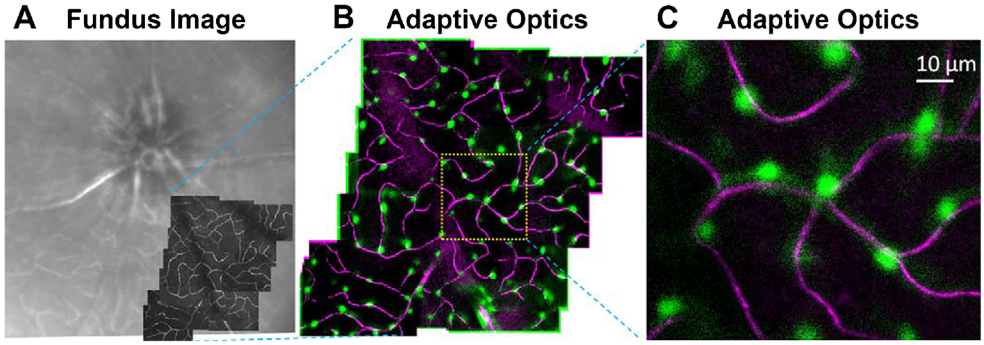

Fig. 1 Simultaneous in vivo imaging of vascular perfusion and fluorescent retinal pericytes using a two-channel, adaptive optics scanning laser ophthalmoscope (AOSLO). **a** Wide-field HRA Spectralis image shows approximately 30° field of the mouse retina. Superimposed on the fundus image are motion-contrast AOSLO fields demonstrating capillary perfusion with micron-level resolution. **b** Two-channel imaging using AOSLO in vivo. Channel 1 collects NIR motion contrast demonstrating capillary perfusion (*magenta*, moving blood cells), while channel 2 simultaneously images DsRed fluorescently labeled pericytes (*green*). **c** AOSLO field (5°) demonstrates the association of pericytes (*green*) with perfused capillaries (*magenta*). Montage is from a single capillary stratification (reprinted from [117])

muscle actin (α-SMA), desmin, and regulator of G protein signaling 5 (RGS-5) [39, 51, 99–105]. Cell surface molecule expressed by pericytes include neuron-glial antigen 2 (NG2), PDGFRβ, and vascular cell adherence molecule 1 (VCAM-1) [49, 51, 99, 106–108]. To date, no specific molecular marker has been identified that will reliably label and differentiate pericytes from other cell types found in the retina. Instead, the markers described above must be used in combination and with contextual information such as species, vessel type, developmental or angiogenic stage, tissue specificity, and/or pathology in order to confirm pericyte identity.

Quantification of pericyte loss has historically been conducted using a method referred to as "trypsin digest" in which the neural components of the retina are digested away, leaving the microvasculature intact [109]. Subsequent optimization of the trypsin digest protocol [110, 111] revealed that elastase, not trypsin, was the active component of the trypsin solution and that, in the fixed retina, elastase preserves the microvasculature structure. Elastase digests can be stained using standard immunohistochemical methods. Furthermore, at least in humans, pericytes and endothelial cells are further distinguishable by their nuclear shape. Pericytes have small, dark-staining round or slightly oval nuclei; although they are completely enveloped by basement membrane, they protrude from the abluminal wall of the capillary [25, 109, 112]. Endothelial cells have larger oval or ellipsoid nuclei that lie in the axis of blood flow [25, 109, 112].

However, distinguishing between pericytes and endothelial cells is not as straightforward in mice, which are widely used to study the role of specific genes in diabetic retinopathy. Moreover, in an elastase digest, the analysis of the microvasculature is performed out of the context as other components of the neurovascular unit including glial cells and neurons are removed to allow visualization. Of great promise are methods like CLARITY, which allows for the transformation of intact biological tissue into an optically transparent and chemically permeable hydrogel-based nanoporous structure that retains structural integrity and relevant molecules such as native antigens, neurotransmitters, soluble and cell membrane proteins, and mRNAs [113]. CLARITY methodology removes lipid bilayers and replaces them with hydrogel monomers that are covalently linked to remaining biomolecules. The resultant tissue preparation can be further visualized and analyzed, enabling intact tissue in situ hybridization, immunohistochemistry, and antibody labeling of the intact tissue or organ. CLARITY has not yet been applied to the eye, but its successful use on tissue of the mouse brain [113, 114] is promising. More recently, another method for the optical clearing of tissues, named CUBIC, has been developed. As for the CLARITY methodology, the CUBIC protocol creates optically transparent tissue while retaining subcellular structures; however, CUBIC has the advantages of utilizing nontoxic water-soluble chemicals and not requiring expensive clearing reagents or specialized devices [115]. Application of these methods, which retain the full tissue architecture, to the eye should help to increase our understanding of how pericyte loss influences the neurovascular unit and how changes in other components of the unit directly affect pericyte homeostasis and function.

In addition to these new advances in tissue processing, a next generation of live imaging is also now being developed. These new imaging technologies can be used to noninvasively visualize retinal pericytes in the living eye. Using a two-channel, adaptive optics scanning laser ophthalmoscope (AOSLO) [116], retinas of transgenic mice expressing fluorescent pericytes (NG2, DsRed) were imaged in vivo (Fig. 1). The first channel imaged vascular perfusion with new infrared light, while the second channel simultaneously imaged fluorescent retinal pericytes [117]. Pericyte morphology and topography observed from in vivo imaging were confirmed using flat mounts with conventional fluorescent microscopy. This is the first demonstration of high-resolution imaging of

retinal pericytes in situ, and this promises to provide the basis to track and quantify pericyte topography, morphology, and function. Because of its noninvasive nature, visualization can be accomplished in the living retina over time, allowing for the progressive monitoring of microvascular disease, like DR. Intravital imaging technology using AOSLO is also being used in the human retinas, allowing for the imaging of vascular wall structures, including nuclei, and for the clear visualization of a variety of retinal vascular and nonvascular changes in subjects with mild to moderate nonproliferative DR [98, 96]. Thus, this new imaging innovation has the potential to revolutionize our understanding of the disease itself.

Conclusion

In summary, the loss of retinal pericytes is likely an early factor contributing to the onset and progression of clinically relevant vascular pathology in DR. Renewed focus on pericyte protection or replacement as a therapeutic goal for the treatment of patients with diabetic retinopathy may one day result in the prevention or delay of vision loss. Translational efforts that combine a focus on cell signaling regulation in conjunction with in vivo imaging studies of pericytes may ultimately lead to the development of innovative approaches to treat diabetic retinopathy.

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Compliance with Ethics Guidelines

Conflict of Interest Joseph F. Arboleda-Velasquez, Cammi Valdez, Christina Kaiser Marko, and Patricia A. D'Amore declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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- • Of importance
- •• Of major importance

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