



CKJ REVIEW

The role of endothelial glycocalyx in health and disease

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ABSTRACT

The endothelium is the largest organ in the body and recent studies have shown that the endothelial glycocalyx (eGCX) plays a major role in health and disease states. The integrity of eGCX is vital for homoeostasis and disruption of its structure and function plays a major role in several pathologic conditions. An increased understanding of the numerous pathophysiological roles of eGCX may lead to the development of potential surrogate markers for endothelial injury or novel therapeutic targets. This review provides a state-of-the-art update on the structure and function of the eGCX, emphasizing the current understanding of interorgan crosstalk between the eGCX and other organs that might also contribute to the pathogenesis of kidney diseases.

Keywords: chronic kidney disease, diabetes mellitus, endothelial glycocalyx

INTRODUCTION

The vascular endothelium is the largest organ in the body, forming an interface between the bloodstream and the blood vessel wall. It lines the inside of all blood vessels in the body. The luminal surface of all vascular endothelial cells is covered by the endothelial glycocalyx (eGCX), which comprises membrane-bound negatively charged proteoglycans, glycoproteins, glycolipids and glycosaminoglycans [1]. It provides the building block needed for a strong and functional vascular endothelium. Over the last few years, the potential possible pathophysiological roles of eGCX have been the focus of intense research. Disruption or dysfunction of the eGCX has been associated with disease states such as diabetes, chronic kidney disease (CKD), inflammatory conditions, sepsis, hypernatraemia, hypervolaemia and ischaemia/reperfusion injury. Also, changes

in the eGCX have been associated with treatment responses in conditions such as sepsis. In this review we summarize evidence regarding the structure and function of the eGCX, alterations of the eGCX in various diseases states and therapeutic options.

eGCX structure

The eGCX lines the luminal surface of the vascular endothelium, interacting with plasma lipids and proteins [2, 3]. The composition and dimensions, permeability and charge of the eGCX interact dynamically with blood flow throughout the vasculature [4, 5]. The eGCX is attached to the endothelial cells through several backbone molecules, mainly proteoglycans and glycoproteins, creating a network in which soluble molecules from either plasma or endothelium are incorporated [1]. Proteoglycans consist of a core protein that can contain one or

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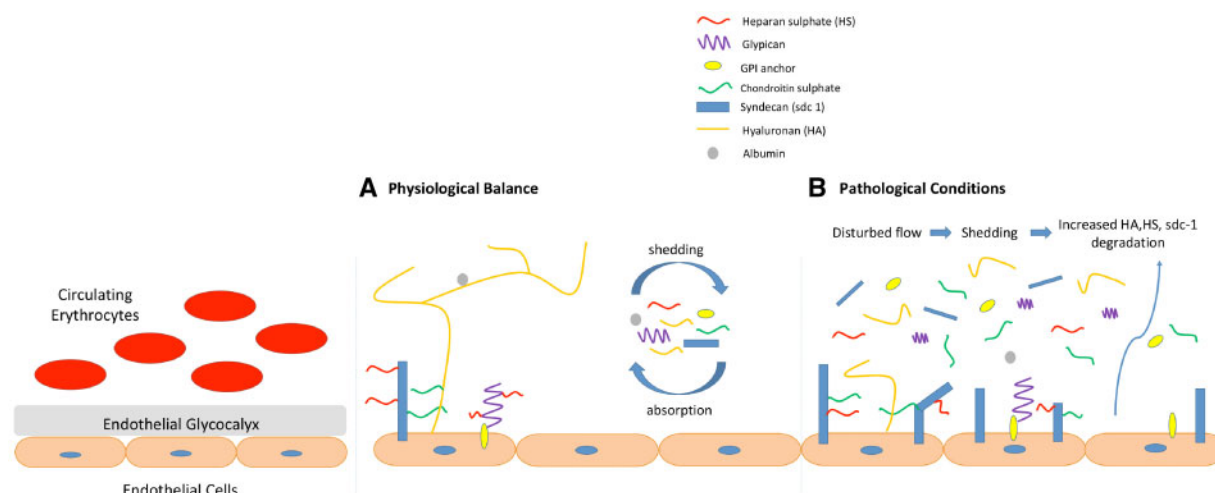


FIGURE 1: Structure and functions of eGCX in physiological and pathological conditions.

more negatively charged glycosaminoglycan (GAG) side chains. eGCX proteoglycans are structurally diverse regarding the size of the core protein, the number and type of GAG side chains and attachment to the endothelial cell membrane. Some core proteins such as syndecan are attached to endothelial cells through a transmembrane domain [6]. Others, such as glypican, are connected to the endothelial cell membrane through a glycosylphosphatidylinositol anchor [7], while others, such as perlecan, decorins, versicans, mimecans and biglycans, are secreted after the attachment and modification of GAG side chains [1].

GAGs are characterized by distinct linear disaccharide polymers of variable length. There are five types of GAGs: hyaluronic acid (HA), heparan, chondroitin, keratin and dermatan sulphates. Among these, heparan sulphate is the dominant type, making up 50–90%. Small modifications in the concentration and organization of GAGs can have a great impact on eGCX function since GAGs contain many distinct binding sites for plasma-derived proteins [1].

HA is a much longer GAG than heparan or chondroitin sulphate. HA is secreted on the endothelial surface and linked to the endothelial surface receptor CD 44 in caveolae. It weaves into the eGCX through its interaction with the CD44 receptor. Even if it is not sulphated, as opposed to the other four types of GAGs, it gets a negative charge from hydration properties supplied by carboxyl groups [8]. HA has been found to provide a direct connection between the endothelium and its extracellular matrix and is involved in the maintenance of eGCX integrity. The loss of HA as a part of eGCX degradation is observed to be closely associated with the endothelial dysfunction, which is an early predictor of many diseases such as diabetes, hypertension, atherosclerosis and dyslipidemia [9].

Endothelial glycoproteins also contribute to preserve eGCX stability. Endothelial glycoproteins contain 2–15 sugar residues arranged as branched carbohydrate side chains and comprise adhesion molecules, such as selectins (E and P), integrins and immunoglobulins, and some components of coagulation, haemostasis and fibrinolysis. Molecules secreted by glycoproteins participate in a wide range of eGCX functions, including endothelial cell adhesion, recognition and recruitment from the bloodstream and cell signalling [1] (Figure 1).

Functions of eGCX

eGCX functions are summarized in Table 1. Tissue fluid balance and microvascular fluid exchange have been classically

explained by the Starling principle since 1896. However, recent developments in our understanding of eGCX functions have increased the need for revising the Starling principle by incorporating the glycocalyx model. The eGCX is also known as the endothelial gatekeeper, summarizing its main physiological role as being the key determinant of vascular permeability, maintaining the balance between fluid filtration and absorption in the capillary lumen [1, 10].

The eGCX, composed of a negatively charged network of GAGs and proteoglycans, coats the luminal surface of the vascular endothelium. The eGCX is not permeable to large molecules such as dextran, and its neutralization increased rat mesenteric artery permeability for fluorescence-labelled dextrans [11]. The negatively charged GAGs also contribute to prevent albumin transport through the vascular wall barrier [12]. Thus vascular permeability is modified by the molecular size, structure and electrostatic charge of the eGCX [13].

The eGCX also modulates interactions between the vessel wall and blood cells. The negatively charged eGCX repels and is impermeable to red blood cells (RBCs), contributing to ensure the enclosed flow of RBCs in the microcirculation, and weakens the interaction of leucocytes and platelets with the vessel wall, thus controlling platelet and leucocyte adhesion and modulating the haemostatic and inflammatory responses [1, 12, 14]. eGCX adhesion molecules, including intercellular adhesion molecules 1 and 2, platelet/endothelial cell adhesion molecule and vascular cell adhesion molecule (VCAM), behave as ligands for integrins on leucocytes and platelets [1] and are induced by the inflammation. After monocyte and neutrophil adhesion via integrins and selectins, adhesion molecules promote cell rolling and leucocyte extravasation [15]. On the other hand, how the glycocalyx (conversely) inhibits leucocyte adhesion as shown by some studies needs further investigation [16, 17].

The eGCX also controls the interaction with the microenvironment by enabling binding of ligands and enzymes that regulate cell signalling, enzyme modifications and vascular protection. Without being exhaustive, fibroblast growth factors and the lipolytic system [lipoprotein lipase and its ligand low-density lipoprotein (LDL)] are dependent on the interactions of their ligands and receptors within the eGCX [1]. Moreover, the eGCX is a binding site for crucial anticoagulant mediators such as heparin cofactor II, antithrombin III, thrombomodulin and tissue factor pathway inhibitor (TFPI). Heparin cofactor II is a

Table 1. Functions of eGCX

Acts as the exclusion zone between blood cells and the endothelium
The exact composition varies greatly according to the local microenvironment
Plays the main role in transvascular fluid exchange and fluid balance (acts as the 'molecular sieve' for plasma proteins, forming the basis of the Starling principle and acting as the origin of the oncotic forces, which control the transcapillary movement of water)
Modulates adhesion of inflammatory cells and platelets to the endothelial surface
Functions as a sensor and mechanotransducer of the fluid shear forces
Maintains blood rheology and damage leads to platelet activation (normally retains anticoagulation factors such as antithrombin)
Plays a role in shear stress-dependent nitric oxide production
Retains protective enzymes (e.g. superoxide dismutase)

plasma protease inhibitor, inhibiting thrombin when in the presence of eGCX dermatan sulphate. Antithrombin III inhibition of coagulation by lysing thrombin, factor Xa and factor IXa is enhanced when it is bound to eGCX heparan sulphate. The endothelial cell surface protein thrombomodulin contains GAGs (chondroitin sulphate) and is a cofactor for thrombin. TFPI is a single-chain polypeptide that can reversibly inhibit factors VIIa and Xa after binding the eGCX heparan sulphates [1, 18, 19].

The eGCX may also protect the endothelium by binding enzymes that metabolize oxygen radicals, such as extracellular superoxide dismutase. These enzymes maintain nitric oxide (NO) bioavailability, decrease oxidative stress and prevent endothelial dysfunction [1, 18, 19]. Furthermore, the eGCX facilitates the release of NO when exposed to shear stress, thus reducing shear stress by dilating vessels [18–20]. Heparan sulphate proteoglycans sense the shearing force of interstitial flow, resulting in mechanotransduction and activation of specific cellular responses [21]. Most mechanical stress is converted to biochemical signals through the solid phase of the eGCX rather than through its fluid phase. The eGCX is also able to redistribute to perform some of its important actions, thus modulating microvessel haemodynamics [22].

Disruption of the eGCX and its role in pathophysiology

The eGCX may be damaged by exposure to shear and oxidative stress in conditions such as diabetes, sepsis, CKD, hypernatraemia, hypervolaemia and ischaemia/reperfusion injury [12, 23–27]. Here we will discuss the suspected roles of the eGCX in these conditions.

DISEASES

Diabetes and hyperglycaemia

Diabetes mellitus (DM) may be complicated by microvascular (retinopathy, neuropathy, nephropathy) and macrovascular [cardiovascular disease (CVD), peripheral arterial disease, stroke] injury. Chronic hyperglycaemia injures the vessel wall, leading to increased endothelial permeability and impaired NO synthase function. However, the precise cause-and-effect relationship between vascular dysfunction and DM complications are not fully understood [7]. Shedding of the eGCX is thought to contribute to DM-induced vascular dysfunction. Assessment of the systemic glycocalyx volume in 10 healthy subjects by comparing the distribution volume of a glycocalyx-permeable

tracer (dextran 40) and a glycocalyx-impermeable tracer showed that the systemic glycocalyx volume was decreased by 50% within 6 h after induction of acute hyperglycaemia [28]. Hyperglycaemia also increased plasma hyaluronan levels and promoted endothelial dysfunction and coagulation activation [28]. The eGCX thickness was 50% lower in DM patients than in healthy controls, while serum hyaluronidase and hyaluronan levels were higher in diabetics [23]. The thinnest eGCX was found in DM patients with microalbuminuria [23]. Furthermore, the eGCX volume was lower in sublingual and retinal vessels from patients with type 2 DM [29]. Overall, these studies revealed that both acute and chronic hyperglycaemia significantly reduced eGCX size, supporting the idea that eGCX disruption may be associated with DM-induced endothelial dysfunction.

Endothelial dysfunction, characterized by faulty vasodilation due to decreased NO availability in response to endothelium-derived relaxing factors, is present in both type 1 and 2 DM patients. It is a well-known risk factor for CVD and is thought to lead to microangiopathy by altering vascular permeability, blood flow and pressure [30–39]. Indeed, endothelial dysfunction predicts early micro- and macrovascular complications of DM. Several other mechanisms may also contribute to endothelial dysfunction in DM, including dyslipidaemia and hypertension. However, hyperglycaemia itself is still thought to be the primary cause of DM-related eGCX disruption and dysfunction [38]. Mediators of hyperglycaemia-induced eGCX damage include reactive oxygen species, advanced glycation end products and the activation of glycocalyx-degrading enzymes such as hyaluronidase (HYAL1) and heparanase [23, 38].

DM-related eGCX shedding correlated with altered serum GAG concentrations, including increased HA, syndecan-1 and chondroitin sulphate and decreased heparan sulphate. HA and syndecan-1 were recently proposed as plasma markers of DM-related eGCX shedding. Plasma syndecan-1 was increased in both type 2 and 1 DM patients with diabetic nephropathy and microalbuminuria. However, further studies are required to determine exactly how DM-induced eGCX loss and dysfunction contributes to vascular injury and dysfunction in DM [38].

Sepsis

Sepsis is a systemic inflammatory response to a documented or presumed infection. The eGCX is damaged early in the course of inflammatory conditions such as systemic inflammatory response syndrome and sepsis. In hamsters, tumour necrosis factor- α (TNF- α), a representative pro-inflammatory cytokine, damaged the eGCX and enhanced macromolecular permeability without increasing leucocyte recruitment [40]. TNF- α induced release of cytokines, heparinase and histamine and proteases by mast cells further disrupted the eGCX [41]. TNF- α induced shedding of syndecan-4 were mediated by matrix metalloproteinase-9 (MMP-9), contributing to eGCX damage [42]. There is also evidence that endothelial signalling is critical to septic glycocalyx degradation via TNF- α [17, 43].

Increased serum eGCX constituent levels correlated with increased mortality in sepsis [41, 44]. Shedding of eGCX constituents, such as endothelial cell adhesion molecules, is thought to induce further inflammation and increase recruitment of leucocytes and platelets contributing to organ dysfunction, such as acute kidney injury (AKI) and respiratory failure [45, 46].

Disruption of the eGCX in sepsis is associated with increased vascular wall permeability to macromolecules, loss of circulating albumin and subsequent fluid extravasation and oedema

[7]. Parenteral albumin did not increase survival rates in sepsis patients as compared with crystalloids alone [47]. Together, these studies suggest that while eGCX disruption persists, effective oncotic pressure cannot be restored by administering albumin since large amounts of albumin will continue to leak into the interstitial space [48].

CVD

Despite therapeutic efforts to tackle the main risk factors (dyslipidaemia, high blood pressure, diabetes, obesity, smoking), CVD remains the leading cause of death globally. Atherosclerosis is a major cause of CVD and dysfunction of vasculo-protective endothelium is a key contributor. Exploration of the vasculo-protective properties of the vessel wall against atherosclerosis may identify novel therapeutic approaches [49]. The eGCX is considered to be protective from atherosclerosis, and eGCX shedding is associated with loss of the vasculo-protective properties of the vessel wall [49]. eGCX shedding increases lipid fluxes that lead to lipid deposition in the vessel wall, a characteristic feature of atherosclerosis, interferes with endothelial cell communication and increases inflammatory cell migration to the vessel wall. Thus disruption of the eGCX leads to decreased endothelial NO synthase expression by endothelial cells, promoting vasoconstriction and dysregulation of vascular tone. Emerging eGCX-targeted approaches could potentially be used to restore eGCX and treat early cases of atherosclerosis [50].

In isolated pig heart, near-complete eGCX shedding occurred after reperfusion following 20 min of warm (37°C) no-flow ischaemia, resulting in increased capillary permeability-induced tissue oedema with enhanced coronary perfusion pressure [51]. These results are consistent with human findings of increased plasma levels of eGCX constituents following coronary artery bypass or peripheral arterial surgery [45]. In pig heart, antithrombin significantly decreased post-ischaemic eGCX shedding, vascular leakage/tissue oedema and coronary perfusion pressure. Protection by antithrombin was potentiated by the addition of colloid [51].

Statins are the most used lipid-lowering medications, reducing LDL cholesterol through inhibition of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase. Statins have pleiotropic actions beyond lowering LDL cholesterol, which depend on inhibition of intracellular signalling requiring HMG-CoA reductase metabolites, such as inflammatory cytokine-induced signalling in endothelial cells, a key driver of eGCX shedding [9]. Additionally, familial hypercholesterolaemia is associated with a profound perturbation of the eGCX. Rosuvastatin for 8 weeks led to the partial recovery of systemic glycocalyx volume in 13 patients with heterozygous familial hypercholesterolaemia [52]. However, uncertainty still exists about whether this is related to a direct statin effect or to improved lipid status [45].

Diazoxide is a potassium channel activator with antihypertensive and hyperglycaemic activities. During coronary artery bypass surgery, diazoxide decreased post-surgical plasma hyaluronan and syndecan-1 levels, which supported the idea that diazoxide treatment may reduce disruption of the eGCX [53].

Oxidative stress is widely recognized as a major contributor to the development of endothelial dysfunction, eGCX disruption and atherosclerosis progression. In experimental metabolic syndrome, combination therapy with olmesartan and azelnidipine successfully reduced oxidative stress via inhibition of nicotinamide adenine dinucleotide phosphate oxidase activity in

aortic endothelial cells and improved endothelial function and maintenance of eGCX integrity [54].

CKD

Intact endothelial cell function is essential for normal kidney function. Patients with CKD have increased eGCX shedding, which is associated with endothelial dysfunction. Plasma levels of two major eGCX components, syndecan-1 and hyaluronan, increased steadily across CKD categories [25]. This is thought to be a consequence of increased shedding, although a lack of urinary excretion of immune reactive degradation products could not be entirely ruled out. Supporting the increased shedding hypothesis, plasma syndecan-1 and HA positively correlated with plasma markers of endothelial dysfunction such as von Willebrand factor, soluble fms-like tyrosine kinase-1, angiopoietin-2 and soluble vascular adhesion molecule-1. Consistently, in a rat model of CKD, plasma syndecan-1 inversely correlated with eGCX thickness, which was significantly decreased in aortic endothelial cells from CKD rats [27]. Overall, this study suggests that eGCX integrity and function are compromised in CKD. However, the specific mechanisms underlying the loss of eGCX integrity in CKD are not yet fully understood. In CKD, endothelial dysfunction is widely acknowledged to be a risk factor for atherosclerosis and cardiovascular events [55, 56]. Further supporting the causative link between CKD and endothelial dysfunction, endothelial function improves after kidney transplantation [55, 57, 58].

To investigate the association between renal function and the eGCX dimension, the perfused boundary region (PBR) was measured in control participants, patients with normal kidney function after kidney transplantation, end-stage renal disease patients and patients with interstitial fibrosis/tubular atrophy by using non-invasive sidestream darkfield (SDF) imaging. Serum levels of eGCX components such as syndecan-1, soluble thrombomodulin and the marker of endothelial activation, angiopoietin-2, were also measured [51]. This study indicated that PBR and the serum levels of syndecan-1 and thrombomodulin were elevated in patients with end-stage renal disease or interstitial fibrosis/tubular atrophy compared with the control participants and stable kidney transplant patients. PBR was also found to be positively correlated with angiopoietin-2. Taken together, these results support the idea that patients with reduced renal function (estimated glomerular filtration rate) have a decreased eGCX dimension [59]. Although diabetic nephropathy induces a decrease in eGCX dimension [60], the link between eGCX and end-stage renal disease is still not fully understood. Oltean et al. [61] demonstrated in a mouse study that vascular endothelial growth factor-A (VEGF-A) played a key role in renal endothelial dysfunction. In a murine diabetic nephropathy model, kidney VEGF-A165b was upregulated in mice without a loss of kidney function but not in those with a loss of kidney function. The VEGF-A165b isoform was shown to be involved in the phosphorylation of VEGF receptor 2 on the glomerular endothelial cells, reducing the degree of diabetic nephropathy vascular endothelial growth factor related eGCX damage and improving glomerular permeability. After observing the functional and histologic benefits of administration of VEGF-A165b in animal models with diabetic nephropathy, VEGF-A165b was tested on isolated diabetic human glomeruli. The permeability function of the human glomeruli was also restored with the administration of VEGF-A165b, protecting the eGCX of renal vasculature from the destructive effects of diabetic nephropathy [61].

Inflammation contributes to eGCx damage during kidney disease. Monocyte chemoattractant protein-1 [MCP-1/chemokine (C-C motif) ligand 2] is the key chemokine recruiting inflammatory cells such as monocytes and macrophages to the kidney and clinical trials targeting MCP-1 support its pathogenic role in humans [62]. Inhibition of MCP-1 with emapticap pegol (NOX-E36) restored the eGCx barrier dimensions in streptozotocin-induced diabetes in Apoe knockout mice. NOX-E36 decreased eGCx degradation by heparinase, which was regulated by macrophage-secreted cathepsin L. This was associated with decreased albuminuria and kidney inflammation in the absence of changes in systemic haemodynamics [63]. Atrasentan, a selective endothelin A receptor antagonist whose clinical trial for diabetic nephropathy was recently terminated, decreased albuminuria by repairing the eGCx barrier in patients with diabetic nephropathy [64].

Evidence of eGCx degradation was also observed in patients suffering from ischaemia/reperfusion injury during vascular surgery [65]. In another study, ischaemic AKI in renal transplantation from donors after cardiac death (DCD) and living donors was investigated by using SDF imaging of peritubular capillaries and serum levels of syndecan-1 and heparan sulphate. DCD kidneys were reported to have reduced capillary flow and higher serum levels of syndecan-1 and heparan sulphate compared with living donor kidneys with minimal ischaemia. These findings provided strong evidence that the eGCx was degraded due to ischaemia and subsequent reperfusion. The loss of endothelial cells and eGCx integrity could contribute to AKI through decreasing tissue perfusion and more inflammation following reperfusion [66].

Administration of GAG-degrading enzymes (hyaluronidase, heparinase and chondroitinase) to mice resulted in increased glomerular vascular permeability to albumin but not to Ficoll (uncharged polymer), indicating that eGCx degradation led to reduced charge selectivity and proteinuria [67]. These results support a role for eGCx damage and systemic endothelial dysfunction in the genesis of albuminuria [68].

Plasma syndecan-1 and HA levels were higher in dialysis patients than in controls, showing that dialysis patients lost eGCx barrier functions and the severity of the loss was correlated with the level of inflammation. The authors also measured the eGCx dimension using the PBR and checked the serum markers of the eGCx components and endothelial dysfunction, such as E-selectin. Interestingly, no correlation was detected between the variables [26].

Peritoneal dialysis requires a healthy peritoneal membrane. It is thought that both a healthy mesothelium and peritoneal vessels are required to preserve the peritoneal barrier. In peritoneal dialysis patients, SDF imaging of the sublingual microvasculature did not disclose any relationship between PBR assessment of systemic eGCx and peritoneal transport parameters [69]. This is not surprising, given that peritoneal vessels receive the bulk of the peritoneal fluid bio-incompatibility challenge [70]. Thus some of the most toxic and highly reactive peritoneal dialysis fluid metabolites, such as 3,4-dideoxyglucose-3-ene readily react with peritoneal membrane components and do not reach the systemic circulation [71].

MOLECULES

Albumin

Albumin is the major serum protein and has multiple important functions. Stabilization of eGCx is thought to be one of them.

Electrostatic interactions occur between negatively charged GAGs in the eGCx and the positively charged arginine in albumin. It is believed that hypoalbuminaemia could facilitate shedding of the eGCx [72].

Albumin transports multiple small molecules, including free fatty acids and sphingosine-1-phosphate (S1P) [73]. Binding of S1P to its receptors resulted in MMP inhibition and eGCx integrity preservation [74]. In this regard, in trauma patients, higher plasma levels of syndecan-1, a sign of eGCx shedding, was significantly correlated with a reduction in plasma colloid osmotic pressure [75]. However, this study sheds no light on whether hypoalbuminaemia facilitated eGCx shedding or eGCx shedding promoted hypoalbuminaemia.

In a recent study, Garsen *et al.* [76] showed that endothelin-1 induces proteinuria by heparanase-mediated disruption of the glomerular glycocalyx in diabetic nephropathy. In this study, the authors demonstrated that in mice, podocyte-specific knockout of the endothelin receptor prevented the diabetes-induced increase in glomerular heparanase expression, consequent reduction in heparan sulphate expression and eGCx thickness and development of proteinuria observed in wild-type counterparts [76].

Thus the relationship between albumin and eGCx seems to be reciprocal. eGCx disruption may facilitate albumin translocation to the interstitial space, while hypoalbuminaemia itself may disturb the function and structure of the eGCx.

Glomerular endothelium, glomerular basement membrane and podocytes are the three key components of the glomerular filtration barrier (GFB). Pathological albuminuria results from increased passage of albumin through the GFB, which exceeds the capacity of proximal tubular cells to reabsorb albumin. Pathological albuminuria is an important indicator of glomerular filtration dysfunction and, as an early sign of progressive cardiovascular and renal disease, is considered as a criterion to diagnose CKD [77]. While classically considered a marker of podocyte injury, albuminuria has been observed in the absence of podocyte changes, confirming the close integration of GFB components and the potential impact of endothelium and basement membrane injury on glomerular albumin permeability. The endothelial surface layer, comprised of the eGCx and adsorbed plasma constituents, restricts the permeability of glomerular capillaries to albumin. Communication between the glomerular endothelium and podocytes also influenced the contribution of podocytes to the albumin permeability of the GFB [78].

Recently a novel sensitive glomerular permeability assay confirmed that eGCx damage leads to increased albumin glomerular permeability in a mouse model. eGCx dysfunction in diabetic mice was associated with increased urinary albumin excretion. Furthermore, eGCx function was restored by angiotensin-1 [79]. Patients with albuminuric CKD had widespread loss of the eGCx, which was hypothesized to lead to increased systemic microvascular permeability, linking albuminuria to systemic vascular disease [78, 80].

Tissue sodium deposition

High salt (NaCl) intake has a blood pressure-independent effect on endothelial function, contributing to the development of vascular diseases, whereas the arterial system benefits from lowering salt intake. The eGCx has been proposed as an effective sodium buffer since positively charged sodium ions can be trapped in the eGCx mesh by negatively charged proteoglycans [81, 82]. Quantitative eGCx analyses showed that chronic

exposure to high ambient sodium weakened the sodium buffer capacity of eGCX, leading to eGCX disruption through reduction of negatively charged heparan sulphate residues [24]. High sodium levels increase the expression of endothelial sodium channels (EnNaCs) in the plasma membrane. Both the collapse of the eGCX and the increased number of EnNaCs allowed sodium to access endothelial cells more freely. Enhanced sodium fluxes into endothelial cells triggers intracellular signalling that may promote endothelial dysfunction through a reduction in NO release and an increase in mechanical arterial stiffness [24, 83, 84]. Arterial stiffness is a well-characterized contributor to the vasculopathy of CKD [85].

Volume

Volume loading is a blood-sparing procedure commonly used to improve haemodynamics and optimize cardiac output. However, it may lead to adverse effects such as tissue oedema, hypervolaemia, hypertension and heart failure [86]. Hypervolaemia is associated with enhanced release of atrial natriuretic peptide (ANP) and with eGCX collapse [87]. ANP itself was found to disrupt the eGCX through a cyclic guanosine monophosphate-linked proteolytic pathway in patients having coronary bypass surgery [88, 89]. Collapse of the eGCX was associated with detrimental interstitial fluid shifting [86]. From that perspective, plasma levels of eGCX degradation components should be explored for their potential as surrogate markers to assess vascular endothelial injury that may provide information about when to limit uncontrolled fluid resuscitation to prevent interstitial oedema [90].

Furthermore, crystalloids and colloids are the two main types of volume expanders. Research indicates that eGCX has an important role in the differential effects of colloids and crystalloids on plasma volume expansion. Crystalloid was considered to be expanded throughout the intravascular space, whereas colloids had no ability to distribute into the glycocalyx [91–93]. This issue is important since, during fluid resuscitation, the integrity of the eGCX should be considered. For example, recent studies have shown that crystalloid versus colloid infusion makes sense when considering the integrity of the eGCX in critical patients [92, 93].

THERAPEUTIC RESTORATION OF THE EGCX

The eGCX may restore itself within 5–7 days of an insult [94]. Accelerating restoration of the eGCX and protecting it from further damage are promising targets for the treatment of chronic vascular disease, as well as management of patients in critical care settings [44]. To date, there is no established drug directly acting on protection and regeneration of the eGCX [95]. However, some agents have been explored in this regard, including sulodexide, albumin, fresh frozen plasma, glucocorticoids and other agents.

Sulodexide is a mixture of heparin sulphate (80%) and dermatan sulphate (20%) extracted from porcine intestinal mucosa that has been unsuccessfully tested in clinical trials for diabetic nephropathy, but is in clinical use for venous insufficiency [96]. In type 2 diabetic patients, sulodexide restored the components of the eGCX in the retinal circulation within 8 weeks [29]. In rats with carotid artery balloon injury, sulodexide restored the eGCX and reduced damage, blood coagulation, lipid metabolism and local inflammation [97]. Sulodexide also accelerated eGCX restoration, decreased vascular permeability and increased survival in septic mice [98].

Albumin and fresh frozen plasma have several clinical indications. In pig heart transplantation-induced ischaemia/reperfusion, albumin decreased interstitial oedema and leucocyte-to-endothelial interaction within coronary arteries, contributing to preserve eGCX integrity [99]. Albumin is a carrier for several bioactive molecules, including S1P [100]. S1P is considered to protect the eGCX by inhibiting syndecan-1 shedding [101].

Fresh frozen plasma also protects eGCX integrity and preserves syndecan-1. In addition to being a source of albumin, fresh frozen plasma may inhibit various proteases that disrupt the eGCX and may induce the release of preformed syndecan-1 from endothelial cells [102].

Glucocorticoids are widely used as anti-inflammatory agents. They preserve the eGCX precisely through anti-inflammatory actions on leucocytes and by reducing cytokine-induced damage on the vascular barrier [103]. In an isolated pig heart model, administration of hydrocortisone protected the eGCX against ischaemia/reperfusion and TNF- α infusion [104]. A better characterization of the molecular basis of these effects may reveal new cellular targets for drug development devoid of the extensive adverse effects profile of glucocorticoids.

Several additional approaches have preserved eGCX volume in animal studies, including NO, TNF- α inhibitors, hyaluronan, TNF-polyethylene glycol, allopurinol, adenosine agonists and heparin [7].

CONCLUSION

Damage to the eGCX during DM and other systemic diseases appears to be a key contributor to endothelial dysfunction. Additional identification of factors regulating the stability and repair of the eGCX under stress conditions and the pathophysiological roles of the eGCX might ultimately lead to the design of new diagnostic and therapeutic strategies for diseases characterized by endothelial dysfunction or vascular injury, including CKD.

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CONFLICT OF INTEREST STATEMENT

None declared.

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