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Endothelial glycocalyx as a potential theriapeutic target in organ injuries

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Abstract

Objective: The endothelial glycocalyx (eGC) is a dynamic and multicomponent layer of macromolecules found at the surface of vascular endothelium, which is largely underappreciated. It has recently been recognized that eGC is a major regulator of endothelial function and may have therapeutic value in organ injuries. This study aimed to explore the role of the eGC in various pathologic and physiologic conditions, by reviewing the basic research findings pertaining to the detection of the eGC and its clinical significance. We also explored different pharmacologic agents used to protect and rebuild the eGC.

Data sources: An in-depth search was performed in the PubMed database, focusing on research published after 2003 with keywords including eGC, permeability, glycocalyx and injuries, and glycocalyx protection.

Study selection: Several authoritative reviews and original studies were identified and reviewed to summarize the characteristics of the eGC under physiologic and pathologic conditions as well as the detection and protection of the eGC.

Results: The eGC degradation is closely associated with pathophysiologic changes such as vascular permeability, edema formation, mechanotransduction, and clotting cascade, together with neutrophil and platelet adhesion in diverse injury and disease states including inflammation (sepsis and trauma), ischemia-reperfusion injury, shock, hypervolemia, hypertension, hyperglycemia, and high Na^+ as well as diabetes and atherosclerosis. Therapeutic strategies for protecting and rebuilding the eGC should be explored through experimental test and clinical verifications.

Conclusions: Disturbance of the eGC usually occurs at early stages of various clinical pathophysiologies which can be partly prevented and reversed by protecting and restoring the eGC. The eGC seems to be a promising diagnostic biomarker and therapeutic target in clinical settings.

Keywords: Endothelial glycocalyx; Permeability; Injuries; Glycocalyx protection

Introduction

The thin endocapillary layer was first proposed in conjunction with the regulation of vascular permeability and visualized as a thin thick "endocapillary layer" in the intravascular lumen under the electron microscopy several decades ago. Due to the advancement in tissue fixation, staining and imaging techniques, there has been an increasing appreciation for the role of endothelial glycocalyx (eGC) *in vivo*. The eGC lines both fenestrated and non-fenestrated capillaries, which is sparse in sinusoidal capillaries whereas continuous in brain tight junctions, forming endothelial barrier in organs.[1] Because of its ubiquitous nature, degradation of the eGC alters the permeability of multiple capillary beds and therefore causes formation of proteinuria in the glomerulus. In humans, the thickness of the eGC can be measured non-invasively and is associated with cardiovascular risk factors, although it remains to be confirmed whether the eGC can be used as an early marker of vascular damage and organ injuries.

In this review, we explored the components of the eGC as a regulator of vascular barriers. We also summarized the emerging evidence that the eGC plays an important role in the regulation of vascular permeability, transmission of shear stress and modulation of vascular homeostasis, including leukocyte and platelet adhesion in vessels, nitric oxide (NO) production and vasodilation, erythrocyte velocity, and hematocrit. The review provides insight into the role of the eGC in pathophysiologic development of the various diseases and sheds light on the promising therapeutic strategies that protect and restore the eGC thereby reducing microvascular endothelial dysfunction and organ injuries.
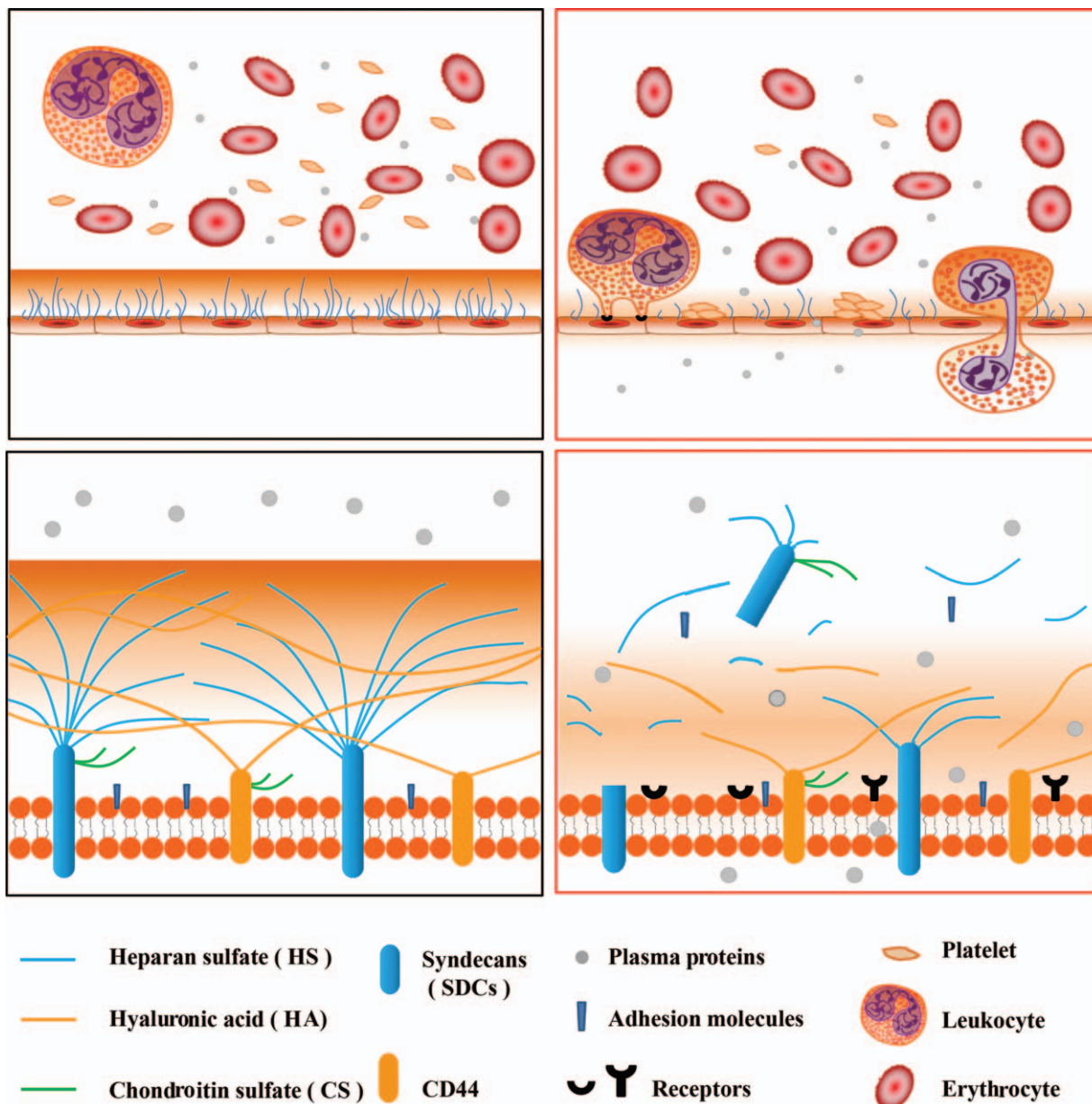
Figure 1: Schematic overall (top) and partial (bottom) view of the endothelial glycocalyx (eGC) under physiologic (left) and pathologic (right) conditions. The eGC plays a major role in endothelial barrier and regulates vascular permeability and exclusion properties, inflammation, and coagulation. The eGC degradation results in higher permeability to water and protein as well as high red blood cell accessibility, leukocyte, and platelet adhesion. In various acute and chronic clinical conditions, including inflammation (sepsis, trauma), ischemia reperfusion, shock, hypervolemia, excessive shear stress, or medical operation process, the main constituents of the eGC, including heparan sulfate (HS) bound to syndecans, and hyaluronic acid bound to, for example, CD44, are shed which are mainly regulated by glycosaminoglycan-specific enzymes heparanase, hyaluronidase, and other proteinases such as cathepsins and Matrix metalloprotease (MMPs). After degradation of the eGC, the inflammatory domains of HS, receptors and adhesion molecules on the endothelial surface are exposed, exacerbating inflammation.

Components of the endothelial glycocalyx

The eGC is a layer of villous polysacchari-protein composite structure located on the apical membrane of endothelial cells between vessel walls and plasma. It contains an array of glycoproteins, proteoglycans, and glycosaminoglycans (GAGs) and covers the luminal surface of vascular endothelium, thereby providing the basis for plasma-endothelial cell interaction [Figure 1]. Proteoglycans are core proteins that carry GAG chains. Many glycoproteins function as receptors on the cell surface, such as selectins, integrins, and members of the immunoglobulin superfamily, with sialic acid residues oligosaccharides chains attached to them. All these elements are weaved into the net of the eGC. The endothelial surface layer (ESL) is a much thicker structure, which also consists of secreted proteoglycans (eg, versican and perlecan) and their adsorbed plasma proteins (eg, orosomucoid and albumin). Together with GAGs and plasma proteins, the ESL as a whole forms a dynamic barrier to circulating cells and soluble biologic macromolecules. So far, the GAG families expressed on the surface of endothelial cells are heparan sulfate (HS), hyaluronic acid or hyaluronan (HA), and chondroitin/dermatan sulfate (CS). The most prominent members expressed on the surface of endothelial cells are HS,

accounting for 50% to 90% of the total GAGs. The core proteins of HS proteoglycans are transmembrane syndecans, membrane-anchored glypicans and the basement matrix-associated perlecans. HA binds transmembrane proteins CD44. The syndecans and CD44 participate in the organization of the cytoskeleton (attached to cortical actin framework) whereas the glypicans bind to the plasma membrane. In terms of molecular structure, there is no much difference among the healthy glycocalyx of capillaries. However, significant morphologic differences of glycocalyx exist in different organs under healthy conditions or pathologic conditions.[1]

Physiologic function

The eGC acts as a major endothelial barrier, regulating vascular permeability and proteinuria formation, shear stress-induced vasodilation in arterioles, and leukocyte and platelet adhesion [Figure 1]. The relative functions of different glycocalyx components have been uncovered in recent decades. HS proteoglycans have been shown to participate in inflammatory cationic peptide-induced signaling that links cytoskeletal reorganization with subsequent barrier dysfunction and mechanotransduction signaling that results in NO/reactive oxygen species (ROS) production, vasodilation and increased permeability.[2,3]- Transmembrane proteins CD44, binds to HA, which interacts with the cortical cytoskeleton that forms barriers to phagocytic receptor engagement.[4] There is a growing consensus that all components of the eGC synergize to maintain vascular integrity and provide organ protection.

Regulation of vascular permeability

Our understanding of the vascular permeability has improved substantially with increasing recognition of the eGC, a major barrier to water and plasma protein exchange. Selective permeability of the endothelial barrier with regard to protein and macromolecule diffusion leads to a regulation of water efflux. Accumulating evidence based on enzymatic removal of the majority of the eGC confirmed that the eGC acts as a primary vascular barrier and regulates hydrostatic and oncotic pressure gradients between the lumen of the blood vessel and the interstitial space.[2,3,5] Rehm et al[6] hypothesized "a double-barrier concept", in which both the eGC and endothelial cells contributed to an intact vascular barrier, which has been reflected in recent research.[7] The eGC plays a role in vascular barrier regulation through overlying cell-cell junction that limits water and protein flux and regulating sheer stress that alters junctional integrity.[3]

Glomerular capillary permeability is characterized by proteinuria. The integrity of the glomerular filtration barrier (GFB) prevents the leaking of albumin and other plasma proteins into the urine, thus preventing proteinuria. The GFB consists of podocytes, the glomerular basement membrane (GBM) and the highly fenestrated endothelium covered with the eGC. Mild (15 min) renal ischemic reperfusion injury induces loss of charged fibers without apparent changes in the podocytes or the GBM resulting in massive proteinuria, suggesting that subtle changes in the eGC play important role in proteinuria formation.[8] Recent

works have highlighted a cross talk between podocytes and glomerular endothelial cells (GEnCs) in the mechanism of proteinuria formation, involving in the eGC.[9,10] Vascular endothelial growth factor (VEGF), angiopoietins, endothelin-1, and transforming growth factor beta (TGF-β) are the major mediators of GEnCs and podocyte communication.[7,9-11] These mediators produced by podocyte regulate microvascular permeability by indirectly modifying of the eGC on GEnCs. GEnCs and the eGC with negative charges regulate glomerular selective permeability and proteinuria formation. Numerous sulphate residues on the GAG chains of the eGC, contribute to the polyanions of GFB, which is the structural basis of charge-dependent glomerular permselectivity.[10] The GAG-specific enzymes heparanase, hyaluronidase or chondroitinase reduce the glomerular eGC thickness, leading to reduce charge selectivity and increase macromolecular passage, resulting in proteinuria.[12] Electron microscopy reveals that long-term removal of the eGC leads to the transendothelial passage of albumin without proteinuria in almost all the glomeruli, coupled with alteration of the glomerular ultrastructure and GBM function. These results indicate that the eGC degradation most likely reflects an early time point in the development of glomerular damage and proteinuria.[13]

Transmission of shear stress

Accumulating evidence indicate that the eGC is involved in endothelial wall-mediated transmission of shear stress. However, the underlying mechanism on how the eGC mediates mechanotransduction remains unclear. Multitude possibilities exist, including cytoskeleton-associated signaling, direct intracellular signaling, and regulation of local concentration gradients as well as transportation of ions, amino acids, and growth factors. When the eGC is intact, the GAGs of glycocalyx act as stress mechanoceptor on the endothelial surface, which senses and transduces shear stress to induce NO production, an effect that depends on the $Ca2^+$ intake via endothelial Transient Receptor Potential (TRP) channels.[14] The GAGs regulate shear stress-induced NO production through ROS or superoxide dismutase (SOD).[2,3] However, Enzymatic degradation of the eGC components had no effect on shear-induced cyclooxygenase-2 (COX-2)/prostaglandin I2 (PGI2) signaling way involving PECAM-1, PI3K, FAK, and p38.[15] These results can be explained in terms of a "bumper-car" model,[16] in which two independent cellular signaling pathways are activated in response to fluid shear stress, one transmitted from focal adhesions and another involved in glycocalyx core proteins. The focal adhesions pathways regulate COX-2/PGI2 signaling. The specific connections of glycocalyx core proteins to the actin cytoskeleton (eg, syndecans) and the plasma membrane (eg, glypicans) mediate specific intracellular signaling (eg, NO production, cytoskeletal reorganization).

Modulation of vascular homeostasis

The eGC modulates vascular homeostasis through its physical barrier properties. The eGC shedding under enzymatic degradation or pathologic condition increases leukocytes and platelets adhesion.[17] A thinner eGC is associated with increased leukocyte-endothelial interactions and vascular permeability in sepsis. Matrix metalloprotease

7 (MMP7) is a proteinase that can specifically cleave syndecan-1 (SDC-1) and the numbers of platelets adherent to MMP7-treated cells were significantly increased in human umbilical vein endothelial cells (HUVECs).[18] Thrombogenicity and platelet aggregates were reduced when the eGC was restored.[19] Glycocalyx degradation is positively correlated with monocyte-endothelial cell adhesion, plasminogen activator inhibitor-1, and intercellular adhesion molecule-1 (ICAM-1) release, and negative correlated with endothelial NOS activity, which compromises the vascular homeostasis.[20] These results illustrate that the eGC plays a significant role in regulating the vascular inflammatory responses and blood clotting function, which are closely related to vascular homeostasis. Furthermore, a thicker eGC maintains a higher level of small conductance potassium channel-3 (SK3) expression which may thus explain the preservation of the endothelium-dependent hyperpolarization vasodilation.[21] Adenosine-mediated regulation of microvascular volume is associated with increased plasma supply to the eGC as a result of impaired barrier properties.[22] According to previous studies-involving the use of sidestream darkfield imaging of the sublingual microvasculature of participants, a thicker glycocalyx was associated with higher efficient perfusion.[23] Therefore, preserving the eGC might improve microcirculatory oxygen distribution.

Glycocalyx dysfunction conditions

The main constituents of the eGC are shed from the vascular endothelial surface under various acute and chronic clinical conditions [Figure 1]. Some of such conditions include sepsis, trauma, inflammation, ischemia-reperfusion injury, shock, hypervolemia, hypertension, hyperglycemia, and high Na^+ as well as diabetes and atherosclerosis.

The composition of the eGC is determined by the balance between shedding and synthesis [Figure 2]. When the eGC is exposed to enzymatic degradation or shear force, the new dynamic balance of restoring and shedding can be rebuild by self-assembly, which allow adaptation to changes in the local environment. After acute glycocalyx shedding, the eGC can restore itself to its native hydrodynamical thickness within 5 to 7 days *in vivo*.[24] In another report, HS restoration after enzymatic degradation occurred on the surface of endothelial cells for 20 h under static conditions *in vitro*.[25] The endogenous mechanisms governing the eGC reconstitution remains unclear. In contrast, Yang *et al*[26] reported that rapid glycocalyx recovery after non-septic degradation depends on the induction of fibroblast growth factor receptor 1 (FGFR1) and HS biosynthetic enzyme, exostosin 1 (EXT1), whereas the FGFR1/EXT1-mediated glycocalyx reconstitution mechanisms are impaired during sepsis. The observation that inhibition of FGFR1 signaling did not completely suppress the eGC recovery suggests the presence of other reparative pathways that merit further investigation. That is, vascular injury and organ dysfunction may occur when the balance of glycocalyx degradation and reconstitution is disturbed.

Sepsis, trauma, and inflammation

The degradation of the eGC occurs in infective and non-infective inflammation, like sepsis and trauma.[17] Tumour

necrosis factor-α (TNF-α) rapidly(within 30 min) induced the cleavage of 65-k heparanase to its active 50-K isoform and subsequent degradation of the eGC in sepsis-induced lung injury.[27] The depth of the eGC in the subpleural microvasculature, the relative densities of HS in the lung microvasculature, and perlecan in renal glomeruli were significantly reduced in sepsis.[28] TNF-α is a major proinflammatory cytokine of the inflammatory cascade, especially in the setting of sepsis. TNF-α treatment decreased glomerular fenestral density and increased fenestral diameter of glomerular capillary endothelium, as well as resulted in the degradation of GEnCs, maybe as result of high heparanase expression.[29] TNF-α may also increase MMP9 expression, which leads to the shedding of SDC-4 coupled with HS from the GEnCs.[30] Sepsis causes disturbance of the eGC and organ injury, not simply through shedding the eGC but delaying the eGC regeneration.[26] Disturbance of the eGC is closely related to the microvascular endothelial dysfunction that is characteristic of sepsis-induced acute respiratory distress syndrome (ARDS).[31]

In a prospective study, SDC-1 was found to be an independent predictor of mortality in trauma patients. High circulating SDC-1 may serve as a marker of eGC shedding and endothelial activation in trauma patients. It may also be associated with sympathoadrenal activation, inflammation, tissue injury, coagulopathy, and mortality.[32,33]

Inflammation leads to perturbation of the eGC, which in turn contributes to inflammatory response cascade.[27] Depending on its cationic charge, the leukocyte heme-enzyme myeloperoxidase (MPO), mediates neutrophil recruitment and activation, which exerts proinflammatory effects on the vascular system. A reduction in eGC thickness was reversed after removal of vessel-bound MPO, suggesting that MPO, via ionic interaction with HS side chains, can cause neutrophil-dependent SDC-1 shedding and physical collapse of the eGC structure.[34] The collapse of the eGC stimulates macrophage recruitment and macrophage phenotype alterations.[35,36] The eGC degradation under flow stimulates endothelial cells altering to proinflammatory phenotypes and increase NF-κB and ICAM-1 activity, thus promoting leukocyte adhesion and focal vascular inflammation.[37] Preserving the eGC effectively suppresses ICAM-1, VCAM-1, and E-selectin in response to TNF-a stimulation and dampens proinflammatory cytokines thus inhibiting the progress of inflammation which prevents vital organ injuries and decreases mortality in sepsis.[28]

Ischemia-reperfusion (I/R) injury, shock

Mounting evidence from isolated guinea pig heart models suggests that I/R injury causes the degradation of glycocalyx, accompanied with postischemic oxidative stress, increased release of histamine and cathepsin B, enhanced coronary perfusion pressure, as well as increased vascular permeability, transudate formation, and inflammation.[38] Oxygen-free radicals may account for damage of the eGC. Moreover, cathepsin may be involved in heparanase activation during enzymatic degradation of glycocalyx. In rats and mice kidney, I/R injury causes a significant alteration to the structure and function of
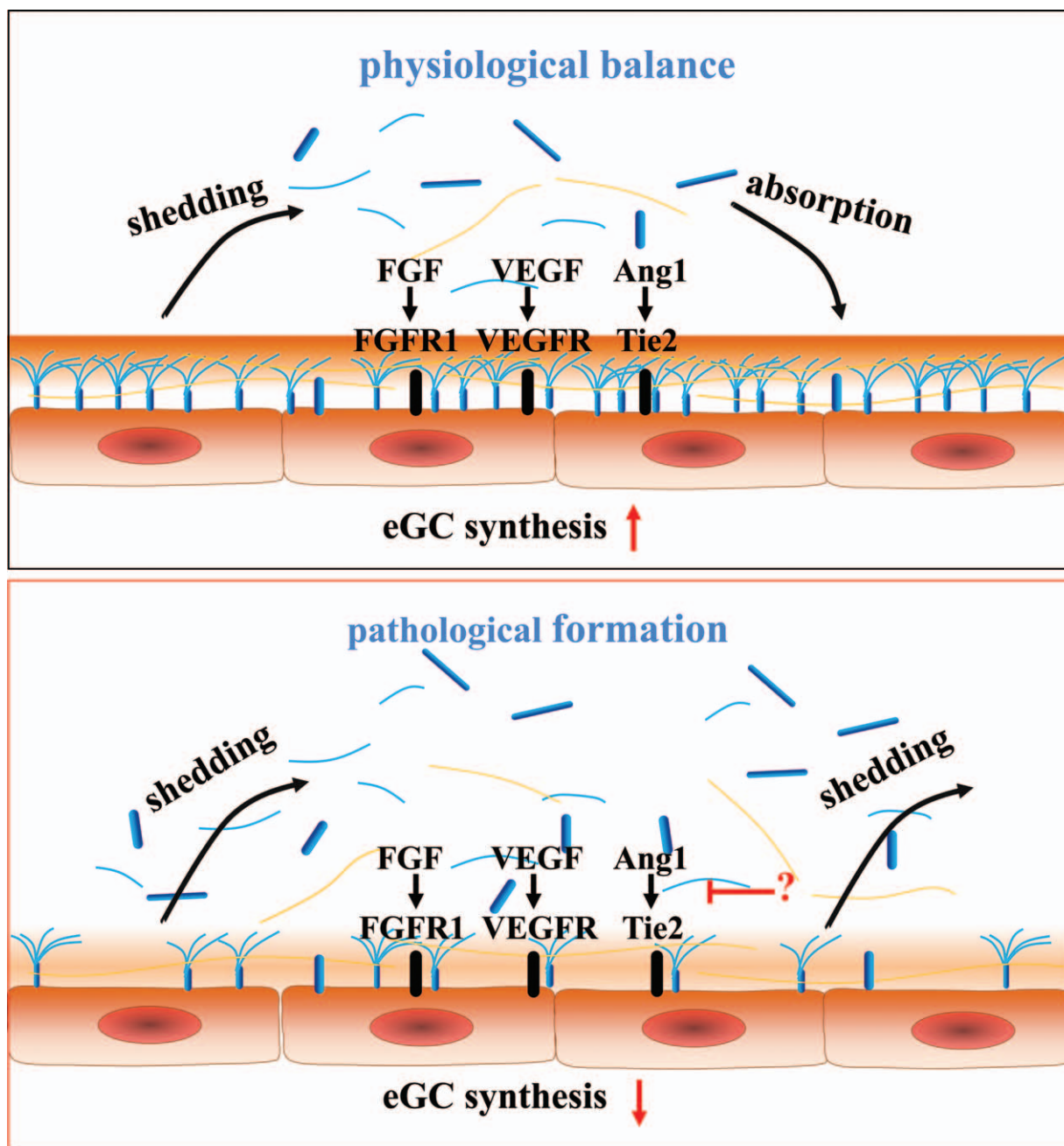
Figure 2: The structural dynamics and pathologic formation of the endothelial glycocalyx (eGC). The self-regulatory mechanism of the eGC under physiologic balance (top) and pathologic formation (bottom). The eGC composition is the result of a balance between shedding of its components after pathologic conditions, the adsorption of components from circulating blood, and synthesis of the eGC. Under pathologic conditions, the eGC integrity is damaged through the shedding of one or more of its components (eg, heparan sulfate, syndecan-1, or hyaluronic acid) into the blood and the self-synthesis mechanism are inhibited, such as sepsis-induced the impairment of the fibroblast growth factor receptor 1/exostosin 1 mechanisms that mediate eGC restoration. The disturbance of the structural dynamics balance results in pathologic alterations.

glomeruli and tubular segments, in which heparanase plays an important deleterious role.[39] It takes only 15 min to impair the glomerular barrier, mainly the eGC.[8] In patients undergoing surgery of the ascending aorta with cardiopulmonary bypass or surgery for infrarenal aortic aneurysm, components of glycocalyx, SDC-1, and HS, are released into the plasma, causing 42- and 10-fold increase in global ischemia with circulatory arrest, 65- and 19-fold increase during regional ischemia of heart and lungs after cardiopulmonary bypass, and 15- and 3-fold increase after infrarenal ischemia.[40]

Acute changes in the vascular microenvironment can trigger the eGC shedding. Accumulating evidence from *in vitro* and *in vivo* indicates that hemorrhagic shock induces the eGC shedding and endothelial injury, accompanied with disrupted junctional integrity.[41,42] It has been demonstrated in patients that a significant decrease in glycocalyx thickness after hemorrhagic shock in trauma and ST-elevation myocardial infarction complicated cardiogenic shock, positively correlated with worse local blood flow microcirculatory density reduction and coagulation.[43,44]

Hypervolemia, hypertension, and high Na+

Hypervolemia and hypertension can induce glycocalyx shedding. Some studies indicate that sufficient hypervolemia-induced release of atrial natriuretic peptides (ANPs) may cause shedding of the eGC layer. The effects of hypervolemia on the ANP-glycocalyx axis remain to be determined. However, there is consensus that ANP concentrations increases in patients undergoing on- and off-pump coronary artery bypass surgery, preceding the shedding of glycocalyx. It is speculated that ANP may lead to perturbation of the eGC in coronary artery bypass surgery.[45] Natriuretic peptide at physiologically relevant concentrations could shed the eGC, increase transudate formation, and extravasation of colloid.[46]

In spontaneously hypertensive and stroke-prone spontaneously hypertensive rats, the deterioration of the eGC occurred at the capillaries; however, it was preserved in the arterioles leading to increased vascular permeability. These findings reveal early changes in the blood-brain barrier precede chronic hypertension.[47] Chronic high Na+-induced the deterioration of the eGC results in breakdown of its barrier function. This may partly account for the eGC deterioration in early stage of salt-sensitive hypertension and the relative cardiovascular diseases. Chronic high Na+ (24 h), as a risk factor for cardiovascular pathologies and inflammation, has recently been demonstrated to increase the shedding of the eGC and release of inflammatory cytokines and adhesion monocytes. In contrast, acute Na+ excess (30 min) did not damage the eGC *in vivo* and *in vitro*.[48] However, a study suggested that acute Na+ excess (60 or 120 min) would cause the eGC degradation further exacerbating shock conditions.[49] It has been demonstrated, in most recent research, that salt and aldosterone induce albuminuria via MMPs dependent damage of the glomerular eGC.[50]

Hyperglycemia and diabetes

Acute hyperglycemia increases glycocalyx degradation and vascular barrier derangements.[51,52] It was demonstrated in 10 healthy volunteers that acute hyperglycemia reduced glycocalyx volume, resulting in increased coagulation. Glycocalyx perturbation was triggered by oxygen radicals, which play an important role in vascular dysfunction during hyperglycemia. Recent research using GEnCs model found that high glucose decreased transendothelial electrical resistance (TEER) and increased albumin flux, all of which impaired the integrity of hyperglycemic GEnCs monolayers.[53] These results imply that compromised GEnC and glycocalyx may precipitate acute hyperglycemia or even diabetes microvascular complications.

Type 1 diabetic patients are characterized by systemic and microvascular glycocalyx damage, the severity of which is increased in presence of microalbuminuria, suggesting that dysfunction of the eGC contributes to kidney injury and may lead to cardiovascular disease or albuminuria.[54] Inhibiting the enzymatic degradation of the eGC or restoring the eGC will reduce albuminuria in diabetic nephropathy.[36] Endothelin-1 increased heparanase expression in podocytes and damaged the eGC *in vivo* and *in vitro*, but

had no effect on cultured mouse GEnCs. This indicates a podocyte-endothelial cross talk in diabetic nephropathy.[10] Recent work in rats, HUVEC and human glomerular microvascular endothelial cells, and human diabetic kidney model, demonstrated that high glucose and diabetes reduced Klf2 expression and contributed to the pathogenesis of diabetic nephropathy, which were associated with increased VEGFA, Flk1, and Ang-2 but reduced Flt1, Ang-1, Tie-2, eNOS, ZO-1, and glycocalyx. These endothelial markers might be regulated by Klf2.[55] The underlying mechanism remains to be determined. It has been demonstrated that VEGF-A165b upregulation in mouse podocytes restores the glomerular eGC through activation of VEGF receptor 2 in GEnCs, which reduces apoptosis and decreases glomerular permeability in early or advanced diabetic nephropathy.[11]

Atherosclerosis

Glycocalyx degradation and the associated endothelial dysfunction can perturb vascular homeostasis, causing arterial wall damage and contribute to early stages of atherosclerosis.[56] Glycocalyx degradation under sheer stress promotes leukocyte adhesion to an inflamed endothelium and is a defining feature of atherosclerosis.[37] Ox-LDL, a risk factor for early stages of atherosclerosis, decreases the amount of HS proteoglycans, and stimulates immobilization of leukocytes at the endothelial surface.[57] In ApoE knockout mice, a thin eGC of large arteries and capillaries increased the thrombotic response and macrophage recruitment.[35] These results suggest that the eGC plays an important role in pathophysiologic development of atherosclerosis.

Detection of the eGC and clinical significance

Accurate assessment of the structural organization of the normal or damaged eGC is challenging due to the highly fragile and unstable nature of the eGC. A wide range of dimensions of the eGC are obtained with different labels, imaging techniques [Table 1]. Furthermore, the thickness of the eGC varies with species and types of the vessel, *in vivo* and *in vitro* as well as cell culture conditions.

Biomarkers produced by the eGC shedding may be used as monitoring and diagnostic tools. During the initial onset of pathologic formation, the eGC is damaged, and circulating levels of the eGC components including HS, SDCs, and HA can be measured and are reportedly useful as biomarkers for diseases. Early indices of urinary GAG fragmentation can predict acute kidney injury and in-hospital mortality in patients with septic shock or ARDS, suggesting correlations between the severity of disease and glycocalyx integrity.[58] Patients undergoing dialysis have an impaired glycocalyx barrier, which was confirmed by presence of glycocalyx fragments in dialysis patients plasma.[59] Johansson *et al*[32,60] conducted two separate prospective observational studies to explore the shedding of glycocalyx major proteins. They found that SDC-1 is significantly associated with inflammation, coagulopathy, sympathoadrenal activation, and increased mortality in trauma patients. Furthermore, circulating SDC-1 can be used to identify trauma patients with endothelial dysfunction and may serve as an independent predictor of mortality[33] and

Table 1: Common detection methods of the endothelial glycocalyx (eGC).

Technique	Methods	Physiologic/ pathologic glycocalyx	Model	References
Transmission electron microscopy (TEM)	Fixed with glutaraldehyde, stained with lanthanum nitrate	200 nm/0 nm	I/R in isolated guinea pig hearts	[70]
	Fixed in glutaraldehyde stained with uranyl acetate and lead citrate solution	30 nm/10 nm	Diabetic nephropathy in mice	[11]
	Labeled with cationic ferritin	80%/40%	Diabetic nephropathy in ApoE KO mice	[36]
	Stained with Alcian Blue, fixed in osmium tetroxide and lanthanum nitrate	50 nm/50 nm	Myocardial arterioles in diabetic mice	[21]
Confocal microscopy	Stained with R18 (cell membrane) and fluorescent-labeled wheat germ agglutinin lectin, WGA (glycocalyx)	200 nm/20 nm	Diabetic nephropathy in rat	[11]
	Stained with CD31 (endothelial cells) and fluorescent-labeled lectin *Lycopersicon esculentum*, LEA (luminal glycocalyx)	1.5 μm/0.7 μm	Diabetic nephropathy in ApoE KO mice	[36]
	Stained with CD31 and LEA or *Bandeiraea simplicifolia* (BSI) (eGC); WGA (podocyte-specific glycocalyx)	0.4–1.0 μm/0.2–0.6 μm	Hyaluronidase treatment on the kidney of mice	[13]
Intravital microscopy	Measuring ESL exclusion of 150 K FITC-dextran from the vessel surface	1.5–1.7 μm /0.2–0.5 μm	Acute lung injury in septic mice	[26]
	Stained with FITC-HRP-WGA	1.07 μm/0.36 μm	Skin arterioles and venules in septic mice	[17]
	Subtracting the width of the red blood cell track from the capillary diameter	1.5 μm/0.8 μm	Acute lung injury in septic mice	[28]
Sidestream darkfield imaging	Perfused boundary region (PBR) ($\neq$glycocalyx thickness): measuring penetration of erythrocytes into glycocalyx	3.3 μm/3.6 μm	The sublingual microcirculation in dialysis patients	[59]
	PBR	2.09–2.18 μm	The sublingual microvasculature in participants	[23]
Atomic force microscopy (AFM)	A triangular cantilever with a mounted spherical tip periodically indents the cells	200 nm/100 nm	Living endothelial cells	[7]
Differences between non-circulating and circulating volume	Urinary ratio of 70- to 40-K dextran	0.3/0.4	Diabetic mice	[21]
Estimates of glycocalyx components	Plasma SDC1 concentrations measured by ELISA	90/16.5 ng/mL	Patients with postoperative sepsis	[61]

ESL: Endothelial surface layer; SDC1: Syndecan 1.

potential biomarker for sepsis and survival after abdominal surgery.[61] The level of eGC thickness and fragments in patients plasma can be used as auxiliary diagnostic indices to assess the severity of coronary atherosclerosis.[62] In transplantation, the eGC fragments may be useful biomarkers to predict organ acceptability and development of transplant outcomes. Therefore, interventions to protect the eGC may improve transplantation outcomes.[63] A new technique using side-stream dark-field imaging is now clinically available for assessing the eGC. This may be a promising method of monitoring microvascular perfusion and oxygen uptake of vital organs as well as guiding infusion solutions volume.[64,65]

Therapeutic strategies targeting the eGC

Fluid management (fresh frozen plasma, plasma albumin, and hydroxyethyl starch)

Perioperative fluid management still remains controversial as a method to optimize volume status and type of fluid to administer. Adequate fluid resuscitation in hypotension and the harmful consequences of fluid overload should be considered.[66] This may avoid glycocalyx shedding induced by hypotension and hypervolemia, as well as interstitial edema resulting in tissue hypoperfusion. When choosing the type of fluid, glycocalyx should also be taken into account to prevent the fluid from penetrating into interstitial space. When an isolated perfused heart of guinea pig was subjected to ischemia, a transient increase in vascular leak occurred with hydroxyethyl starch (HES) and saline, but not with albumin. Electron microscopy examination revealed an intact eGC without interstitial edema in the albumin group.[67] After hemorrhage and resuscitation with different fluids, a series of quantitative relationship frameworks are evaluated with measurements of microvascular diameter, blood flow, vascular permeability, glycocalyx thickness, and plasma levels of glycocalyx shedding biomarkers, coupled with evaluation of systemic parameters. Thus monitoring plasma SDC-1 or HS as biomarkers of glycocalyx shedding guides resuscitation strategies following hemorrhage. The relationship framework showed that blood and plasma, but not colloid resuscitation or crystalloid resuscitation, support vascular stabilization via restoring the eGC after hemorrhage.[41] Crystalloid resuscitation can prolong clotting time and lower platelet counts, which is responsible for hemodilution. Otherwise, plasma based resuscitation preserves the eGC and maintains endothelial integrity, improved blood flow and coagulation.[68] Hemorrhagic shock sheds the eGC and disrupts junctional integrity, which were improves with fresh frozen plasma but not lactated ringers, and are in agreement with the quantitative relationship frameworks.[69] These results demonstrate that assessment of glcocalyx might be incorporated in guiding fluid management.

Anesthetics (sevoflurane)

It has been proved that inhalation of anesthetic sevoflurane attenuates glycocalyx release into the coronary effluent of isolated guinea pig heart in myocardial I/R injury model. Electron microscopy revealed a massive destruction of the eGC without sevoflurane and an almost intact glycocalyx with 2% sevoflurane either for 15 min pre-, post-ischemia, or consecutive.[38] Sevoflurane reduces leukocyte and platelet adhesion induced by I/R which protects the eGC in isolated guinea pig heart.[70] Sevoflurane protects the endothelium from I/R injury *in vivo*; however, anions release and HS shedding increase significantly over time after I/R injury in propofol-anesthetized pigs.[71] Furthermore, a propofol overdose significantly reduced the eGC in vital organs of mice and human microvascular endothelial cells with systemic hyperpermeability, which is associated with the reduction of ATP production. More experiments need to compare the effect of different anesthetic on the eGC and organ injuries so that proper anesthesia managements are applied during surgery operations.[72]

Glucocorticoid (hydrocortisone and dexamethasone)

Chappell *et al*[73] demonstrated that hydrocortisone protects the eGC, thus maintaining the vascular barrier and reducing interstitial edema in I/R injury in isolated guinea pig heart. Hydrocortisone treatment reduced degradation of glycocalyx, increased the 7-day survival rate, improved neurologic outcome after cardiac arrest and cardiopulmonary resuscitation in rats.[74] TNF-α increased rapidly coronary resistance, vascular permeability, tissue edema, the release of lactate, uric acid, purines, and histamine which were accompanied with severe degradation of the eGC, and these effects were inhibited by hydrocortisone treatment.[75] Preconditioning with hydrocortisone preserved the eGC and mitigated postischemic polymorphonuclear neutrophils (PMN) adhesion, thereby alleviating vascular leakage, tissue edema, and inflammation.[76] Dexamethasone suppressed the expression of MMPs and rescued the expression of ZO-1 and syndecan-1 in aortic of septic rats, suggesting that dexamethasone may prevent endothelial perturbation and glycocalyx shedding in sepsis by inhibiting MMPs.[77]

Anticoagulant drugs (antithrombin, heparin, and heparinoids)

It has been demonstrated that antithrombin promotes the endothelial release of PGI2 by interacting with the cell surface heparin-like GAGs, which prevents leukocyte activation by inhibiting TNF-α/NF-κB activation in kidney I/R injury.[78] The I/R-induced release of SDC-1 and HS in isolated guinea pig heart was decreased to basal levels and electron microscopic examination revealed an intact glycocalyx following pre-treatment with antithrombin. Moreover, immunohistologic stainings indicated that antithrombin is located both on and within the eGC, suggesting that antithrombin significantly reduces glycocalyx shedding and tissue edema. Additional application of colloid (6% HES) augmented these effects of antithrombin.[79] Schmidt *et al*[27] demonstrated in sepsis that heparin or non-anticoagulant heparanase inhibitor N-desulfated/re-N-acetylated heparin (NAH) maintain the eGC thickness and the inhibition of neutrophil adherence, inflammation, and acute lung injury. Unfractionated heparin reduced sepsis-induced increase in the shedding of SDC-1 and HS in beagle dogs, which correlated with IL-6 and TNF-α.[42] Blocking of inflammatory N- and 6-O-sulfated HS domains on endothelium with heparinoids (tinzaparin and enoxaparin) or specific anti-HS antibodies significantly reduces the number of rolling and adherant leukocytes under dynamic flow conditions in mice kidney and human GEnCs.[80] These results suggest that heparin and heparinoids may protect the eGC by interacting with N- and 6-O-sulfated HS domains independent of anticoagulant effect.

Supply of the eGC components and inhibition of relevant enzyme

As previously mentioned, the structural imperfection of the eGC is the pathophysiologic basis of various diseases. Cells that are deficient in HS or with low HA synthesis are significantly more sensitive to histone than the normal cells. Plasma inter-α inhibitor protein (IAIP) and HA,

neutralize the cytotoxic effects of extracellular histones, partly maybe through restoration of the eGC, and these effects are attributed to the negatively charged GAGs CS and high molecular-weight HA moieties of IAIP complex.[81] Enzymatic removal of HS not only alters the organization of gap junction proteins, but also closes inter-endothelial gap junction channel activity. After 24 h, exogenous HS alone, sphingosine-1-phosphate (S1P) alone, or HS together with S1P led to HS regeneration; however, only exogenous or self-recovery of HS can restore gap junction proteins and activity *in vitro*.[82]

MMPs and endogenous HS-specific heparanase are important enzymes that degrade the eGC. *In vitro*, S1P inhibits the MMP activity by activating S1P1 receptor which restores the eGC through the PI3K pathway.[83,84] S1P inhibits MMP7-induced SDC-1 shedding and S1P-mediated upregulation of SDC-1 reduces platelet adherence.[18,85] Application of MMPs inhibitor (ortho-phenanthroline) inhibited glycocalyx shedding, but increased transudate flow in isolated guinea pig heart in the presence of ANP. This seemingly discordant result may be explained by the fact that MMPs somehow attenuate ANP-induced loosening of endothelial cell cohesion.[46] Based on this point, non-specific inhibitor of MMPs is inappropriate for humans. In another research, pretreatment with MMP9 inhibitor (batimastat) in GEnCs or specific MMP9 knockdown settings prevented the shedding of SDC-4 and HS in response to TNF-α.[30] In most recent research, specific gelatinase inhibitor targeting MMP2 and 9 preserved the eGC, blocked the rise in glomerular sieving coefficient, and prevented albuminuria.[50] These effects deserve more cell and animal experiments in-depth study.

Heparanase, *n*-specific enzyme that cleaves HS, is involved in the loss of glycocalyx structural integrity and pathologic changes. However, it remains to be determined whether heparanase has the protective or deleterious effect *in vivo*. HS degradation arises from post-translational heparanase activation (ie, cleavage of 65-k proenzyme to 50-K active form). Heparanase is activated in I/R injury. Renal I/R injury induced significant deteriorative renal injury was more prominent in the heparanase-overexpressing mice. Upregulation of endogenous heparanase along with overexpression of proinflammatory and profibrotic cytokines in I/R injury, which was attenuated by heparanase inhibitor (PG545).[39] Furthermore, hyaluronidase 1 deficiency prevents glycocalyx HA shedding during diabetes and affords protection against diabetes-induced glomerular barrier dysfunction.[21] Glycocalyx-specific enzymatic inhibitors remain to be explored as the new therapeutic strategies to protect vital organs.

Diabetes management (sulodexide, atrasentan, and metformin)

The effects of sulodexide on protecting the eGC may be due to having similar GAG structure to glycocalyx. In patients with type 2 diabetes, sulodexide administration before and after 2 months increased both the sublingual and retinal glycocalyx dimensions and decreased the transcapillary escape rate of albumin and plasma hyaluronidase in patients with diabetes.[86] Recent work in balloon-injury

rat carotid artery model showed that sulodexide reconstructed the eGC and recovered the normal cytoarchitecture, attenuating the inflammatory expression, blood coagulation, and lipid metabolism.[87] Atrasentan is an antagonist of endothelin-1. Endothelin-1 releases due to endothelial activation and induces heparanase expression in podocytes, which damages the endothelium and the eGC, resulting in proteinuria and renal failure.[10] Atrasentan reduces proteinuria by protecting the glomerular eGC, and this may be the mechanism by which atrasentan reduces the expression of glomerular heparanase and cathepsin-L.[36] Two weeks treatment of metformin significantly recovered the eGC barrier, without changing blood glucose levels. Cardiovascular benefits of metformin in diabetes may account for the reconstruction of the eGC.[88] The mechanism for this side effect of metformin independent of blood glucose levels remains unclear and need to be explored in further experiments.

Accelerating angiogenesis (FGFR1, VEGFR, and Tie2)

It has been demonstrated in lung that the eGC self-recovery depends on reparative pulmonary endothelial FGFR1 induction *in vivo* and *in vitro*.[26] The studies on GEnCs has shown that both VEGFA and VEGFC increase HA synthesis, VEGFC metabolizes more highly charged GAGs and VEGFA induces the shedding of charged GAGs.[89] VEGF-A165b restores the glomerular eGC, in addition to reducing glomerular permeability and apoptosis by activating VEGF receptor 2 in GEnCs.[11] ABTAA (Ang-2-blocking and Tie2-activating antibody), like Ang1, triggers long-term Tie2 activation, which suppresses heparanase and preserves the eGC, thus enhancing vascular integrity in vital organs by reducing sepsis-induced inflammation to gain long term survival in sepsis.[28] Ang-2 is a negative regulator of the eGC that depend on Tie2, leading to increased permeability and edema formation *in vivo*.[7] Upregulation of Ang-2 levels may partly account for blood-brain barrier disruption in patients suffering from stroke. Increased permeability and stroke size were rescued by restoration of Tie2 signaling.[90] In conclusion, it is promising to restore the eGC and microvascular barrier by accelerating angiogenesis.

Nanomaterials and glycocalyx-mimetic biomaterials

Nanotechnology and biomaterials engineering have been recently applied to protect glycocalyx in experimental models. Degradation of the eGC promoted interactions of the nanoparticles with microvascular endothelial cells under pathologic condition and targeted delivery of the nanoparticles to the site of injury.[91] Therefore, nanomaterials have high diagnostic and therapeutic potential. Based on *in vitro* and *in vivo* experiments, a one-step technique to coat endothelial linings with corline heparin conjugate, a unique structure resembling that of a proteoglycan, protect the vasculature in thrombotic disorders and in organ transplantation.[92] HS analogs and cationic copolymer have been shown to prevent acute lung injury by interacting with glycocalyx in animal experiments.[93] Carbohydrate-modified surfactant polymers are essential for application in biomedicine as a glycocalyx-mimetic biomaterials.[94]

Chinese herbs

Neferine (Nef), a bisbenzylisoquinoline alkaloid and Berberine (BBR), an isoquinoline alkaloid, both extracted from different Chinese herbs, have multiple pharmacologic activities. Nef has been shown to exert protective effects in several diseases by modulating signal transduction, cell proliferation, cell apoptosis, and cell autophagy.[95,96] BBR exerts therapeutic effects on sepsis, diabetes mellitus, atherosclerosis, and myocardial infarction by regulating in signaling pathways, anti-inflammatory, anti-oxidation, and anti-apoptosis processes.[97,98] As referred before, the role of the eGC in these diseases has generated increasing attention. In recent reports, it has been proved that Nef and BBR can alleviate the eGC degradation and promote the eGC restoration, which may be due to inhibiting the factors that damage the eGC, including ROS, MMP9, and heparanase in sepsis-induced ARDS.[99,100] The therapeutic effects of these Chinese herbs, such as whether they involve changes in the eGC, remain to be further studied. This provides a new research direction on therapeutic strategies targeting the eGC.

Conclusions and perspectives

In recent decades, it has been shown that glycocalyx plays a major role as a microvascular endothelial barrier that protect against organ injuries including myocardial ischemia, lung injury, brain edema, and kidney diseases.[74,79,101-103] Structural alterations in the eGC lead to multiple pathophysiologic changes such as increased vascular permeability (interstitial edema and proteinuria), attenuated vascular responses to shear stress, platelet and leukocyte adhesion, generation of a prothrombotic environment, and altered microvascular rheology. Anti-inflammation, volume restriction, effective circulating oxygen supply, lung protective mechanical ventilation, and perioperative hemodynamics stability should be considered to reduce the factors that damage the eGC such as TNF-α, ANP, hypoxia, and sheer stress during perioperative surgery. Intraoperative glycocalyx thickness monitoring using sidestream dark-field imaging may be a promising non-invasive technique to monitor microvascular perfusion and oxygen uptake of vital organs and guide infusion solutions volume. Monitoring and protecting the eGC in patients, especially those complicated with chronic disease, may lower cardiovascular comorbidities risk and improve the surgical outcome. Current pharmacologic therapies aimed at protecting (underlying the mechanism of inhibiting multiple adverse factors and enzymatic attacks) and restoring (underlying the mechanism of reassembling components and accelerating angiogenesis) of glycocalyx, have only been tested in animals with the aim of protecting organs and improve outcome via reducing vascular responses. But the effectiveness of such strategies remain to be determined in clinical experiments while accounting for unpredictable compensatory responses. Glycocalyx, also expressed on the surface of circulating tumor cells (CTCs) which foster cancer metastasis via adhering to the luminal surface of microvasculature, physically enhances the availability of CTC-receptors interaction with ligands sheltered by the vascular eGC.[104] The tumor cell glycocalyx can also regulate the ability of therapeutic ligands to bind to CTCs in the bloodstream. In future, therapeutic strategies could be explored that focus on protecting the vascular eGC and disrupting the glycocalyx on CTCs to prevent tumor metastasis following oncologic surgery and improve the effectiveness of cancer drugs.

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Conflicts of interest

None.

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