Brief Review

Endothelial Glycocalyx as a Shield Against Diabetic Vascular Complications

Involvement of Hyaluronan and Hyaluronidases

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Abstract—The endothelial glycocalyx (EG), which covers the apical surface of the endothelial cells and floats into the lumen of the vessels, is a key player in vascular integrity and cardiovascular homeostasis. The EG is composed of PGs (proteoglycans), glycoproteins, glycolipids, and glycosaminoglycans, in particular hyaluronan (HA). HA seems to be implicated in most of the functions described for EG such as creating a space between blood and the endothelium, controlling vessel permeability, restricting leukocyte and platelet adhesion, and allowing an appropriate endothelial response to flow variation through mechanosensing. The amount of HA in the EG may be regulated by HYAL (hyaluronidase) 1, the most active somatic hyaluronidase. HYAL1 seems enriched in endothelial cells through endocytosis from the bloodstream. The role of the other main somatic hyaluronidase, HYAL2, in the EG is uncertain. Damage to the EG, accompanied by shedding of one or more of its components, is an early sign of various pathologies including diabetes mellitus. Shedding increases the blood or plasma concentration of several EG components, such as HA, heparan sulfate, and syndecan. The plasma levels of these molecules can then be used as sensitive markers of EG degradation. This has been shown in type 1 and type 2 diabetic patients. Recent experimental studies suggest that preserving the size and amount of EG HA in the face of diabetic insults could be a useful novel therapeutic strategy to slow diabetic complications. One way to achieve this goal, as suggested by a murine model of HYAL1 deficiency, may be to inhibit the function of HYAL1. The same approach may succeed in other pathological situations involving endothelial dysfunction and EG damage.

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Overview of the Endothelial Glycocalyx

Endothelial Glycocalyx: A Unique Extracellular Matrix

Eukaryotic cells are endowed with a complex external layer of PGs (proteoglycans), glycoproteins, and glycolipids called extracellular matrix (ECM), which regulates outside-in signaling, protects the cell from exterior aggression, and maintains tissue integrity. The composition and thickness of this ECM vary according to cell types. In the vascular system, a polymeric sugar-rich network covers the apical surface of the endothelial cells (ECs), in a structure that floats into the lumen of the vessels. This particular ECM, commonly called endothelial glycocalyx (EG), was observed for the first time in 1966 in capillaries of rat intestinal mucosa using electron microscopy combined with ruthenium red staining.[1] Other more recent methods have shown this structure in several types of vessels and estimated its structural or functional thickness to anything between 0.5 and 5 µm, depending on the method and the vessel caliber. The methods most often used probe the EG accessibility to macromolecules, red blood cells, or white blood cells; analyze the velocity of microparticles near the vessel wall; or directly observe the structure through labeling.[2–8]

Despite uncertainty about its size and access properties, the importance of the EG in vascular integrity and cardiovascular homeostasis has progressively been recognized.[7,9–13] Some of its essential roles are creating a space between blood and the endothelium; controlling vessel permeability through withholding or slowing down protein and macromolecule passage, which leads to regulation of water efflux; restricting leukocyte and platelet adhesion to the endothelium, thus moderating inflammation and thrombosis; and allowing an appropriate EC response to flow variation through mechanosensing. These functions are described in more detail in this review before turning to the damaging effects of diabetes mellitus on the EG.

EG Composition and Homeostasis

The term EG is often used to describe a dense—although nonuniform—layer adjacent to ECs, particularly when observed in vitro. In vivo, the loose luminal layer covering

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the endothelium is sometimes called endothelial surface layer.[14] In this review, the term EG will be used to describe the structure of this coat, without distinguishing the different layers and without stressing any difference between the in vitro and in vivo situations.

The EG is a porous, hair-like, regularly organized layer composed of PGs, glycoproteins, glycolipids, and glycosaminoglycans (GAGs).[14,15] The main PGs in the EG are sdc (syndecan)-1, -2 and -4 and gpc1 (glypican-1), all firmly bound to the cell plasma membrane. Syndecans are transmembrane proteins, whereas glypicans stick to lipid rafts via a glycosylphosphatidylinositol anchor.[16] Other endothelial PGs such as mimecan and perlecan are soluble, secreted proteins present in both EG and blood.[17]

Various GAGs fit in the EG: heparan sulfate (HS), chondroitin sulfate, hyaluronan (HA), and to a lesser extent dermatan sulfate and keratan sulfate. At physiological pH, the EG is negatively charged. HS, covalently bound to proteins, is the most abundant GAG in the EG, with an average content of 50% to 90% of all GAGs. Chondroitin sulfate and dermatan sulfate, also covalently linked to proteins, are the second GAGs in abundance. HA, the only nonsulfated GAG in the EG, is less abundant and does not covalently bind to proteins. However, contrary to other GAGs, HA has a very large degree of polymerization so it can reach a molecular weight >1 million Dalton. HA incorporates deeply inside the EG, in the denser layer adjacent to the ECs.[9,14] Various investigations using enzymatic treatments have confirmed the nonuniform distribution of GAGs within the EG. Actually, the impact of GAG-degrading enzymes on EG thickness is highly variable because of the nature of the vessel, the method used to analyze EG thickness, and the treatment itself (amount and duration of exposure). For example, bacterial heparinase (which degrades HS) injected into rat mesenteric postcapillary venules, and analyzed using intravital microscopy, induces a 43% decrease in EG thickness, whereas chondroitinase and hyaluronidase can decrease EG thickness by 34% and 26%, respectively.[14] The results variability is particularly high for testicular hyaluronidase, which induces a decrease of EG thickness of 58% in rat myocardial capillaries, when observed through electronic microscopy[3] and of 25% in mouse carotid arteries when observed using 2 photon laser scanning microscopy.[18] In vitro, hyaluronidase produces a mere 15% decrease in EG thickness of bovine lung microvascular ECs measured by atomic force microscopy.[19] The in vitro versus in vivo compositions of the EG may differ.

Plasma proteins such as albumin, orosomucoid, antithrombin III, extracellular superoxide dismutase, lipases, growth factors (vascular endothelial growth factor, fibroblast growth factor), and chemokines integrate and loosely associate with this network of GAGs and PGs.[7] Altogether, they form the looser layer called endothelial surface layer.

EG Dynamics Under Physiological and Pathological Conditions: The Role of Sheddases

During vessel development, the EG is present on ECs as soon as a flow is initiated.[20] In adult life, or on cultured cells, the EG remains as long as ECs are subjected to flow. The EG is often absent from cultivated ECs under static conditions,[21] although recent studies were able to observe this structure on glomerular ECs.[22]

Under physiological conditions, the EG is a stable structure resulting from a balance (Figure 1) between (1) shedding of its components, following continuous passage of the blood flow (shear stress); (2) adsorption of components from circulating blood; and (3) synthesis of new components, including HA, by ECs.[7,9,10,23,24] The type of flow imposed on ECs also influences the thickness and composition of the EG. A thinner EG is observed in vascular tree areas that are predisposed to develop atherosclerosis,[25] for example, at arterial bifurcations where flow is disturbed. In these areas, the amount of HA and HS decreases and LDLs (low-density lipoproteins) rapidly accumulate in the intima.[26] On the other hand, a pulsatile laminar flow imposed on cultured ECs mimics healthy intravascular conditions and raises HA synthesis through the HA synthase HAS2 (HA synthase 2).[27] Shear stress also increases HA incorporation into the EG.[28] Therefore, HA seems to be systematically associated with favorable conditions for the endothelium.

Several pathological conditions and various molecules are known to induce shedding of ≥1 components of the EG (eg, sdc1, HS, or HA) in the blood and eventually the urine as follows: inflammation,[29] hyperglycemia,[30] septicemia, ischemia-reperfusion,[24] cardiopulmonary bypass surgery and ensuing lack of oxygen,[31] abnormal shear stress,[28] hypertension, viral

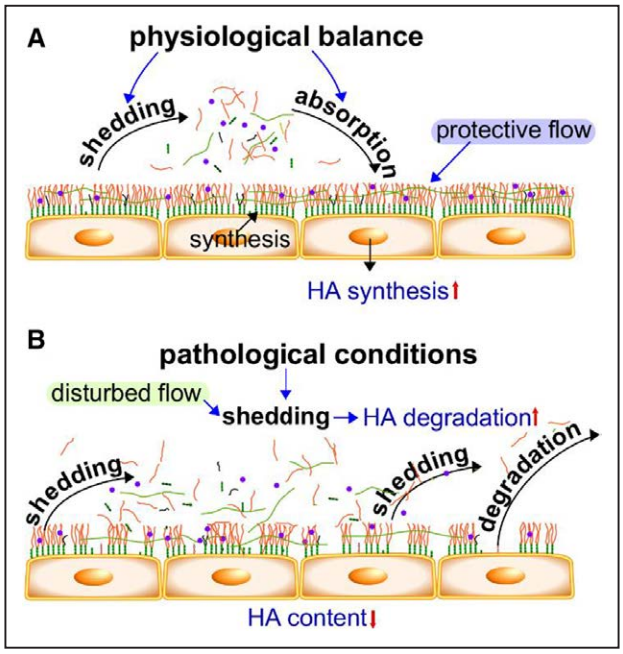

Figure 1. Endothelial glycocalyx structure dynamics. Endothelial glycocalyx (EG) structure (**A**) under physiological conditions and (**B**) in pathological conditions. In light green: hyaluronan (HA), in red: heparan sulfate (HS), in dark: chondroitin sulfate, in purple: plasma proteins, in dark green: glycoproteins and proteoglycans such as sdc (syndecan)-1. **A**, EG structure and composition is the result of a balance between shedding of its components after shear stress, the adsorption of components from circulating blood, and synthesis of new components by endothelial cells (ECs). HA synthesis increases under protective flow (pulsatile laminar flow). B, Under pathological conditions, EG integrity is damaged through the shedding of one or more of its components (eg, sdc1, HS, or HA) into the blood. Under disturbed flow (such as in vascular tree bifurcations areas), HA EG content decreases.

infections,[12] oxidized LDLs,[32] and TNF-α (tumor necrosis factor α).[33] The size of the EG also decreases in collateral arteries after vascular occlusion of the main branch.[34]

The cellular mechanisms involved in this EG degradation are not well known but key enzymes, such as heparanase, released by mast cells and podocytes, and HYAL1 (hyaluronidase 1), a hyaluronidase that is concentrated in, and perhaps released by, ECs,[35,36] seem to be the main candidates of a group that can be called sheddases. For instance, heparanase activity increases in blood and tissues during respiratory failure associated with septicemia[37] or following angiopoietin-2 stimulation during sepsis.[38] Similarly, in the atherosclerosis-prone ApoE (apolipoprotein E) deficient mice made diabetic, atrasentan, an endothelin receptor antagonist, or inhibition of monocyte chemotactic protein-1 protects against glomerular EG alteration via a decrease of heparanase expression.[39,40] Recently, we showed that HYAL1-deficient mice display a thicker EG and are protected from EG alterations and endothelial dysfunction in early diabetes mellitus.[41]

Other sheddases have also been suggested. (1) MMPs (matrix metalloproteases) seem to be responsible for the degradation of EG in viral infections such as dengue or hantavirus.[42–44] In cultured ECs, TNF-α activates MMP-9, which in turn cleaves sdc4 and HS.[45] (2) Thrombin and plasmin, involved in the thrombotic and fibrinolytic processes, respectively, also cleave sdc4 expressed on the surface of human umbilical vascular ECs.[46] (3) Cathepsin B and tryptase levels increase in coronary blood after cardiac ischemia, in parallel with EG degradation.[47,48] (4) Serine proteases, such as elastase and proteinase 3, released by endothelium-adherent leukocytes, can also participate in EG degradation.[37] (5) In cultured ECs, HYAL2 attached to the external side of the plasma membrane is implicated in glycocalyx impairment under low shear stress.[49]

Functions of the EG

In all arterial or venous blood vessels of any caliber, the negatively charged EG participates in the selective permeability of the endothelial barrier, acting as a charge and size barrier against protein and macromolecule diffusion from blood to the interstitial space. Early, in-depth EG studies have reported that its enzymatic degradation leads to increased vascular permeability,[50] a fact that is not disputed.

As it delimits a space between circulating blood and ECs, the EG also participates in endothelial protection and homeostasis. Under physiological conditions, the GAG chains form a screen in front of adhesion molecules and sterically prevent binding of leukocytes or platelets to adhesion receptors such as ICAM1 (intercellular adhesion molecule 1), VCAM1 (vascular cell adhesion molecule 1), and von Willebrand factor. Therefore, EG degradation impairs a major protection against endothelial activation and leads to increased leukocyte adhesion and possibly thrombosis.[51]

The EG functions go far beyond steric protection of the endothelium. The EG has also been identified as a vascular protector through regulation of the vessel microenvironment, according to the following mechanisms. (1) The EG catches several enzymes, inhibitors, or agonists within its structure. For example, the EG may interact with antithrombin III and thrombomodulin, thereby contributing to the antithrombotic properties of a healthy endothelium.[7,52] (2) The EG also binds cytokines and modulates the inflammatory response. For example, sdc1, wearing HS chains, creates a chemotactic interleukin-8 gradient which guides neutrophil recruitment to the appropriate places.[53] (3) The EG globally reduces oxidative stress through superoxide dismutase retention.[7] (4) Finally, one of the main functions of the EG is mechanosensing (aka mechanotransduction), together with shear stress transduction.

EG and Mechanotransduction

Mechanotransduction is a complex process, which is not completely understood. It means that extracellular sensory stimuli such as shear stress, compression, or cell tension induce ≥1 intracellular signals via activation of specific receptor types, such as tyrosine kinase and G protein–coupled receptors.[54] Mechanotransduction is initiated on the cell surface and transmitted to the cytoskeleton, in the direction of intercellular junction sites and cell-matrix adhesion sites, as well as toward the nucleus for transcription regulation. Being in close contact with blood flow in the vessel lumen and directly attached to the cytoskeleton actin on the other side, EG extensions are ideally located to detect shear stress and transmit it to the surrounding intracellular structures.[55,56] In fact, the EG has been proposed for many years as a key actor in flow-induced vasorelaxation.[57] However, to this date, the actual intermediate signals of flow-induced, shear stress–related mechanotransduction have not been elucidated, and the role of EG in this regard is unresolved. What is known is that enzymatic degradation of the EG by neuraminidase, heparinase, or hyaluronidase decreases flow-induced nitric oxide (NO) production,[55,58] confirming that sialic acids, HA, and HS-containing GAG chains play an important role in this signal transmission. One of the main roles of the EG in mechanotransduction may thus be initiation of NO-dependent vasorelaxation by shear forces acting directly on the EC surface.[11] NO-dependent relaxation can also be induced by various agonists such as acetylcholine acting on their respective EC receptors. EC alignment with the direction of flow is also inhibited after EG destruction by heparinase treatment,[59] bringing further evidence to the role of the EG in EC response to blood flow.

Core proteins of EC PGs, such as sdc1, sdc2, sdc4, and gpc1, also take part in the process of mechanotransduction. The cytoplasmic tails of syndecans associate with the cytoskeleton, although the secondary structure of their ectodomains predicts a flexible molecule.[60] Fluid shear stress transmitted to the core proteins of the EG and the connection to the actin cytoskeleton mediate specific cell signaling such as NO production and cytoskeletal reorganization. Sdc1 or sdc 4 depletion alter flow sensing and the cell alignment that should follow.[61,62] In the absence of sdc1, ECs exposed to an atheroprotective flow abolish key signaling events such as Akt phosphorylation and exhibit an inflammatory and atherosclerotic phenotype.[63] Silencing of the gpc1 gene inhibits shear stress–induced activation of the endothelial NO synthase.[61]

Various elements other than the EG also participate in mechanotransduction. However, most of them are directly associated with the EG.[64] These elements comprise the following. (1) Integrin-type cell adhesion receptors: these

transmembrane proteins interact on one side with the ECM components, whereas their cytoplasmic domain links to the cytoskeleton. Some integrins present in the EG are involved in flow-induced vasodilation and arterial pressure regulation.[65,66] (2) Mechanosensor microdomains called caveolae: these 50- to 100-nm deep plasma membrane invaginations are enriched in cholesterol, sphingolipids, and different signaling molecules such as receptors with tyrosine kinase activity, G protein–coupled receptors, kinases, phosphatases, and ion channels.[67] Clustering of these molecules in caveolae optimizes signal transduction. Caveolae are closely related to the EG because they contain the HA receptor CD44 and essential EG PGs such as gpc1. (3) The primary cilium on ECs: this 1- to 5-µm-long protrusion into the vessel lumen contains cytoskeleton microtubules connected to the cytoplasm and may thus play a critical role in mechanotransduction. For instance, primary cilia on embryonic heart ECs induce a shear response through Kruppel-like factor-2 activation, which positively regulates the endothelial NO synthase and downregulates endothelin production.[68] These sensory primary cilia are exclusively present in areas of low and disturbed blood[69,70] and appear as a self-regulatory mechanism for ECs to modulate their response to shear stress. The cilia may take over mechanotransduction in poor EG areas.[68]

Several mechanisms have been proposed to explain the mechanosensor function of the EG, although none of them is well demonstrated. The 2 main proposals are as follows: (1) the EG may reorganize under shear stress, thanks to the mobility of gpc1, attached to the plasma membrane through a glycosylphosphatidylinositol anchor. This would lead to a clustering of HS and activation of intracellular signals[16,71]; and (2) the HS and HA molecules may deform (torque), allowing them to interact with some elements of the cytoskeleton and activate NO synthesis.[72,73]

Significance of HA for EG Functions

Despite the importance of HA in the EG, the way HA attaches to the plasma membrane and integrates the EG remains unclear. HA is either bound to its receptor CD44[74–76] or remains attached to the extracellular part of the HA synthases, which are located at the inner face of the EC plasma membrane and extrude their reaction product extracellularly through a pore.[77,78] HA can also interact with chondroitin sulfate on sdc1[54] or can link to the EG through HA-binding proteins.[79] Finally, HA may self-assemble to form a fibrous network.[80]

From the earliest studies of EG enzymatic degradation, it appeared that both HA and HS are involved in EG integrity and functions.[81,82] Optical and fluorescence microscopy studies have suggested that HS, concentrated in the upper layer of the EG, is responsible for maintaining its structure, whereas HA and chondroitin sulfate, present in the denser layer adjacent to the ECs, would ensure the selective permeability of the EG.[14] This hypothesis remains untested.

EG degradation through hyaluronidase treatment revealed an involvement of HA in most of the functions described for EG (Figure 2; Table 1).

EG in Diabetic Pathology

EG Destruction During Diabetes Mellitus

Chronic hyperglycemia induces both micro- and macrovascular complications that affect various organs like the skin, muscles, heart, brain, eyes, and kidneys. Microvascular complications comprise retinopathy, nephropathy, and neuropathy. The main macrovascular complications result from atherosclerotic plaques and include ischemic heart disease, stroke, and peripheral artery disease. All these macrovascular events may also occur independently of diabetes mellitus, so it may be difficult to disentangle the effects of diabetes mellitus and

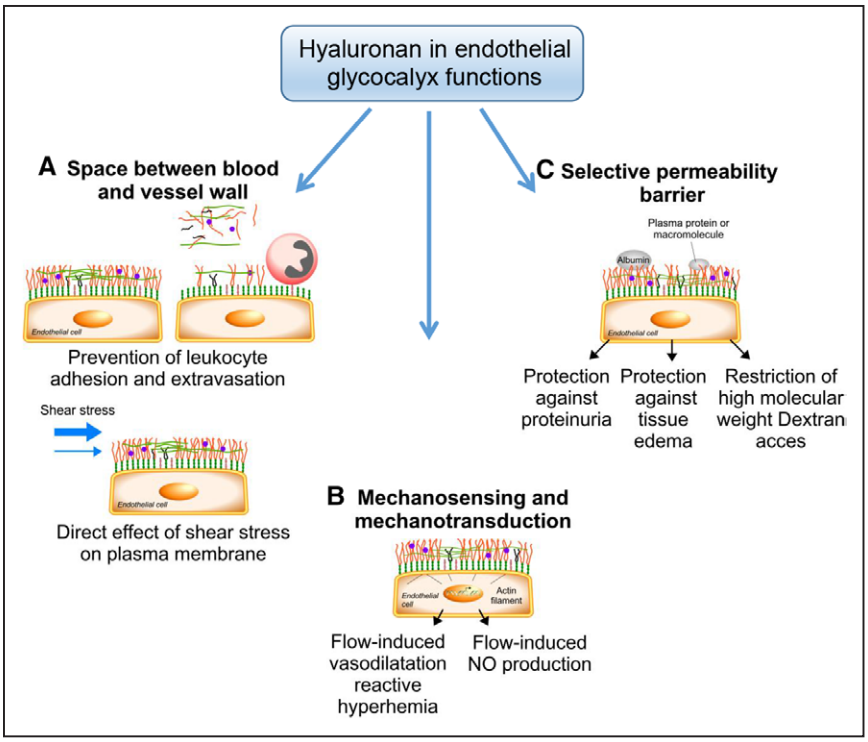

Figure 2. Hyaluronan (HA) involvement in endothelial glycocalyx (EG) functions. **A**, Protection and homeostasis of the endothelium: EG as a screen in front of adhesion molecules, preventing binding of leukocytes to adhesion receptors and reducing endothelial activation by limiting the direct effect of shear on EC plasma membrane. **B**, Mechanotransduction: signal transduction from the blood to the cytoskeleton of the EC: NO production induced in response to shear stress, and vasodilation during reactive hyperemia. **C**, Selective permeability of the endothelial barrier with regard to protein and macromolecule diffusion leading to a regulation of water efflux. Protection against proteinuria and fluid leakage.

Table 1. Evidence of HA Involvement in Many Key Functions of the Endothelial Glycocalyx

Domain	Glycocalyx Functions	HA Involvement in These Functions (References)
Permeation properties	Size and charge barrier against protein and macromolecule movement, protection against proteinuria	14,82,167–169
	Protection against microvascular fluid loss, prevention of tissue edema	3
Space between blood and endothelium	Reduction of blood shear stress on ECs surface	56
	Screen in front of adhesion molecules (ICAM1, VCAM1)/protection against leukocyte adhesion and extravasation	34
Vessel microenvironment regulation	Antithrombotic properties (by catching ATIII and thrombomodulin)	152
	Modulation of inflammation (by catching cytokines)	Role of HA not determined for this function
	Decrease of oxidative stress (by catching superoxide dismutase)	Role of HA not determined for this function
Mechanosensing	Alignment of ECs with the flow direction, reorganization of cytoskeleton	170
	Regulation of EC proliferation and migration under laminar flow	170
	Regulation of VE cadherin in cell junctions	Not implicated[171]
	Flow-induced NO production	56,58,172,173
	Flow-dependent vasodilation	174
	Reactive hyperemia	167,175

ATIII indicates Antithrombin III; ECs, endothelial cells; HA, hyaluronan; ICAM1, intercellular adhesion molecule 1; VCAM1, vascular cell adhesion molecule 1; and VE cadherin, vascular endothelial cadherin.

atherosclerosis per se. In fact, diabetes mellitus–induced atherosclerosis seems to follow the same histological course as in nondiabetic conditions, including endothelial injury, smooth muscle cell proliferation, foam cell development and infiltration, platelet activation, and increased inflammation. In diabetes mellitus, all these pathogenic steps occur in an accelerated way, in more diffuse localizations, with higher vascular remodeling and more plaque ruptures.[83–86] Some key factors in the biochemical pathways involved in the progression of atherosclerosis in diabetic patients have been identified. They comprise overproduction of reactive oxygen species (ROS), increased formation of advanced glycation end products (AGEs) and activation of AGE receptors, enhanced polyol and hexosamine fluxes, PKC activation, and chronic vascular inflammation.[85]

A large body of evidence supports the link between the development of atheromatous lesions and a thin EG overlying local thickening of the intima in bifurcation and branching areas.[87,88] Although this vascular phenomenon has not been specifically studied in the case of diabetes mellitus, it is clear that most diabetic micro- and macrovascular complications, if not all, are also coupled with damaged EG. We will focus our discussion on diabetes mellitus–related EG damage.

The involvement of EG damage in diabetic pathophysiology, which had been suspected for a long time, was actually demonstrated only recently. Intravital microscopy revealed a decrease in EG systemic volume both in type 1 diabetes mellitus (T1DM) patients and during induced acute hyperglycemia.[89,90] A decrease of EG volume in sublingual and retinal vessels was also observed in type 2 diabetes mellitus (T2DM) patients.[91] Damage to the EG has also been reported in animal models. In rat and mouse T1DM models of retinopathy, the size of the EG of retinal vessels is significantly decreased.[92,93] In db/db mice, simulating T2DM, the EG of brain microvessels is altered.[94] Mouse models of diabetic nephropathy, because of either streptozotocin-induced or congenital (Akita mice) diabetes mellitus, also show a damaged glomerular EG.[95–97]

In terms of functional consequences, EG deterioration during diabetes mellitus is clearly associated with many typical phenomena of micro- and macrovascular complications, such as decreased arterial vasodilation[98] (a typical step in endothelial dysfunction), increased endothelial permeability to macromolecules,[30] and, in the retinal vessels, fluid leakage, loss of pericytes, and neovascularization.[93] EG damage during diabetes mellitus is also associated with activation of coagulation,[90] increased leukocyte adhesion,[92] development of albuminuria,[95–97] and deterioration of the blood-brain barrier.[94]

Endothelial Dysfunction and Vascular Complications of Diabetes Mellitus

Endothelial dysfunction is generally defined as a decrease in the capacity of the vessel to dilate in response to an endothelium-dependent vasodilator (eg, acetylcholine or bradykinin) or in response to blood flow.[99,100] Other signs of endothelial dysfunction are changes in adhesion molecules (selectin, ICAM1) and proinflammatory molecules expression, decrease in platelet aggregation inhibition (procoagulation status), and modification in the regulation of smooth muscle cell proliferation (proliferative status).

Endothelial dysfunction is universally present in T1DM and T2DM patients and in experimental diabetes mellitus models.[101] It is an early predictor of micro- and macrovascular complications related to diabetes mellitus. However, the link between diabetes mellitus and the pathogenesis of vascular complications cannot be summarized by a single mechanism because in diabetic patients, especially T2DM, hyperglycemia can also be coupled with hypertension, lipid metabolism deregulation, or low systemic inflammation. However,

hyperglycemia remains primordial in the cause of diabetes mellitus–related endothelial dysfunction.[102]

Mechanisms of EG Alteration During Hyperglycemia

The mechanisms of EG degradation in diabetes mellitus, or in other diseases for that matter, are not well known.[103] It was suggested that hyperglycemia modifies the sulfation of HS in the Golgi apparatus, but investigations of this hypothesis yielded conflicting results.[104,105] Other authors suggested a decreased amount of GAGs on PGs, which could then modify the barrier properties of the EG.[106] The relevance of these in vitro observations remains uncertain. More convincingly, the involvement of 3 factors in EG deterioration during diabetes mellitus has received experimental support. These factors are ROS, AGEs, and activation of the sheddases heparanase and HYAL1.

The involvement of ROS in diabetic complications is well established. An overproduction of O_2^- (superoxide anion) and its resulting increased oxidative stress systematically accompany hyperglycemia. This pathway has been implicated in all 4 main mechanisms pointed out to explain the impact of hyperglycemia on ECs: activation of the polyol and hexosamine pathways, protein kinase C activation, and AGE formation.[107] Antioxidant treatment can partially avoid EG damage.[90,108] Finally, ROS are able to depolymerize GAGs and more particularly HA.[109–111] AGEs, another systematic consequence of hyperglycemia, are also responsible for high–molecular weight HA degradation.[112]

Besides ROS and AGEs, usual suspects in all diabetic complications, the roles of heparanase and the hyaluronidase HYAL1 have recently been exposed. Heparanase is secreted by macrophages and endothelin-activated podocytes and seems directly implicated in the process of EG degradation leading to diabetic nephropathy.[39,113,114] For instance, inhibition of monocyte chemoattractant protein-1 restores the EG in a mouse model of combined diabetic/atherosclerotic nephropathy through reduction of heparanase expression by macrophages.[40]

Evidence on the implication of HYAL1 in EG degradation is also substantial. The amount of HYAL1 in the plasma, measured by ex vivo enzymatic activity, increases during diabetes mellitus in mice, rats, and humans.[41,89,91,115,116] Furthermore, our laboratory has shown that streptozotocin-induced diabetic mice lacking HYAL1 display a thicker EG and are protected from HA shedding, endothelial dysfunction, and microalbuminuria in early diabetes mellitus, when compared with HYAL1 competent mice.[41] Together, these observations point to HYAL1 as a major actor of early diabetic vascular (and possibly renal) complications.

Role of Hyaluronidases

Like all vertebrate hyaluronidases, HYAL1 is an endo-β-*N*-acetyl-hexosaminidase that acts through hydrolysis of its substrate.[117] It is the most active enzyme in HA degradation and homeostasis, as shown by comparative HA-degrading activities of various hyaluronidases (HYAL2 is much less active[118]) and by 40- to 100-fold elevations of plasma HA levels in HYAL1-deficient humans.[119] A theoretical model for HA catabolism in somatic tissue proposes internalization of HA through the coupled action of the HA receptor CD44 and HYAL2 on the cell surface, in an acidic microenvironment created by the Na^+/H^+ exchanger-1 proton pump. This allows cleavage of HA into 20-kDa fragments that are then routed to the lysosomes in which HYAL1 continues the degradation of HA into tetrasaccharides.[120,121]

Despite this model, the way HYAL1 could cleave HA in the EG is not known. One possibility is that ECs could endocytose HYAL1 from the plasma, where it circulates without being active (the pH being too high). HYAL1 could then reach its optimal pH of activity (ca. 3.7) in the EC lysosomes after endocytosis. In support of this scheme, uptake of intravenously injected HYAL1 into liver sinusoidal ECs has been demonstrated.[35] In addition, several cell types including RAW264.7 macrophages,[122] Hu22RV1 from prostate adenocarcinoma,[123] and EA.hy926 ECs (S. Dogné unpublished data, 2014) display an active endocytosis of HYAL1. The supply of HYAL1 from the blood to ECs could, therefore, increase during diabetes mellitus. This hypothesis is attractive but several gaps in knowledge remain. For example, what is the source of increased plasma HYAL1 in diabetes mellitus? Is increased plasma HYAL1 a cause or a consequence of EG degradation? Is there, in addition, an increased acidic microenvironment in the diabetic EG that could allow increased HA endocytosis into ECs (perhaps through HYAL2) and thus increased HA degradation? All these elements remain to be explored.

HYAL2, the other main somatic hyaluronidase implicated in HA metabolism, may also play a role in the shedding of EG HA during diabetes mellitus because HYAL2 is a glycosylphosphatidylinositol-anchored enzyme located at the surface of the ECs as in many other cell types.[124] Indeed, HYAL2 has been suggested as an actor of EG damage in cultured ECs under low shear stress.[49] Furthermore, HYAL2-deficient mice raised in our laboratory have a thicker EG than their wild-type counterparts.[125] However, to our knowledge, no evidence of increased HYAL2 activity in diabetes mellitus or implication of HYAL2 in EG degradation besides cultured ECs has been provided. The role of HYAL2 in diabetic complications remains speculative.

Plasma Markers of EG Shedding

Signs of EG degradation in clinical conditions can be evaluated by measuring the elevation of some EG components in plasma, usually sdc1, HS, or HA. Which of these components is altered depends on the pathology.

During ischemia/reperfusion procedures in vascular surgery (global or regional ischemia), drastic increases in sdc1 (ca. 40×) and HS (10×) can be measured in the blood.[31] In immediate survivors of cardiac arrest, significant increases in sdc1, HS, and HA plasma levels are observed.[126] Ischemia-reperfusion performed in vivo on rat liver or on isolated guinea pig heart increases the release of HS and sdc1. This elevation is associated with a decrease of EG thickness, as observed by electron microscopy.[47,127]

In sepsis, sdc1 and HS plasma levels serve as blood markers of EG disruption[128] and strongly correlate with the disease

severity.[129] Recently, HA plasma levels have also proved to be elevated in sepsis and to represent a prognostic marker for morbidity and survival.[130] Association of EG degradation with inflammation is observed in a dog septic shock model where sdc1 and HS plasma levels correlate with those of IL (interleukin)-6 and TNF-α.[131]

Sdc1 and HS plasma levels are also indirect markers of EG degradation in hemorrhaged rats after resuscitation fluids.[132] After prolonged moderate-intensity endurance training, the same markers have been proposed to evaluate EG integrity.[133]

In chronic kidney disease, sdc1 and HA plasma levels increase continuously with the severity stages of the disease[134]; they are also increased in dialysis patients.[135] A rat model of chronic kidney disease shows increased sdc1 plasma levels coupled to a significant decrease in EG thickness, as measured by atomic force microscopy.[134]

Finally, in diabetes mellitus, an alteration in blood GAG concentration (increased HA, decreased HS, and chondroitin sulfate) was reported as early as 1973.[136,137] A slightly different alteration in serum GAG concentration (increased chondroitin sulfate and dermatan sulfate, decreased HS) was later reported in T2DM.[138] More recently, HA and sdc1 were proposed as blood or plasma markers of EG shedding in diabetes mellitus.[89,90,139,140] HA is discussed below. Sdc1 plasma levels increase in T2DM patients[139] and in T1DM patients with diabetic nephropathy, in parallel with microalbuminuria.[140,141]

In summary, plasma markers of EG shedding such as sdc1 and HA are available and have been validated in several cardiovascular diseases and in diabetes mellitus but to date have not made their way into the clinic, perhaps because the importance of EG degradation besides other markers of severity of diabetes mellitus is not yet fully recognized.

Table 2. Therapeutic Approaches to Protect or Improve the Endothelial Glycocalyx During Diabetes Mellitus

Therapeutic Molecules or Genetic Model	Diabetes Mellitus Subjects or Animal Models (Reference)	Benefits (or Absence Thereof) Observed	Potential Mechanisms
Sulodexide (GAG complex extracted from porcine intestinal mucosa)	T1DM and T2DM mice[176,177]	Decreased albuminuria	Enhanced GAG precursor abundance for GAG synthesis
			Reduction of heparanase expression (mRNA transcript and protein level)
	T2DM patients[91]	EG size restoration in retinal and sublingual vessels	Enhanced GAG precursor abundance for GAG synthesis
			Reduction of GAG metabolism
			Inhibition of heparanase and possibly hyaluronidase activity
	T2DM patients[165]	Lack of long-term renal protection	Unknown
Metformin	T2DM mice[178]	Improvement of endothelial barrier function (70 kDa dextran exclusion)	Unknown
Atrasentan	Diabetic ApoE$^{-/-}$ mice, model of diabetic nephropathy[39]	Increased glomerular EG coverage	Reduction of glomerular heparanase expression, preservation of HS levels
		Decreased albuminuria	
		Increased nitric oxide amount in the kidney	
Spiegelmer emapticap pegol (NOX-E36)	Diabetic ApoE$^{-/-}$ mice, model of diabetic nephropathy[40]	Increased glomerular EG coverage	Reduction of glomerular heparanase expression, preservation of HS levels
		Decreased albuminuria	
ETA and ETB receptor deficiency (podocytes)	Diabetic podETRKO mice[166]	Preservation of EG size	Inhibition of heparanase expression and secretion by podocytes, preservation of HS levels
		Decreased albuminuria	
HYAL1 deficiency	Diabetic HYAL1-deficient mice[41]	Increased EG thickness	Decreased degradation of EG HA
		HA shedding prevention	
		Improvement of glomerular endothelial barrier (70 kDa dextran permeation)	
		Decreased albuminuria	
		Improvement of endothelial function (vasodilation)	
HYAL1 inhibitors	Not tested yet		

ApoE indicates apolipoprotein E; EG, endothelial glycocalyx; ETA, endothelin receptor A; ETB, endothelin receptor B; GAGs, glycosaminoglycans; HA, hyaluronan; HS, heparan sulfate; HYAL1, hyaluronidase 1; MMPs, matrix metalloproteinases; podETRKO, podocyte-specific ETA/ETB deficient; T1DM, type 1 diabetes mellitus; and T2DM, type 2 diabetes mellitus.

Shedding of EG HA During Diabetes Mellitus

As previously described, hyperglycemia is responsible for EG deterioration through several mechanisms, 3 of which (ROS, AGEs, and hyaluronidase activity) are capable of depolymerizing HA. Shrinkage, alteration, or degradation of the EG is associated with development of cardiovascular diseases.[142] During acute hyperglycemia or in patients with T1DM, the size of the EG is reduced by 50% to 80% and plasma HA concentration increases by 30% to 80%.[89,90,116] This HA shedding contributes to increasing vascular permeability, overactivated coagulation, and decreasing flux-induced vasodilation. A degraded EG coupled with an increased plasma HA concentration is also observed in T2DM patients[91] and in rodent models of T1DM.[41,115] Furthermore, in HYAL1-deficient mice, an unusual increase in EG thickness coupled with a decrease in HA shedding results in a protection of endothelial function and glomerular barrier during early diabetes mellitus.[41] Shedding of HA further to alteration of the EG can therefore be considered as a solid observation in the pathogenesis of diabetic vascular and renal complications.

Dual and Opposing Roles of HA in Vascular Complications

Because HA localizes in the ECM of all tissues, it is also implicated in diabetic tissue remodeling, notably in vessel walls and kidneys, leading to micro- and macrovascular complications. In diabetic rat glomeruli, abnormal deposits of HA combine with infiltration of macrophages and monocytes.[143,144] In addition, neosynthesized HA accumulates within mesangial cells and induces the release of HA aggregates in the extracellular space, forming cables which are adhesive for monocytes.[145] This HA accumulation within the mesangium could participate in the development of diabetic glomerulosclerosis. Information on HA accumulation in diabetic vessels is limited. In the aortic intima of diabetic pigs, for instance, HA accumulates only in the presence of hyperlipidemia.[146] On the other hand, abnormal HA deposition, especially in vessel walls and plaques, has been implicated in several models of atherosclerosis in the absence of diabetes mellitus.[147–151]

There is, however, a striking example in an atheromatic disease model, the ApoE$^{-/-}$ mice, where the suppression of HA deposits in the tissues (including vessel walls) during atherogenesis does not produce beneficial effects, on the contrary, because of simultaneous disappearance of EG HA. Indeed the inhibition of HA synthesis using 4-methylumbelliferone in these ApoE$^{-/-}$ mice led to altered relaxation in response to acetylcholine and to a rise in plaque areas.[152] The thickness of EG was reduced by the HA synthesis inhibitor. Whether a true protection of vessel integrity would be afforded by maintaining EG HA in the face of diabetic insults remains to be demonstrated.

Prevention of EG Degradation: A New Therapeutic Target

Several recent studies and reviews have concluded to the therapeutic necessity to avoid EG degradation or to promote its synthesis and recovery in both atherosclerotic disease and diabetes mellitus.[12,13,153] EG recovery after experimental enzymatic degradation, using hyaluronidase or heparinase injections, was the subject of several early studies showing that recovery was relatively fast and could be accelerated by infusion of a mixture of HA and chondroitin sulfate or by supplementation with exogenous HS, respectively.[51,82] Prevention of EG degradation during pathologies is undoubtedly more complex, although some successes have been observed in animal models. Studies in humans are rare.

The main examples can be summarized as follows. (1) In rats, using plasma instead of saline for resuscitation after hemorrhagic shock increases sdc1 expression and restores EG thickness.[154] (2) In guinea pigs, albumin prevents EG degradation (shedding) during cardiac transplantation, decreasing fluid leakage and preventing leukocyte adhesion to the endothelium.[155] This EG stabilization seems to be mediated by sphingosine-1 phosphate naturally linked to albumin.[156] (3) The antioxidants superoxide dismutase and catalase preserve EG HS amount and barrier property in cultured glomerular ECs submitted to ROS.[157] These antioxidants also maintain EG thickness and inhibit leukocyte adhesion in

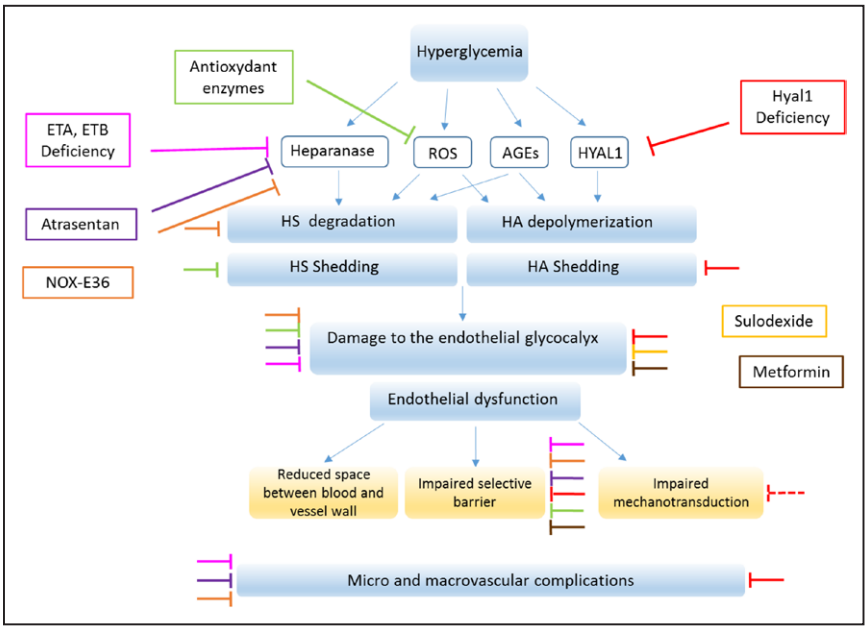

Figure 3. Summary of the main approaches tested to prevent endothelial glycocalyx (EG) degradation or to restore it in diabetes mellitus. Effects of each tested component (various colors) are pointed out with a closed tip arrow, at every stage where benefits have been observed. Only sulodexide has been tested in humans but found to not provide sufficient clinical efficacy for limiting long-term diabetic renal complications. In animal models, inhibitors of heparanase and HYAL1 (hyaluronidase 1) are suggested as new therapeutic tools. AGE indicates advanced glycation end product; ETA, endothelin receptor A; ETB, endothelin receptor B; HA, hyaluronan; HS, heparan sulfate; and ROS, reactive oxygen species.

hamster cremaster muscle capillaries after oxidized LDL challenge.[158] (4) Doxycycline decreases EG shedding and resulting leukocyte adhesion on ECs during ischemia-reperfusion in rats.[159] (5) On the isolated guinea pig heart, hydrocortisone and antithrombin also preserve EG thickness, maintaining its antiadhesive and barrier properties, alleviating vascular leakage (edema) and inflammation.[160–162] (6) Intravenous injection of exogenous HA during ischemia-reperfusion in mice avoids thinning of EG and damage to the permselectivity barrier of the EG.[5] (7) Intravenous injection of wheat germ agglutinin lectin, which adsorbs to the EG, in rats with chronic kidney disease reduces albuminuria.[163] (8) Finally, in patients with hypercholesterolemia, rosuvastatin also preserves EG thickness and prevents HA shedding through normalization of the LDL levels.[164]

On diabetic pathology, the main approaches tested to date are listed in Table 2 and illustrated in Figure 3. Only one of the potential therapeutic products (sulodexide) has been tested in humans but found to not provide sufficient clinical efficacy for limiting long-term diabetic renal complications.[165] In animal models, inhibitors of heparanase and HYAL1 have been suggested as new therapeutic tools.[39–41,166]

Conclusions

Damage to the EG during hyperglycemia seems to be an initial and critical step in provoking endothelial dysfunction. Although the mechanisms of this degradation are not well understood, preserving some key components of the EG seems a good proposal to try and prevent diabetic micro- and macrovascular complications, most of which are initiated by endothelial dysfunction. This dysfunction may result in large part from EG damage. HA is a major component of the EG and was shown to be required in various functions of this protective layer. HA may also buffer ROS and AGE attacks during hyperglycemic episodes. Although several therapeutic molecules have been tested for EG protection and restoration in animal models of vascular diseases and even diabetes mellitus, none of them has been shown to have clinical effectiveness. Preserving the size and amount of EG HA in the face of diabetic insults could be a useful novel therapeutic strategy to slow diabetic complications. One way to achieve this goal, as suggested by a murine model of HYAL1 deficiency, may be to inhibit the function of HYAL1, the main circulating hyaluronidase that seems enriched in the endothelial layer and may participate in HA removal from, and collapse of, the EG during diabetes mellitus.

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Disclosures

None.

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Highlights

- The endothelial glycocalyx (EG) covers the apical surface of the endothelial cells and is essential to vascular integrity and cardiovascular homeostasis.
- Hyaluronan, a key glycosaminoglycan of the EG, is implicated in most of the functions described for EG. The amount of hyaluronan in the EG may be regulated by the HYAL (hyaluronidase) 1.
- Diabetes mellitus induces damage to the EG, including shedding of hyaluronan into the plasma and increases in plasma hyaluronidase. This is an early sign of vascular dysfunction.
- Preserving the size and amount of EG hyaluronan, for example, through HYAL1 inhibition, is an attractive novel therapeutic strategy to slow diabetic vascular complications.