Minireview

Implications of fibrotic extracellular matrix in diabetic retinopathy

Henry A Resnikoff[1], Charles G Miller[2] and Jean E Schwarzbauer[1]

[1]Department of Molecular Biology, Princeton University, Princeton, NJ 08544-1014, USA; [2]Department of Ophthalmology, Scheie Eye Institute, University of Pennsylvania, Philadelphia, PA 19104, USA
Corresponding author: Jean E Schwarzbauer. Email: jschwarz@princeton.edu

Impact Statement

Diabetic retinopathy (DR) is a leading cause of blindness that is currently difficult to prevent and treat; we highlight the early and important role of fibrosis, in order to direct interest and investigation into the extracellular matrix in DR. We consider evidence from humans, animal models, and cell culture models to support our hypothesis that highlights fibrosis as a crucial driver of capillary dysfunction. The combination of clinical and basic science consideration contributes clarity to the field, in which the fibrotic process in the diabetic retina is well-documented but often overlooked. Our work presents a likely model that is both illuminating and inviting of further investigation, and helps explain current barriers to successful treatment.

Abstract

Fibrosis is an accumulation of extracellular matrix (ECM) proteins and fibers in a disordered fashion, which compromises cell and tissue functions. High glucose-induced fibrosis, a major pathophysiological change of diabetic retinopathy (DR), severely affects vision by compromising the retinal vasculature and ultimately disrupting retinal tissue organization. The retina is a highly vascularized, stratified tissue with multiple cell types organized into distinct layers. Chronically high blood glucose stimulates certain retinal cells to increase production and assembly of ECM proteins resulting in excess ECM deposition primarily in the capillary walls on the basal side of the endothelium. This subendothelial fibrosis of the capillaries is the earliest histological change in the diabetic retina and has been linked to the vascular dysfunction that underlies DR. Proteins that are not normally abundant in the capillary basement membrane (BM) matrix, such as the ECM protein fibronectin, are assembled in significant quantities, disrupting the architecture of the BM and altering its properties. Cell culture models have identified multiple mechanisms through which elevated glucose can stimulate fibronectin matrix assembly, including intracellular signaling pathways, alternative splicing, and non-enzymatic glycation of the ECM. The fibrotic subendothelial matrix alters cell adhesion and supports further accumulation of other ECM proteins leading to disruption of endothelial cell–cell junctions. We review evidence supporting the notion that these molecular changes in the ECM contribute to the pathogenesis of DR, including vascular leakage, loss of endothelial cells and pericytes, changes in blood flow, and neovascularization. We propose that the accumulation of ECM, especially fibronectin matrix, first around the vasculature and later in extravascular locations, plays a critical role in DR and vision loss. Strategies for DR prevention and treatment should consider the ECM a potential therapeutic target.

Keywords: Diabetic retinopathy, extracellular matrix, fibronectin, fibrosis, basement membrane, neovascularization

***Experimental Biology and Medicine* 2022; 247: 1093–1102. DOI: 10.1177/15353702221087175**

Introduction

Diabetes is a metabolic disorder characterized by abnormally high blood glucose. In 2018, the Centers for Disease Control and Prevention (CDC) estimated 10.5% of people in the United States are diabetic, with rates expected to rise in the coming decades. Diabetics are at risk of serious complications that are responsible for significant disease burden, especially in the kidney, eye, and nerves.[1] Diabetic complications are the leading cause of blindness,[2] kidney failure,[3] and non-traumatic amputation[4] in adults. There is an urgent need to define the molecular pathways that contribute to these complications, in order to develop targeted therapies to prevent them.

Chronically elevated blood glucose has significant effects at the cellular level. Changes in cell metabolism and activation of intracellular signaling are accompanied by stimulation of gene expression and downstream increases in cell proliferation and migration.[5] Major classes of proteins produced in response to changes in glucose include secreted factors such as cytokines and chemokines that promote inflammation and extracellular matrix (ECM)-associated proteins and their receptors that cause fibrosis. Inflammation is well-accepted as a cause for diabetic disease progression

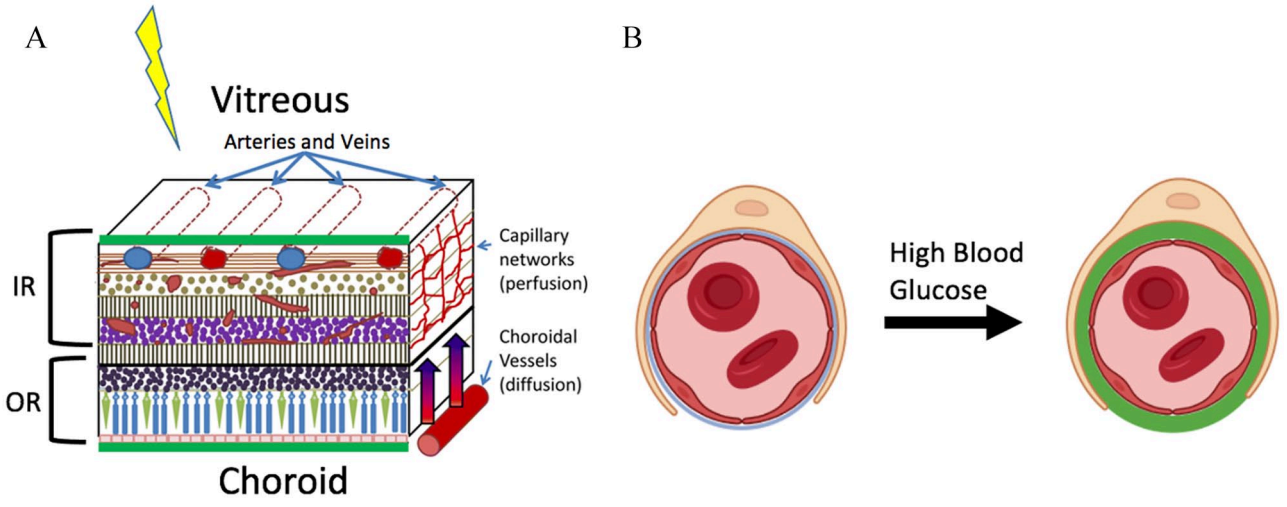

Figure 1. (A) The cartoon depicts the layers of the retina including the capillary networks. Light passes through the vitreous and inner (IR) and outer retinal (OR) layers as indicated on the left. The OR contains photoreceptor cells that respond to light. The IR contains interneurons that transduce signals to neurons that project axons from the IR to the brain. Specialized ECMs (green) called the inner limiting membrane (ILM, top) and Bruch's membrane (bottom) separate the IR from the vitreous and the retinal pigmented epithelium in the OR from the choroid, respectively. Blood vessels feeding the layers are shown on the right. The OR is nourished by diffusion from the choroidal vasculature, while the IR contains a capillary network that extends throughout the layers, branching from arteries and veins shown under the ILM. (B) Cross-section of normal (left) and fibrotic (right) retinal capillaries. In normal conditions, the capillary wall is composed of a monolayer of endothelial cells (red) on a thin laminar basement membrane (gray). In conditions of chronically high blood glucose, the basement membrane becomes fibrotic and thickens as much as 20-fold (green). Pericytes wrap around the capillary wall (tan cells). Red disks inside the capillary represent the red blood cells. (A color version of this figure is available in the online journal.)
Source: Adapted from Gameiro *et al.*[10] (http://creativecommons.org/licenses/by/4.0/).

and several recent reviews describe its role.[5–8] While it is understood that ECM protein accumulation and fibrosis are a critical part of diabetic complications, the idea that ECM plays a causative role is under-appreciated. In this review, we will discuss the significance of glucose-induced changes in the ECM and how these changes affect cell functions and drive disease progression, with a focus on diabetic retinopathy (DR).

Normal versus diabetic retina

The retina is a highly stratified organ composed of specialized cell types organized into functionally distinct layers (Figure 1).[9] Light travels through the vitreous and the inner retina to stimulate photosensitive cells in the outer retina. These cells then transduce signals that reach ganglion cells which extend projections across the inner retina and coalesce to form the optic nerve. Photosensitive cells vary in their identity and density; for example, the macula, located near the center of the retina, allows for high-resolution color vision due to the high density of specialized photoreceptors. As many of the retinal cell layers are located between the light source and the photoreceptor cells, vision can be disrupted by obstructions that form in any of the retinal layers and impede light from reaching the photoreceptors.

The high metabolic demands of the retina require an extensive vascular network. The inner capillary network supplies blood to the inner retina including the neurons that carry impulses to the brain, while the choroidal vessels below the retinal pigmented epithelium supply the outer retina including the light sensitive rods and cones[11] (Figure 1(A)). The integrity of these capillaries depends on endothelial cells forming strong connections to neighboring cells (through adherens and tight junctions) and to the underlying basement membrane (BM) (through ECM adhesions) (Figure 1(B)). Pericytes adhere around the circumference of the capillary and are involved in regulating endothelial cell function and blood flow.[12,13] Together cells and ECM provide the capillaries in the inner retina with selective permeability, forming a blood-retinal barrier that controls the flow of solutes into retinal tissue, thereby controlling the flow of water.[14] Two other types of ECM are present in the retina, the inner limiting membrane (ILM) and Bruch's membrane, which are specialized ECM sheets that separate the retina from the vitreous and the choroid, respectively.

In DR, overt changes in retina structure/function can be detected by histology, fluorescein angiography, and ophthalmoscopy. The earliest histological change in DR is the thickening of capillary BMs.[15] Changes in the ECM precede many of the clinical manifestations of DR that are indicative of capillary dysfunction.[16] These include leaky capillaries, identified on angiography by diffusion of fluorescein dye out of the vessels, and more advanced retinal changes such as patchy capillary non-perfusion, microaneurysms, venous bleeding, lipid exudate, and edema. Later in the disease process, extravascular accumulation of ECM deposits and growth of new, incompetent vessels disrupt the architecture of the retinal layers and interfere with the path of light, impacting vision relative to the size and location of the obstructions. The prevalence of changes in the retinal ECM throughout the progression of DR makes it critical to define how these changes affect blood vessel structure and function in diabetes.

ECM alterations in DR

The ECM is a complex network of proteins and polysaccharides that serves as a scaffold for cell organization, attachment, and movement and is an important regulator of cell

Table 1. Retinal vascular ECM proteins.

Protein	Normal	Diabetes
Laminin	– Major glycoprotein in the basement membrane. – Binds to specific integrins and to other BM proteins.[19] – Important for cell attachment, BM assembly, and structural integrity.	– Increased in the fibrotic subendothelial matrix of galactose-fed rats[20] but not found to increase in diabetic human retina capillary matrix.[21,22]
Type IV collagen	– Forms a planar network. – Binds to laminin via nidogen. – Cells express COL IV binding integrins.[17]	– Increased in the subendothelial matrix of galactose-fed rats and of diabetic humans.[20,22] – Binds FN fibrils in culture.[23]
Perlecan	– Major HSPG of the BM. – Serves important linkage functions. – Binds certain growth factors.[17]	– No evidence that perlecan concentration increases in DR.[24]
Nidogen	– Binds both laminin and COL IV. – Aids in assembly of BMs by linking these two networks.[19]	– No evidence that nidogen concentration increases in DR.
Fibronectin	– Scarce in the normal BM.[21,25] – Present at pericyte foot processes.[12]	– Increased in DR subendothelial matrix.[20,21,26,27] – Accumulation may disrupt the normal architecture of the BM. – Signaling can affect cell–cell junctions.
Tenascin-C	– Adhesion modulatory matricellular protein. – Normally not present in the basement membrane.	– Abundant in the diabetic subendothelial matrix.[21] – Alternatively spliced by cells cultured in high glucose.[28] – Plays a role in regulating FN assembly.

ECM: extracellular matrix; BM: basement membrane; FN: fibronectin; DR: diabetic retinopathy; COL IV: type IV collagen; HSPG: heparan sulfate proteoglycan.

behavior. The ECM is a dynamic structure and is constantly being remodeled, leading to alterations in ECM structure and composition that are accentuated in tissue damage and disease states.[15] The primary type of ECM in the retina is BM, surrounding capillaries on the basal side of the endothelial cells and forming the ILM and Bruch's membrane, at the vitreous and choroid boundaries, respectively. BMs are composed mainly of type IV collagen, the glycoproteins laminin and nidogen, and the heparan sulfate proteoglycan perlecan (Table 1), and these proteins are highly organized into a thin, laminated sheet of ECM.[17] This sheet-like structure provides a platform for endothelial and epithelial cells, promotes interactions of adherent cells with their neighbors through cadherin cell–cell junctions, and induces a polarized cell morphology.[17] Interaction of cells with the ECM is largely mediated by integrin receptors, a family of heterodimeric transmembrane proteins that bind to specific ECM proteins and connect them with the actin cytoskeleton.[18] Integrin–ECM interactions also activate signaling pathways involved in diverse cell functions including migration and proliferation.[18]

Altered capillary ECM is detected prior to other histological and functional changes of DR. Histologically, the capillary BMs, which are normally 50–100 nm thick, become significantly expanded sometimes up to as much as 2000 nm.[21] The term BM thickening is used to describe this altered ECM, implying that while the ECM might thicken, it maintains BM characteristics. This is misleading, as the subendothelial ECM deposited in diabetes lacks the laminar organization of a normal structure, contains certain ECM proteins not found in a normal BM (Table 1), and has altered properties that affect ECM–cell interactions. The fibrotic ECM of DR capillaries has a fibrous architecture by electron microscopy, normal BM components like type IV collagen are distributed across the width rather than as a single thin layer, and interstitial matrix proteins such as fibronectin (FN) and tenascin-C are prominent components.[21] Therefore, as the normal BM beneath the capillary endothelial cells is replaced by fibrotic ECM, subendothelial fibrosis is a more accurate description than BM thickening.

Dysregulated assembly and accumulation of ECM proteins causes fibrosis in many diseases.[29] FN, a foundational matrix protein with numerous roles in normal and pathological ECM assembly,[30] is a significant component of fibrotic deposits. FN is a large dimeric glycoprotein composed of 250 kDa subunits. The liver produces FN that circulates in the blood, and numerous cell types in most tissues secrete FN locally.[30] FN is essential to blood vessel development,[31] and plays a role in blood clotting[32] and wound healing.[33] A major function of FN is matrix assembly in which it is actively organized into fibrils by cells.[34] This process is dependent on integrins, which bind soluble FN and induce conformational changes that promote FN–FN binding and fibrillogenesis. The growth of FN fibrils leads to crosslinking and the formation of insoluble FN matrix.[34]

FN matrix is essential for the assembly of various collagens,[23,35,36] and is required for matrix incorporation of many other ECM proteins including many involved in the development of fibrosis. Ablation of FN matrix by knockout of FN expression or by inhibition of FN fibrillogenesis also causes a loss of collagen I and IV fibrils,[23,37] of deposition of glycoproteins like tenascin-C,[38] and of growth factors such as latent transforming growth factor (TGF)-β binding protein,[39] vascular endothelial growth factor (VEGF),[40] among others.[41] Although FN is normally scarce in the BM,[12] diabetic subendothelial matrix contains abundant FN.[28] The accumulation of FN fibrils provides a foundation for deposition of other proteins. In fact, staining of the fibrotic subendothelial matrix in a diabetic retina not only shows extensive deposition of FN but also high levels of type IV collagen and tenascin-C,[21] two ECM proteins that increase in high glucose conditions.[22,42,43] Changes in composition can affect the ultrastructure and properties of the matrix. The ECM deposited by retinal endothelial cells cultured in high glucose is stiffer by atomic force microscopy[44] and also more permeable to fluorescent 43 kDa dextran[45] than in normal

glucose conditions. FN's foundational role in the assembly of other proteins makes it a key mediator of fibrosis and an interesting candidate for therapeutic modulation.

Mechanisms of ECM modulation in elevated glucose

Cell regulation of ECM assembly

Changes in glucose concentration affect gene expression. High glucose conditions increase FN1 transcription, but the change is modest and insufficient to explain the dramatic increase in matrix FN.[23,43,46] Because plasma FN transits from the bloodstream into tissues,[47] plasma FN provides a source of protein for assembly without changes in gene expression. Changes in intracellular pathways have also been linked to FN matrix assembly in high glucose.[23] It is well-established that high glucose levels activate protein kinase C (PKC) in tissues[48] which will then turn on PKC-dependent pathways. A downstream target of PKC signaling is α5β1 integrin, the primary receptor for FN matrix assembly, and stimulation of integrin receptor activity can dramatically enhance FN assembly independently of gene expression.[23] Intracellular increases in lysine acetylation, which also occur with elevated glucose levels, affect integrins and associated focal adhesion proteins to stimulate FN assembly.[49] Endothelial cells assemble FN basally[50] and it is likely that the initial enhancement of FN fibril formation induces further FN matrix assembly as has been observed with mammary epithelial cells.[51]

Under chronically high glucose conditions, proteins become modified by non-enzymatic glycation and these modifications, called advanced glycation end products (AGEs), have been used as markers of protein alterations due to glucose levels.[52] The ECM is a major target for non-enzymatic glycation and these modifications can make the ECM resistant to degradation by proteases.[53] Furthermore, kidney cell interactions with an AGE-modified ECM stimulate FN matrix assembly.[54] By these mechanisms, non-enzymatic glycations of the matrix are likely playing a feed forward role in DR, inducing the assembly of more matrix proteins which, when glycated, increase the pool of AGEs to further stimulate assembly.

Finally, changes in alternative splicing of ECM proteins can differentially affect protein deposition. Multiple FN isoforms arise by alternative splicing at three sites in the transcript.[55] Mice constitutively lacking the EDA isoform have impaired endothelium-dependent vasodilation in high glucose conditions, compared to wildtype and constitutive EDA^+ mice.[56] Alternative splicing of the tenascin-C transcript also changes in high glucose conditions switching the protein from a small to a large isoform. The small isoform binds FN in the matrix where it is positioned to regulate FN interactions with cell receptors whereas the large isoform is excluded from the matrix thus potentially allowing uncontrolled FN accumulation.[43]

Fibrotic potential of excess FN matrix

It is important to consider how assembly of FN actually alters the architecture of the capillary ECM. Endothelial cells express integrins on their basal surface[50] where laminin-binding integrins assemble and maintain a layer of polymerized laminin that serves as a scaffold for other BM proteins.[19] Enhanced assembly of FN at the interface between endothelial cells and the BM could supplant the laminin layer, providing a distinctly different matrix for cell interaction and negatively affecting the organization of major BM proteins. Evidence to support this suggestion comes from the staining pattern of a thickened diabetic capillary in the retina. FN was observed diffusely distributed throughout the thickened BM while a layer of type IV collagen, a marker of the BM, was localized to the outer rim of the capillary, away from the endothelial layer.[21] While more work is needed to confirm the displacement of the normal BM by FN-rich fibrotic matrix, this theory would help explain endothelial behavioral changes that occur after ECM alterations; as the ECM proteins available for endothelial cell attachment change, the types of activated integrins would also change, further altering the assembly of matrix proteins and other endothelial cell behaviors.

While FN can act as an instigator of matrix reorganization, molecular changes downstream of FN assembly have the potential to further perturb ECM structure and prevent ECM turnover. The diabetic subendothelial matrix has much higher abundance of type IV collagen,[57] which is also organized differently than in a normal BM.[58,59] Collagen fibrils are stabilized by covalent crosslinking and expression of two crosslinking enzymes, lysyl oxidase and peroxidasin, is higher in mesangial cells cultured in high glucose conditions (unpublished observations). Lysyl oxidase is also upregulated by glucose in retinal endothelial cells.[60] Peroxidasin, a type IV collagen crosslinker,[61] has a critical role in eye development and mouse mutants exhibit ECM defects, early onset glaucoma, and retinal dysgenesis.[62] Crosslinking of excess ECM proteins will stabilize insoluble, and possibly defective, fibrils and block protease-dependent turnover. These fibrotic changes downstream of FN matrix assembly could further exacerbate vascular dysfunction.

Retinal defects caused by ECM alterations

Cell adhesion

The integrity of the capillary wall depends on three types of interactions: endothelial integrin interactions with the normal BM (primarily laminin), cadherin-mediated endothelial cell–cell interactions, and intra-BM protein interactions among laminin, type IV collagen, nidogen, and perlecan.[17,19,63] Under normal conditions, the BM controls endothelial cell shape, density, and polarity through baso-lateral cell adhesions. In the diabetic condition, all three types of interactions are perturbed. As mentioned above, accumulation of FN on the basal side of the endothelium will engage a different set of integrins and initiate different intracellular signals to affect cell phenotype. FN can also disrupt or displace normal intra-BM protein interactions to change the thin laminar organization of the BM into a thick fibrotic protein network.

Lateral interactions between endothelial cells are mediated by vascular endothelial (VE)—cadherin, a homophilic

receptor that clusters to form adherens junctions and uses catenins bound to cadherin cytoplasmic domains to organize the actin cytoskeleton.[64] Adherens junctions support tight junctions which are essential for the barrier function of the capillary wall.[65] Together, lateral junctions and basal cell–ECM adhesions determine cell polarity.[66,67]

Disruption of cell junctions or ECM adhesions has a significant impact on cell survival. It is well-established that defects in cell adhesion promote anoikis, cell death induced by loss of attachment to the ECM and/or neighboring cells.[68] During DR progression, there may be insufficient FN-binding integrins during the proposed switch from laminin to FN as a subendothelial substrate, which could weaken endothelial cell attachment and allow anoikis. Intracellular signals downstream of ECM binding might also affect survival. For example, FN binding to α5β1 integrin is known to activate Src kinase,[69] which can phosphorylate VE-cadherin to destabilize cell–cell junctions,[70] also allowing anoikis. Thus, FN deposition in DR could explain, at least in part, the appearance of acellular capillaries in diabetic rat retinas.[71] In the normal capillary, what little FN exists in the BM is concentrated at the pericyte foot process, where it may regulate endothelial cell–pericyte interactions[12] and stabilize cell adhesion. This raises another intriguing possibility that alterations in BM architecture and accumulation of fibrotic matrix due to increased FN deposition push pericytes further away from the endothelial cells, creating false attachment sites for pericytes, promoting apoptosis, and thus, promoting pericyte dropout in DR.

Permeability

Vessel walls of retinal capillaries tightly control transport of molecules between blood and tissue, forming the blood-retinal barrier. This selective permeability depends on proper formation and functioning of endothelial cell–cell and cell–ECM adhesions which in turn are controlled by BM properties.[72] Molecules that diffuse through the capillary wall must pass through the endothelial cell layer, either by paracellular or transcellular transport, and the BM. Supporting this selective permeability are pericytes that mechanically interface with endothelial cells and astrocytes that elaborate factors to tighten the endothelial cell barrier.[13]

Capillary permeability by paracellular transport is at the center of DR pathophysiology. Paracellular transport is governed by the organization of junctional proteins, VE-cadherins, and tight junction proteins like ZO-1 and occludin.[63] Intracellular pathways regulate the integrity of these junctions.[73,74] Thus, in high glucose conditions, the reduced integrity of endothelial cell junctions leads to increased paracellular transport[13] and allows numerous molecules to extravasate into the retinal tissue in a dysregulated fashion. Due to the osmotic pull of these molecules, fluid accumulates within the layers of the retina, leading to diabetic macular edema and severe visual sequelae when the architecture of the macula, the site of central vision, is disrupted.[14]

High glucose induces endothelial cell permeability *in vivo*[75] and *in vitro*.[13] One route is through activation of signaling downstream of FN-integrin binding. Note that FN-binding integrins activate Src, while laminin-binding integrins deactivate Src,[76] making Src an important mediator of endothelial cell permeability. Cell–cell junctions could then be destabilized by Src acting on VE-cadherin, β-catenin, or occludin.[70] Phosphatidylinositol (PI)-3-kinase and AKT are also downstream of FN-binding integrins and can downregulate tight junction proteins.[77,78] These pathways offer insights into how FN buildup beneath endothelial cells could induce permeability.

The ECM alterations in DR are a likely mediator of increased paracellular transport. Interestingly, while certain signaling pathways, including PKC, are activated soon after endothelial cells are cultured in high glucose,[79] increased permeability develops over time,[13] in correlation with the deposition of increased FN and type IV collagen. Given that endothelial cell behavior is governed by the surrounding matrix proteins,[76,80] mediated by the amount and sort of integrins that are bound by the ECM, it is reasonable to conclude that FN aberrantly deposited in the BM could be inducing signaling that downregulates the integrity of adherens and tight junctions.

While FN has the capacity to directly signal to the endothelial cells and pericytes, it could also lead to disruption of the normal BM architecture,[71] altering the properties of the matrix. The ECM deposited by endothelial cells in high glucose is more permeable[45] suggesting that subendothelial matrix changes further reduce barrier function allowing molecules that have bypassed the endothelial cells to extrude into the retina. It is possible that there is a ramping-up effect of cell and matrix permeability working in concert. Potentially, increased ECM protein production and integrin activation in high glucose leads to FN deposition in the BM making it more porous, which then induces cell signaling that decreases junctional integrity. This allows large molecules including FN to leave the lumen of the capillary via paracellular transport, and interact with the BM, providing a significant source of FN to be assembled into the BM and accumulate as subendothelial fibrosis. We propose that, as more FN is assembled, the architectural change of the BM and its impact on endothelial cell function worsen, further disrupting endothelial cell behavior and allowing the BM to become more porous, increasing capillary leak.

Blood flow/ECM compliance

Changes in BM composition and thickness affect its compliance and ECM stiffness has been shown to control many cell functions including cell adhesion,[81] differentiation,[82] matrix assembly,[83] and migration.[84] ECM changes that can modulate compliance include increases in collagen and other fibrous proteins, disorganized ECM protein polymerization, covalent crosslinking by extracellular enzymes (e.g. lysyl oxidase or transglutaminases), and reduced turnover by extracellular proteases. BM stiffness is also affected by accelerated non-enzymatic glycation of ECM proteins with chronically elevated glucose.[21] In DR, a fibrotic subendothelial matrix would have an impact on endothelial cell and pericyte functions that in turn contribute to the dysregulation of blood

flow through the retinal vasculature. Capillaries are responsible for modulating a drop in pressure at sites of diffusion, as well as altering blood flow to meet the demands of the tissue. Normally, pericytes and endothelial cells modulate capillary blood flow by mechanically regulating the size of the retinal vessels in proportion to oxygen demand by the neural tissue.[12,85] This regulation requires capillary distensibility, as well as endothelial cell and pericyte contraction to manipulate the size of the capillary.[12,85] The rate of blood flow through the capillary determines the amount of O_2 and CO_2 exchange and reduced efficiency of exchange leads to hypoxia. In diabetes, a loss of normal capillary blood flow regulation decreases capillary transit time leading to impaired offloading of oxygen.[86] The altered capillary matrix in diabetes could impede normal mechanical manipulation of the capillary by preventing pericyte and endothelial cell contraction. Furthermore, a thicker and stiffer subendothelial matrix leads to a smaller, less distensible capillary lumen, increasing the pressure, and thus causing faster blood flow through the capillary. Interestingly, high blood pressure exacerbates DR severity, suggesting that capillary pressure is likely important to the disease process.[87]

Neovascularization

New blood vessels form by endothelial sprouting which requires endothelial shape changes, BM degradation and re-assembly, as well as cell proliferation and migration in response to extracellular signals such as VEGF.[88,89] Related to the ECM, regulation of proteases that degrade the BM and synthesis of the proteins and receptors needed to re-establish a functional BM will be deficient in DR because of the imbalances in ECM homeostasis discussed above. Furthermore, FN in particular is a pro-proliferative and pro-migratory ECM protein and its presence in excess is likely to over-stimulate the endothelial cells once sprouting has been initiated. FN could be playing a dual role in directing this pathological process. First, as FN becomes overly abundant in DR,[21] it could promote neovascular growth. FN is essential for vascular development, it helps guide vascularization of the developing retina, and it induces capillary sprouting during wound healing.[31,88] VEGF has an early role in sprouting by inducing formation of a tip cell and subsequently is involved in stimulating endothelial cell proliferation.[88] VEGF binds to FN,[90] and bound VEGF in an FN matrix has been shown to direct vascular development.[91] Thus, the upregulation of VEGF in DR combined with increased matrix binding sites due to accumulation of FN in the subendothelial matrix provides a pro-angiogenic environment to promote neovascularization in the retina, supporting progression to proliferative DR. Since VEGF is also a permeability factor,[92] the complex of FN and VEGF in the thickened BM may produce new vessels that are prone to leakage, further complicating DR pathology.

Most treatment options for DR focus on preventing neovascularization of the retina through photocoagulation and VEGF inhibition. While these techniques prevent vision loss in some patients, others progress despite treatment and develop partial or total vision loss.[93] It is possible that the variable response to these treatments stems from the multifaceted nature of vascular dysfunction in DR, with the fibrotic matrix acting as a distinct driver of pathology or as a partner of VEGF in stimulating neovascularization.[92] Notably, αvβ3 integrin, which is activated by FN, is known to act synergistically with the VEGF receptor to stimulate pathways that induce angiogenesis.[94] Furthermore, as VEGF binds FN, it is possible that the fibrotic matrix also modifies VEGF availability, worsening pathology.[90]

ECM alterations as a driver of DR

The purpose of this review is to highlight the ways in which the ECM is a driver of DR pathology. We contend that, in high glucose conditions, FN deposition increases, both changing the BM properties and promoting further assembly of ECM. These changes affect cell adhesion, blood vessel stiffness, capillary permeability, and the appearance of acellular capillaries. Capillary dysfunction leads to leakage, initiating edema and spilling more FN into the retinal tissue. This FN supports neovascularization and the formation of FN-rich fibrotic tissue on the surface of the retina (Figure 2). Evidence implicating FN as essential for subendothelial fibrosis and capillary malfunction is based on FN's presence at the site of such defects *in vivo*, the effects of manipulating FN expression in the retina, and direct measurements of cell and ECM changes using cell culture models. Furthermore, the time course of pathological changes implicates FN and fibrotic subendothelial matrix in the development of capillary dysfunction. Vascular leakage and loss of the blood-retinal barrier are critical changes in DR, and occur because the subendothelial matrix, a physical part of the capillary barrier, is defective and becomes more permeable in high glucose. The fibrotic subendothelial matrix is also a driver of increased capillary pressure, inducing structural defects in the microvasculature and interfering with normal gas exchange, inducing ischemia. Beyond the capillary BM, fibrosis in the ILM[21] could also be encouraging neovascularization, and the abundance of FN in epiretinal membranes[95] suggests both a structural role as well as a pro-contractile role. Interestingly, DR displays a feature known as metabolic memory, where retinopathy worsens for a time even after glucose control is achieved.[96] There are numerous theories to explain this phenomenon, such as the suggestion that epigenetic changes caused by high glucose are slow to reverse. We propose that matrix changes induced by high glucose are slow to turn over, and continue to drive capillary dysfunction even following attenuation of high blood glucose levels.

Of course, DR does not progress simply because of the ECM. The inflammatory response also has a key role, but it is likely that the two work together. For example, alterations to the ECM in DR may aid in inflammatory cell invasion. AGEs attached to ECM proteins by non-enzymatic glycation alter ECM assembly by binding their receptor for advanced glycation end products (RAGE), but RAGE engagement can also induce an inflammatory response that appears to play a role in advancing DR pathology.[97]

Beyond DR, it is likely that subendothelial fibrosis also impacts the development of other complications of diabetes. FN can modulate the development of atherosclerotic lesions,

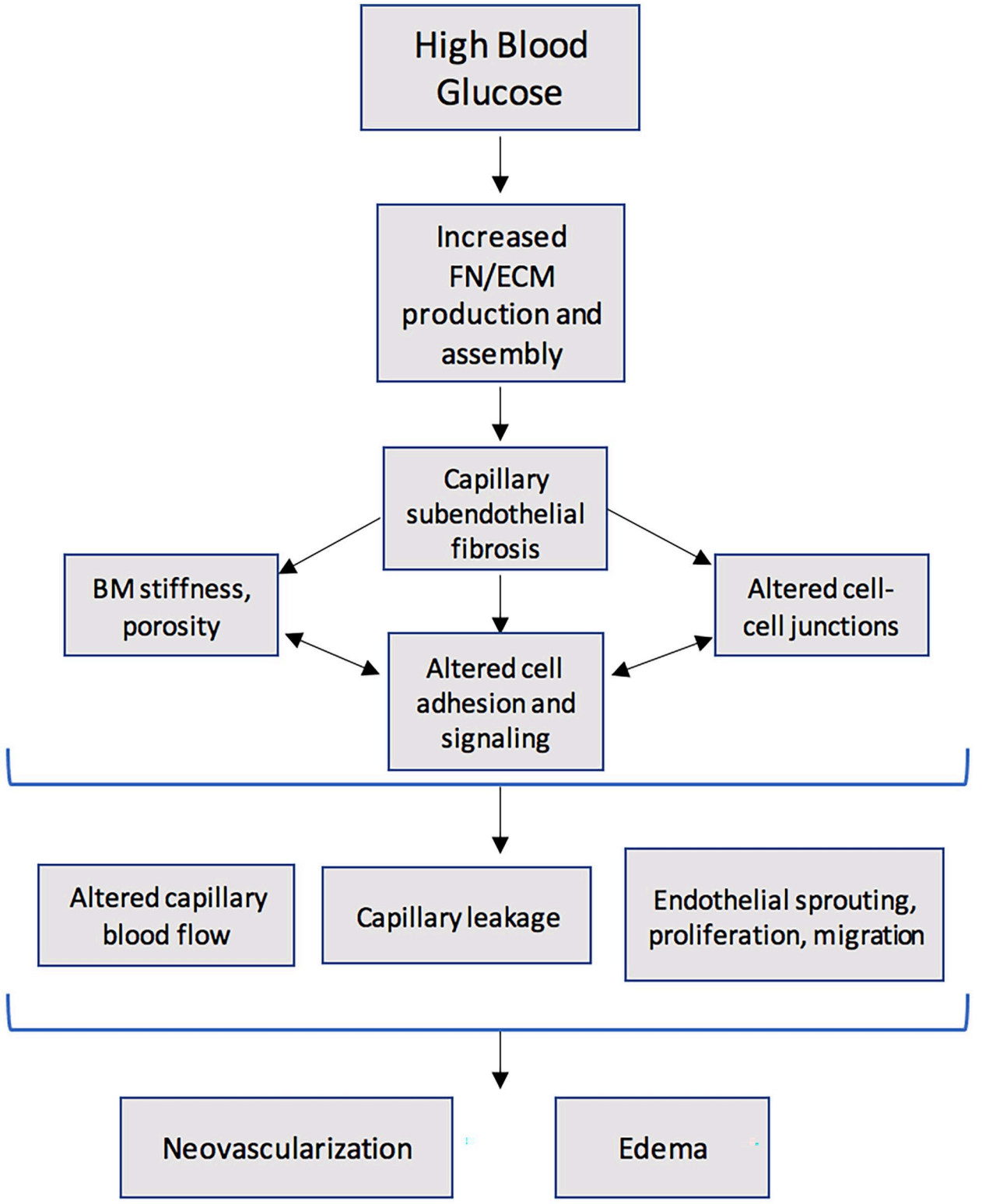

Figure 2. The relationship between high glucose, ECM, fibrosis, and pathology in DR is outlined in this flowchart (see text for details).

a macrovascular complication of diabetes.[1,98] Furthermore, ECM also accumulates in the diabetic kidney,[99] and around diabetic nerves,[100] demonstrating the wide reaching impact of dysregulated matrix assembly in diabetes. As illustrated in this review, high glucose conditions induce significant ECM changes *in vivo* and *in vitro*, but most studies overlook the ECM when investigating models of DR. Understanding the role of FN and the ECM in diabetic complications will be crucial to understanding and treating these multifactorial sequelae.

AUTHORS' CONTRIBUTIONS

All authors participated in developing the topic and determining the breadth of the minireview. All participated in writing and editing the text. H.A.R. and J.E.S. made the figures and table.

DECLARATION OF CONFLICTING INTERESTS

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

FUNDING

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: The authors are grateful for funding from the National Institutes of Health (NIH) the National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS) R01AR073236, the Sud Cook '39 Fund, and the Foundation for Health Advancement.

ORCID ID

Jean E Schwarzbauer https://orcid.org/0000-0003-1012-7593

REFERENCES

1. Fowler MJ. Microvascular and macrovascular complications of diabetes. *Clin Diabetes* 2008;**26**:77–82
2. Beckman JA, Creager MA. Vascular complications of diabetes. *Circ Res* 2016;**118**:1771–85
3. Shahbazian H, Rezaii I. Diabetic kidney disease; review of the current knowledge. *J Renal Inj Prev* 2013;**2**:73–80
4. Rathnayake A, Saboo A, Malabu UH, Falhammar H. Lower extremity amputations and long-term outcomes in diabetic foot ulcers: a systematic review. *World J Diabetes* 2020;**11**:391–9
5. Antonetti DA, Silva PS, Stitt AW. Current understanding of the molecular and cellular pathology of diabetic retinopathy. *Nat Rev Endocrinol* 2021;**17**:195–206
6. Duh EJ, Sun JK, Stitt AW. Diabetic retinopathy: current understanding, mechanisms, and treatment strategies. *JCI Insight* 2017;**2**:e93751
7. Wang W, Lo ACY. Diabetic retinopathy: pathophysiology and treatments. *Int J Mol Sci* 2018;**19**:1816
8. Wong TY, Cheung CMG, Larsen M, Sharma S, Simó R. Diabetic retinopathy. *Nat Rev Dis Primers* 2016;**2**:16012
9. Yan W, Peng YR, van Zyl T, Regev A, Shekhar K, Juric D, Sanes JR. Cell Atlas of the human fovea and peripheral retina. *Sci Rep* 2020;**10**:9802
10. Gameiro GR, Jiang H, Liu Y, Deng Y, Sun X, Nascentes B, Baumel B, Rundek T, Wang J. Retinal tissue hypoperfusion in patients with clinical Alzheimer's disease. *Eye Vis* 2018;**5**:21
11. Campbell JP, Zhang M, Hwang TS, Bailey ST, Wilson DJ, Jia Y, Huang D. Detailed vascular anatomy of the human retina by projection-resolved optical coherence tomography angiography. *Sci Rep* 2017;**7**:42201
12. Dessalles CA, Babataheri A, Barakat AI. Pericyte mechanics and mechanobiology. *J Cell Sci* 2021;**134**:jcs240226
13. Fresta CG, Fidilio A, Caruso G, Caraci F, Giblin FJ, Leggio GM, Salomone S, Drago F, Bucolo C. A new human blood-retinal barrier model based on endothelial cells, pericytes, and astrocytes. *Int J Mol Sci* 2020;**21**:1636
14. Klaassen I, Van Noorden CJ, Schlingemann RO. Molecular basis of the inner blood-retinal barrier and its breakdown in diabetic macular edema and other pathological conditions. *Prog Retin Eye Res* 2013;**34**:19–48
15. Yue B. Biology of the extracellular matrix: an overview. *J Glaucoma* 2014;**23**:S20–3
16. Lee SE, Ma W, Rattigan EM, Aleshin A, Chen L, Johnson LL, D'Agati VD, Schmidt AM, Barile GR. Ultrastructural features of retinal capillary basement membrane thickening in diabetic swine. *Ultrastruct Pathol* 2010;**34**:35–41
17. Yurchenco PD. Basement membranes: cell scaffoldings and signaling platforms. *Cold Spring Harb Perspect Biol* 2011;**3**:a004911
18. Hynes RO. Integrins: bidirectional, allosteric signaling machines. *Cell* 2002;**110**:673–87
19. Hohenester E, Yurchenco PD. Laminins in basement membrane assembly. *Cell Adh Migr* 2013;**7**:56–63
20. Das A, Frank RN, Zhang NL, Samadani E. Increases in collagen type IV and laminin in galactose-induced retinal capillary basement membrane thickening—prevention by an aldose reductase inhibitor. *Exp Eye Res* 1990;**50**:269–80
21. To M, Goz A, Camenzind L, Oertle P, Candiello J, Sullivan M, Henrich PB, Loparic M, Safi F, Eller A, Halfter W. Diabetes-induced morphological, biomechanical, and compositional changes in ocular basement membranes. *Exp Eye Res* 2013;**116**:298–307
22. Halfter W, Moes S, Asgeirsson DO, Halfter K, Oertle P, Melo Herraiz E, Plodinec M, Jenoe P, Henrich PB. Correction: diabetes-related changes in the protein composition and the biomechanical properties of human retinal vascular basement membranes. *PLoS ONE* 2018;**13**:e0195772
23. Miller CG, Pozzi A, Zent R, Schwarzbauer JE. Effects of high glucose on integrin activity and fibronectin matrix assembly by mesangial cells. *Mol Biol Cell* 2014;**25**:2342–50
24. Roy S, Kim D. Retinal capillary basement membrane thickening: role in the pathogenesis of diabetic retinopathy. *Prog Retin Eye Res* 2020;**82**:100903
25. Boselli JM, Macarak EJ, Clark CC, Brownell AG, Martinez-Hernandez A. Fibronectin: its relationship to basement membranes. I. Light microscopic studies. *Coll Relat Res* 1981;**1**:391–404
26. Ljubimov AV, Burgeson RE, Butkowski RJ, Couchman JR, Zardi L, Ninomiya Y, Sado Y, Huang ZS, Nesburn AB, Kenney MC. Basement membrane abnormalities in human eyes with diabetic retinopathy. *J Histochem Cytochem* 1996;**44**:1469–79
27. Roy S, Cagliero E, Lorenzi M. Fibronectin overexpression in retinal microvessels of patients with diabetes. *Invest Ophthalmol Vis Sci* 1996;**37**:258–66
28. Roy S, Sala R, Cagliero E, Lorenzi M. Overexpression of fibronectin induced by diabetes or high glucose: phenomenon with a memory. *Proc Natl Acad Sci USA* 1990;**87**:404–8
29. Distler JHW, Gyorfi AH, Ramanujam M, Whitfield ML, Konigshoff M, Lafyatis R. Shared and distinct mechanisms of fibrosis. *Nat Rev Rheumatol* 2019;**15**:705–30
30. Pankov R, Yamada KM. Fibronectin at a glance. *J Cell Sci* 2002;**115**:3861–3
31. Astrof S, Hynes RO. Fibronectins in vascular morphogenesis. *Angiogenesis* 2009;**12**:165–75
32. Corbett SA, Lee L, Wilson CL, Schwarzbauer JE. Covalent cross-linking of fibronectin to fibrin is required for maximal cell adhesion to a fibronectin-fibrin matrix. *J Biol Chem* 1997;**272**:24999–5005
33. Lenselink EA. Role of fibronectin in normal wound healing. *Int Wound J* 2015;**12**:313–6
34. Singh P, Carraher C, Schwarzbauer JE. Assembly of fibronectin extracellular matrix. *Annu Rev Cell Dev Biol* 2010;**26**:397–419
35. Sottile J, Hocking DC. Fibronectin polymerization regulates the composition and stability of extracellular matrix fibrils and cell-matrix adhesions. *Mol Biol Cell* 2002;**13**:3546–59
36. Velling T, Risteli J, Wennerberg K, Mosher DF, Johansson S. Polymerization of type I and III collagens is dependent on fibronectin and enhanced by integrins α11β1 and α2β1. *J Biol Chem* 2002;**277**:37377–81
37. Saunders JT, Schwarzbauer JE. Fibronectin matrix as a scaffold for procollagen proteinase binding and collagen processing. *Mol Biol Cell* 2019;**30**:2218–26
38. Chung CY, Zardi L, Erickson HP. Binding of tenascin-C to soluble fibronectin and matrix fibrils. *J Biol Chem* 1995;**270**:29012–7
39. Dallas SL, Sivakumar P, Jones CJ, Chen Q, Peters DM, Mosher DF, Humphries MJ, Kielty CM. Fibronectin regulates latent transforming

growth factor-beta (TGF beta) by controlling matrix assembly of latent TGF beta-binding protein-1. *J Biol Chem* 2005;**280**:18871–80
40. Mitsi M, Forsten-Williams K, Gopalakrishnan M, Nugent MA. A catalytic role of heparin within the extracellular matrix. *J Biol Chem* 2008;**283**:34796–807
41. Hynes RO. The extracellular matrix: not just pretty fibrils. *Science* 2009;**326**:1216–9
42. Halfter W, Moes S, Asgeirsson DO, Halfter K, Oertle P, Melo Herraiz E, Plodinec M, Jenoe P, Henrich PB. Diabetes-related changes in the protein composition and the biomechanical properties of human retinal vascular basement membranes. *PLoS ONE* 2017;**12**:e0189857
43. Vega ME, Finlay JB, Vasishtha M, Schwarzbauer JE. Elevated glucose alters global gene expression and tenascin-C alternative splicing in mesangial cells. *Matrix Biol Plus* 2020;**8**:100048
44. Yang X, Scott HA, Monickaraj F, Xu J, Ardekani S, Nitta CF, Cabrera A, McGuire PG, Mohideen U, Das A, Ghosh K. Basement membrane stiffening promotes retinal endothelial activation associated with diabetes. *FASEB J* 2016;**30**:601–11
45. Chronopoulos A, Trudeau K, Roy S, Huang H, Vinores SA, Roy S. High glucose-induced altered basement membrane composition and structure increases trans-endothelial permeability: implications for diabetic retinopathy. *Curr Eye Res* 2011;**36**:747–53
46. Cagliero E, Maiello M, Boeri D, Roy S, Lorenzi M. Increased expression of basement membrane components in human endothelial cells cultured in high glucose. *J Clin Invest* 1988;**82**:735–8
47. Moretti FA, Chauhan AK, Iaconcig A, Porro F, Baralle FE, Muro AF. A major fraction of fibronectin present in the extracellular matrix of tissues is plasma-derived. *J Biol Chem* 2007;**282**:28057–62
48. Koya D, King GL. Protein kinase C activation and the development of diabetic complications. *Diabetes* 1998;**47**:859–66
49. Vega ME, Kastberger B, Wehrle-Haller B, Schwarzbauer JE. Stimulation of fibronectin matrix assembly by lysine acetylation. *Cells* 2020;**9**:655
50. Christopher RA, Kowalczyk AP, McKeown-Longo PJ. Localization of fibronectin matrix assembly sites on fibroblasts and endothelial cells. *J Cell Sci* 1997;**110**:569–81
51. Park J, Schwarzbauer JE. Mammary epithelial cell interactions with fibronectin stimulate epithelial-mesenchymal transition. *Oncogene* 2014;**33**:1649–57
52. Singh VP, Bali A, Singh N, Jaggi AS. Advanced glycation end products and diabetic complications. *Korean J Physiol Pharmacol* 2014;**18**:1–14
53. Mott JD, Khalifah RG, Nagase H, Shield CF 3rd, Hudson JK, Hudson BG. Nonenzymatic glycation of type IV collagen and matrix metalloproteinase susceptibility. *Kidney Int* 1997;**52**:1302–12
54. Pastino AK, Greco TM, Mathias RA, Cristea IM, Schwarzbauer JE. Stimulatory effects of advanced glycation endproducts (AGEs) on fibronectin matrix assembly. *Matrix Biol* 2017;**59**:39–53
55. Schwarzbauer JE, DeSimone DW. Fibronectins, their fibrillogenesis, and in vivo functions. *Cold Spring Harb Perspect Biol* 2011;**3**:a005041
56. Gortan Cappellari G, Barazzoni R, Cattin L, Muro AF, Zanetti M. Lack of fibronectin extra domain a alternative splicing exacerbates endothelial dysfunction in diabetes. *Sci Rep* 2016;**6**:37965
57. Roy S, Maiello M, Lorenzi M. Increased expression of basement membrane collagen in human diabetic retinopathy. *J Clin Invest* 1994;**93**:438–42
58. Song B, Kim D, Nguyen NH, Roy S. Inhibition of diabetes-induced lysyl oxidase overexpression prevents retinal vascular lesions associated with diabetic retinopathy. *Invest Ophthalmol Vis Sci* 2018;**59**:5965–72
59. Jing C, Zhang G, Liu Z, Xu Q, Li C, Cheng G, Shi R. Peroxidasin promotes diabetic vascular endothelial dysfunction induced by advanced glycation end products via NOX2/HOCl/Akt/eNOS pathway. *Redox Biol* 2021;**45**:102031
60. Chronopoulos A, Tang A, Beglova E, Trackman PC, Roy S. High glucose increases lysyl oxidase expression and activity in retinal endothelial cells: mechanism for compromised extracellular matrix barrier function. *Diabetes* 2010;**59**:3159–66
61. Bhave G, Cummings CF, Vanacore RM, Kumagai-Cresse C, Ero-Tolliver IA, Rafi M, Kang JS, Pedchenko V, Fessler LI, Fessler JH, Hudson BG. Peroxidasin forms sulfilimine chemical bonds using hypohalous acids in tissue genesis. *Nat Chem Biol* 2012;**8**:784–90
62. Yan X, Sabrautzki S, Horsch M, Fuchs H, Gailus-Durner V, Beckers J, Hrabě de Angelis M, Graw J. Peroxidasin is essential for eye development in the mouse. *Hum Mol Genet* 2014;**23**:5597–614
63. Bazzoni G, Dejana E. Endothelial cell-to-cell junctions: molecular organization and role in vascular homeostasis. *Physiol Rev* 2004;**84**:869–901
64. Vestweber D. VE-cadherin: the major endothelial adhesion molecule controlling cellular junctions and blood vessel formation. *Arterioscler Thromb Vasc Biol* 2008;**28**:223–32
65. Campbell HK, Maiers JL, DeMali KA. Interplay between tight junctions & adherens junctions. *Exp Cell Res* 2017;**358**:39–44
66. Nelson WJ. Adaptation of core mechanisms to generate cell polarity. *Nature* 2003;**422**:766–74
67. Lee JL, Streuli CH. Integrins and epithelial cell polarity. *J Cell Sci* 2014;**127**:3217–25
68. Gilmore AP. Anoikis. *Cell Death Differ* 2005;**12**:1473–7
69. Schlaepfer DD, Broome MA, Hunter T. Fibronectin-stimulated signaling from a focal adhesion kinase-c-Src complex: involvement of the Grb2, p130cas, and Nck adaptor proteins. *Mol Cell Biol* 1997;**17**:1702–13
70. Rahimi N. Defenders and challengers of endothelial barrier function. *Front Immunol* 2017;**8**:1847
71. Roy S, Sato T, Paryani G, Kao R. Downregulation of fibronectin overexpression reduces basement membrane thickening and vascular lesions in retinas of galactose-fed rats. *Diabetes* 2003;**52**:1229–34
72. Baeten KM, Akassoglou K. Extracellular matrix and matrix receptors in blood-brain barrier formation and stroke. *Dev Neurobiol* 2011;**71**:1018–39
73. Brembeck FH, Rosário M, Birchmeier W. Balancing cell adhesion and Wnt signaling, the key role of beta-catenin. *Curr Opin Genet Dev* 2006;**16**:51–9
74. Fanning AS, Little BP, Rahner C, Utepbergenov D, Walther Z, Anderson JM. The unique-5 and -6 motifs of ZO-1 regulate tight junction strand localization and scaffolding properties. *Mol Biol Cell* 2007;**18**:721–31
75. Oshitari T, Polewski P, Chadda M, Li AF, Sato T, Roy S. Effect of combined antisense oligonucleotides against high-glucose- and diabetes-induced overexpression of extracellular matrix components and increased vascular permeability. *Diabetes* 2006;**55**:86–92
76. Davis GE, Senger DR. Endothelial extracellular matrix: biosynthesis, remodeling, and functions during vascular morphogenesis and neovessel stabilization. *Circ Res* 2005;**97**:1093–107
77. Gonzalez-Mariscal L, Tapia R, Chamorro D. Crosstalk of tight junction components with signaling pathways. *Biochim Biophys Acta* 2008;**1778**:729–56
78. Velling T, Nilsson S, Stefansson A, Johansson S. beta1-Integrins induce phosphorylation of Akt on serine 473 independently of focal adhesion kinase and Src family kinases. *EMBO Rep* 2004;**5**:901–5
79. Hempel A, Maasch C, Heintze U, Lindschau C, Dietz R, Luft FC, Haller H. High glucose concentrations increase endothelial cell permeability via activation of protein kinase C alpha. *Circ Res* 1997;**81**:363–71
80. Tilling T, Korte D, Hoheisel D, Galla HJ. Basement membrane proteins influence brain capillary endothelial barrier function in vitro. *J Neurochem* 1998;**71**:1151–7
81. Lo CM, Wang HB, Dembo M, Wang YL. Cell movement is guided by the rigidity of the substrate. *Biophys J* 2000;**79**:144–52
82. Engler AJ, Sen S, Sweeney HL, Discher DE. Matrix elasticity directs stem cell lineage specification. *Cell* 2006;**126**:677–89
83. Carraher CL, Schwarzbauer JE. Regulation of matrix assembly through rigidity-dependent fibronectin conformational changes. *J Biol Chem* 2013;**288**:14805–14
84. Pelham RJ Jr, Wang Y. Cell locomotion and focal adhesions are regulated by substrate flexibility. *Proc Natl Acad Sci USA* 1997;**94**:13661–5
85. Gardner TW, Davila JR. The neurovascular unit and the pathophysiologic basis of diabetic retinopathy. *Graefes Arch Clin Exp Ophthalmol* 2017;**255**:1–6
86. Ostergaard L, Finnerup NB, Terkelsen AJ, Olesen RA, Drasbek KR, Knudsen L, Jespersen SN, Frystyk J, Charles M, Thomsen RW, Christiansen JS, Beck-Nielsen H, Jensen TS, Andersen H. The effects of capillary dysfunction on oxygen and glucose extraction in diabetic neuropathy. *Diabetologia* 2015;**58**:666–77

87. Liu L, Quang ND, Banu R, Kumar H, Tham YC, Cheng CY, Wong TY, Sabanayagam C. Hypertension, blood pressure control and diabetic retinopathy in a large population-based study. PLoS ONE 2020;**15**: e0229665
88. Gerhardt H, Golding M, Fruttiger M, Ruhrberg C, Lundkvist A, Abramsson A, Jeltsch M, Mitchell C, Alitalo K, Shima D, Betsholtz C. VEGF guides angiogenic sprouting utilizing endothelial tip cell filopodia. *J Cell Biol* 2003;**161**:1163–77
89. Muhleder S, Fernandez-Chacon M, Garcia-Gonzalez I, Benedito R. Endothelial sprouting, proliferation, or senescence: tipping the balance from physiology to pathology. *Cell Mol Life Sci* 2021;**78**:1329–54
90. Martino MM, Hubbell JA. The 12th–14th type III repeats of fibronectin function as a highly promiscuous growth factor-binding domain. *FASEB J* 2010;**24**:4711–21
91. Trujillo S, Gonzalez-Garcia C, Rico P, Reid A, Windmill J, Dalby MJ, Salmeron-Sanchez M. Engineered 3D hydrogels with full-length fibronectin that sequester and present growth factors. *Biomaterials* 2020; **252**:120104
92. Dvorak HF. Reconciling VEGF With VPF: the importance of increased vascular permeability for stroma formation in tumors, healing wounds, and chronic inflammation. *Front Cell Dev Biol* 2021;**9**:660609
93. Cheung N, Wong IY, Wong TY. Ocular anti-VEGF therapy for diabetic retinopathy: overview of clinical efficacy and evolving applications. *Diabetes Care* 2014;**37**:900–5
94. Mezu-Ndubuisi OJ, Maheshwari A. The role of integrins in inflammation and angiogenesis. *Pediatr Res* 2021;**89**:1619–26
95. Grisanti S, Heimann K, Wiedemann P. Origin of fibronectin in epiretinal membranes of proliferative vitreoretinopathy and proliferative diabetic retinopathy. *Br J Ophthalmol* 1993;**77**:238–42
96. Kowluru RA. Diabetic retinopathy, metabolic memory and epigenetic modifications. *Vision Res* 2017;**139**:30–8
97. Zong H, Ward M, Stitt AW. AGEs, RAGE, and diabetic retinopathy. *Curr Diab Rep* 2011;**11**:244–52
98. Babaev VR, Porro F, Linton MF, Fazio S, Baralle FE, Muro AF. Absence of regulated splicing of fibronectin EDA exon reduces atherosclerosis in mice. *Atherosclerosis* 2008;**197**:534–40
99. Thomas MC, Brownlee M, Susztak K, Sharma K, Jandeleit-Dahm KA, Zoungas S, Rossing P, Groop PH, Cooper ME. Diabetic kidney disease. *Nat Rev Dis Primers* 2015;**1**:15018
100. Hill R. Extracellular matrix remodelling in human diabetic neuropathy. *J Anat* 2009;**214**:219–25