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The Endothelial Glycocalyx as a Double-Edged Sword in Microvascular Homeostasis and Pathogenesis

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Expressed on the endothelial cell (EC) surface of blood vessels, the glycocalyx (GCX), a mixture of carbohydrates attached to proteins, regulates the access of cells and molecules in the blood to the endothelium. Besides protecting endothelial barrier integrity, the dynamic microstructure of the GCX confers remarkable functions including mechanotransduction and control of vascular tone. Recently, a novel perspective has emerged supporting the pleiotropic roles of the endothelial GCX (eGCX) in cardiovascular health and disease. Because eGCX degradation occurs in certain pathological states, the circulating levels of eGCX degradation products have been recognized to have diagnostic or prognostic values. Beyond their biomarker roles, certain eGCX fragments serve as pathogenic factors in disease progression. Pharmacological interventions that attenuate eGCX degradation or restore its integrity have been sought. This review provides our current understanding of eGCX structure and function across the microvasculature in different organs. We also discuss disease or injury states, such as infection, sepsis and trauma, where eGCX dysfunction contributes to severe inflammatory vasculopathy.

Keywords: inflammation, microvascular homeostasis, permeability, endothelium, glycocalyx

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INTRODUCTION

The vascular endothelial surface is coated by the GCX matrix that confers important functions in circulatory homeostasis (Weinbaum et al., 2007). The endothelial GCX (eGCX), first visualized in the late 1960s after the invention of transmission electron microscope (Luft, 1966), is mainly formed by proteoglycans and glycoproteins, core proteins anchored to the EC membrane that serve as a foundation for the rest of the glycocalyx constituents. Proteoglycans, principally syndecans and glypicans, are decorated by glycosaminoglycan (GAG) chains such as heparan sulfate and chondroitin sulfate (Li et al., 2012). GAGs are characterized by long linear polysaccharides of repeating disaccharide units with a hexosamine and either an uronic acid or a galactose

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Abbreviations: BBB, blood–brain barrier; DAMP, danger-associated molecular pattern; EC, endothelial cell; eNOS, endothelial nitric oxide synthase; eSOD, endothelial superoxide dismutase; GAG, glycosaminoglycan; eGCX, endothelial glycocalyx; LPS, lipopolysaccharide; NO, nitric oxide; ROS, reactive oxygen species; SAE, sepsis–associated encephalopathy; TRPs, transient receptor potential channels.

(Esko et al., 2009). The amount of GAG chains, length and molecular modifications by sulfation and/or (de)acetylation provide the eGCX an extensive source of structural rearrangements. Notably, heparan sulfate proteoglycans are the most prominent members expressed on the surface of the endothelial cells, accounting for 50–90% of the total endothelial proteoglycans (Ihrcke et al., 1993). The majority of the interactions between syndecans and extracellular matrix molecules, growth factors and cell adhesion molecules seem to be mediated by their heparan sulfate chains through electrostatic interaction (Bernfield et al., 1992; Stringer and Gallagher, 1997). Unlike other eGCX constituents, hyaluronic acid is a linear, non-sulfated GAG that interacts with the cell surface receptor CD44, a glycoprotein (Aruffo et al., 1990). The glycoproteins are highly branched short carbohydrate chains (2–15 sugar residues) capped with sialic acid or a fucose, which mainly function as either endothelial adhesion molecules or components of the coagulation system (e.g., selectins, immunoglobulins, and integrins) (**Figure 1**). Further detailed structure and specific components of the eGCX are reviewed elsewhere (Pries and Kuebler, 2006; Tarbell and Pahakis, 2006; Reitsma et al., 2007; Weinbaum et al., 2007; Esko et al., 2009). It is worth noting that the eGCX composition is subject to a highly dynamic regulation and constant replacement or re-arrangement of molecules, ranging from enzymatic degradation ("shedding") to *de novo* biosynthesis of new molecules and to recruitment of circulating molecules from the blood.

In the following sections, we will focus our discussion on the eGCX as an active component of the EC barrier, its functions, and structural variations within the vascular tree and across organs. Furthermore, we will also summarize the new findings from eGCX research with respect to how eGCX degradation leads to certain vascular pathologies.

The eGCX: An Active Layer Without a Passive Role

The eGCX matrix is an integral component of the vascular wall. Apart from being a physical barrier, the eGCX also plays an effective role in modulating vascular homeostasis. Historically, the eGCX was considered to function as an additional physical barrier between the vessel lumen and the EC membrane (Curry and Adamson, 2012); however, solid experimental evidence has shown an important physiological role for the eGCX in performing a variety of microvascular functions such as regulating vascular permeability, mechanotransduction and leukocyte transmigration (Ihrcke et al., 1993; Davies, 1995; Baldwin and Thurston, 2001; Constantinescu et al., 2003; Curry, 2005; Tarbell and Ebong, 2008; Lopez-Quintero et al., 2009; Lennon and Singleton, 2011; Curry and Adamson, 2012).

The eGCX is one of the major determinants in maintaining endothelial barrier function by acting as an additional molecular filter for the endothelium. The eGCX modulates vascular permeability and hydraulic conductivity by limiting the flux of water and macromolecules (Curry and Michel, 1980; Adamson, 1990; Curry, 2005; Lennon and Singleton, 2011; Curry and Adamson, 2012). It also acts as a vascular barrier through modulation of molecular binding to the EC surface due to the high density of anionic charges on its GAGs side chains. The net negative charge of the eGCX carried by sulfate residues along the GAG chains favors the docking (adsorption) of positively charged molecules (Schnitzer, 1988; Lieleg et al., 2009). Thus, the eGCX regulates vascular permeability by restricting circulating molecules from strongly attaching to the endothelium based on their net charge. Importantly, the molecular size (70–kDa cutoff) is also relevant in determining the penetration of molecules into the eGCX layer, as much as chemical binding (Henry and Duling, 1999; Vink and Duling, 2000; Curry and Adamson, 2012).

Previous studies using perfusion models or intravital microscopy techniques found that eGCX damage by heparinase causes microvascular leakage (Rehm et al., 2004; Jacob et al., 2006). Similar results were found using genetic knock down of a specific eGCX component (Voyvodic et al., 2014). In this regard, increased hydraulic conductivity (*Lp*) of microvessels after removal of the eGCX or plasma proteins has also been shown (Huxley and Curry, 1985; Adamson and Clough, 1992; Weinbaum et al., 2007).

The eGCX plays a pivotal role in mechanotransduction together with other sensors in the endothelium, including G–protein–coupled receptors (Zou et al., 2004; Mederos y Schnitzler et al., 2008), Piezo and transient receptor potential (TRP) channels (Martinac, 2004; Coste et al., 2010; Dragovich et al., 2016), caveolar structures (Rizzo et al., 1998), and integrins and focal adhesions (Ringer et al., 2017). Blood flow exerts mechanical tangential forces to the endothelial surface such as shear stress, which is sensed by the eGCX and triggers the production of nitric oxide (NO), an important modulator of vascular tone (Davies, 1995; Dimmeler et al., 1999; Tarbell and Ebong, 2008; Fu and Tarbell, 2013; Zeng et al., 2018). The ability of the eGCX to reorganize the actin cytoskeleton under shear forces has been demonstrated in studies using EC monolayers as well as *in vivo* approaches. The eGCX core protein syndecan-1 interacts with cytoskeletal proteins through a highly conserved tyrosine residue in the syndecan family (Carey et al., 1996). Also, syndecan-4 acts synergistically with integrins to assemble and rearrange actin stress fibers to orchestrate cell adhesion and focal contact formation (Echtermeyer et al., 1999; Bass et al., 2007; Multhaupt et al., 2009). Interestingly, while syndecans are the main effector in cell adhesion or shape changes via their interaction with the cytoskeleton, glypicans mediate flow–induced endothelial NO synthase (eNOS) activation, based on their location at the endothelial membrane microdomains where caveolae reside (Ebong et al., 2014; Zeng and Liu, 2016; Bartosch et al., 2017). Prior studies with cultured ECs have shown that breakdown of heparan sulfate alters shear stress and impairs NO production (Florian et al., 2003); similar responses were also observed *in vivo* on canine femoral and rabbit mesenteric arteries, where infusion of hyaluronidase (to degrade hyaluronic acid GAGs) or neuraminidase (to remove sialic acid residues), respectively, reduced flow–dependent vasodilation, which is mediated by NO release (as in the majority of vascular beds) (Pohl et al., 1991; Mochizuki et al., 2003).

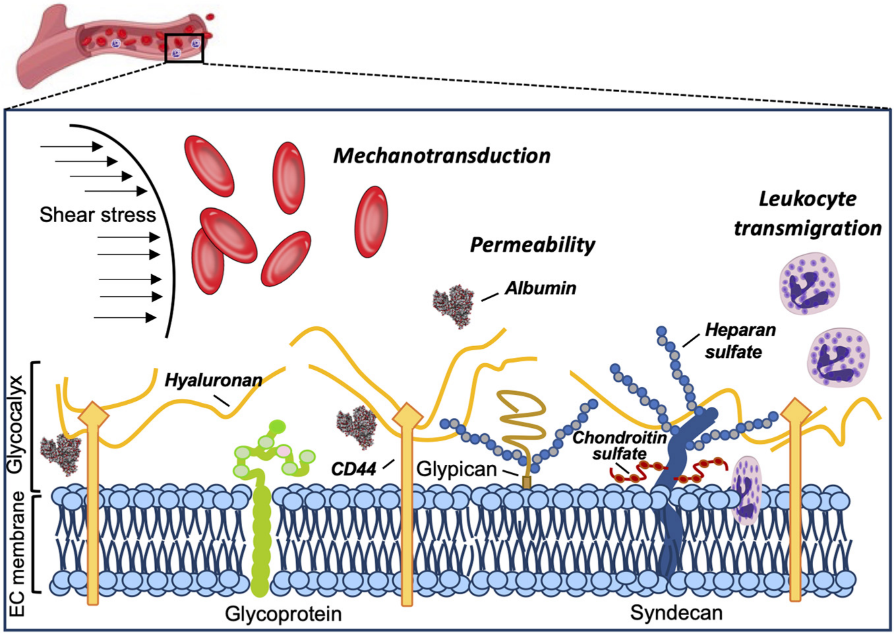

FIGURE 1 | Structure and functions of the eGCX. Schematic representation of the main components and functions of the endothelial glycocalyx. The eGCX is composed of proteoglycans, with long glycosaminoglycan side-chains (GAG-chain) and glycoproteins, with short branched carbohydrate side-chains. The eGCX modulates coagulation, inflammation and mechanotransduction processes.

Additionally, the eGCX also controls the interaction between the endothelium and circulating cells by preventing the latter from approaching the endothelium under basal conditions. Upon inflammatory stimulation, the glycans are shed from the EC surface allowing slow rolling and adhesion of leukocytes (Constantinescu et al., 2003; Lipowsky et al., 2011). Similarly, breakdown of the eGCX increases platelet–vessel wall interactions, further demonstrating an anti-coagulant effect by the eGCX layer (Vink et al., 2000).

The Endothelium Is Heterogenous, So Is the eGCX

The morphology of the microvascular endothelium and associated gene expression vary across different vascular beds in different tissues, therefore showing a remarkable heterogeneity (Aird, 2007; Jambusaria et al., 2020). Likewise, different GAG chain arrangements and eGCX compositions result in great biochemical or structural variations, further contributing to the eGCX heterogeneity. With reference to the thickness and microstructure of the eGCX, it is now well established that both vary across different species, vascular beds, organs and shear stress rates.

The estimation of the eGCX thickness extends from 0.2 to 0.5 μm in capillaries (van den Berg et al., 2003) and venules (Yoon et al., 2017), to 2–3 μm in small arteries (van Haaren et al., 2003; Yen et al., 2015), and 4.5 μm in conductance arteries (Megens et al., 2007). These studies used different methods of eGCX visualization and measurements, including alcian blue staining for transmission electron microscopy, dye–exclusion of different sized tracers, and fluorescently labeled lectins for microscopic imaging (Roth, 1983; Vink and Duling, 2000; van den Berg et al., 2003). Still, there is a large discrepancy when it comes to reporting eGCX thickness, making experimental observations particularly difficult to be reconciled. The reason for this variability, which might not be entirely attributed to differences in the microstructure and composition of the eGCX, might rather be due to a poor preservation of such a fragile structure during fixation and tissue handling (de Mesy Bentley, 2011; Ebong et al., 2011). Comparatively, direct *in vivo* measurements using bright-field microscopy also embody challenges. The close optical refractive index of the eGCX to the surrounding blood makes it very difficult to visualize the eGCX limits, also contributing to bias in the results. *In vitro*, ECs in culture exhibit slightly different eGCX in comparison to the complex structure found in *in vivo* vessels (Potter and Damiano, 2008; Potter et al., 2009). Recently, super resolution fluorescence microscopy (STORM) has been applied to identify the spatio-chemical organization of the eGCX *in vitro* (Fan et al., 2019). Also, glycomic analysis by liquid chromatography coupled to mass spectrometry has emerged as a novel method providing a more detailed and comprehensive characterization of eGCX in cells and tissues (Li et al., 2019, 2020; Riley et al., 2020).

A close view of the eGCX using both scanning and transmission electron microscopy has revealed different eGCX thickness among continuous, fenestrated and sinusoidal capillaries in the heart, kidney, and liver, respectively (Okada et al., 2017). The eGCX layer in both continuous and fenestrated capillaries is thicker than in the sinusoids. In the heart, the eGCX covers the entire luminal endothelial surface. In the kidney, the eGCX appears to occlude the endothelial pores of the fenestrated capillaries. In the hepatic sinusoids, however, the eGCX covers

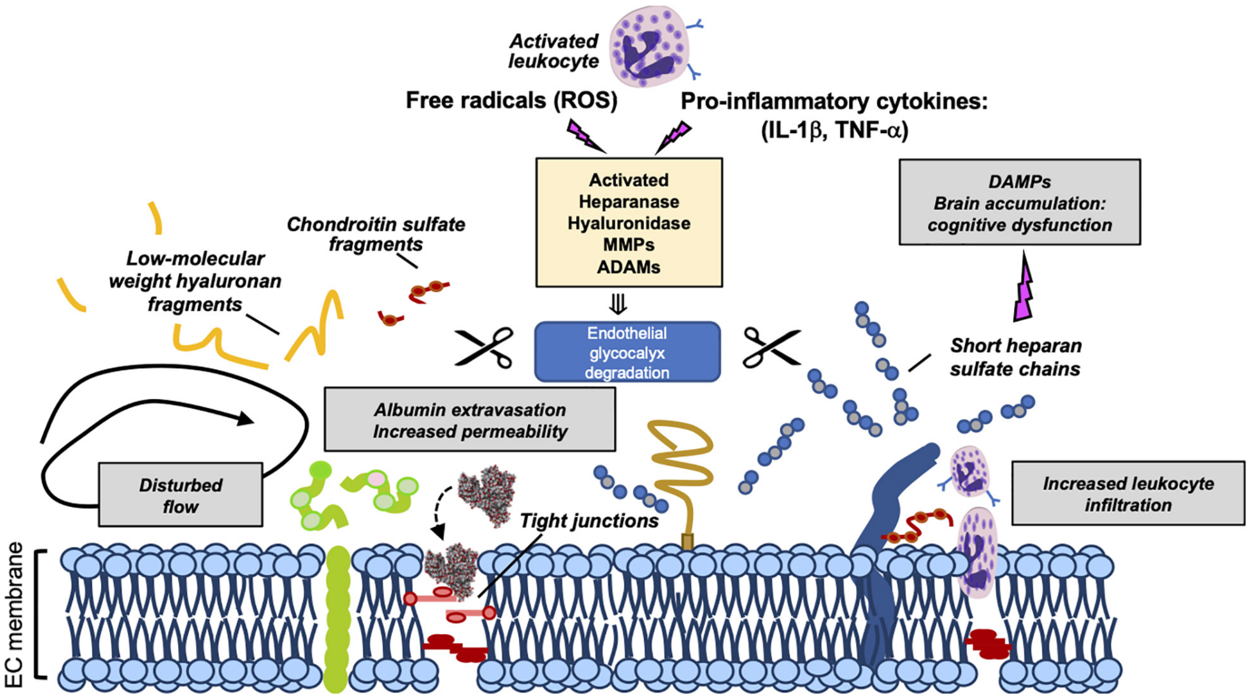

FIGURE 2 | Mechanisms of eGCX degradation and pathogenic consequences of released GCX fragments. Representation of enzymatic degradation of GCX components. The structure of the eGC is the result of a balance between the enzymatic degradation and *de novo* biosynthesis of new molecules and adsorption of circulating components from blood. Several enzymes mediate this degradation. Heparinase, hyaluronidase, MMPs and ADAMs are activated by pro-inflammatory cytokines and ROS promoting the damage and shedding of one or more of its components. This degradation releases eGCX components (such as short heparan sulfate chains, low-molecular weight hyaluronan fragments, and chondroitin sulfate fragments) into the circulation. As a result of its degradation, the eGC becomes thinner allowing the extravasation of albumin, leukocyte adhesion and dysregulated vasodilation. Once in circulation, eGCX components such as heparan sulfate fragments can act as DAMPs leading to cognitive impairment (Hippensteel et al., 2019a). Gray box areas summarize major pathophysiologic features of eGCX degradation. DAMPs, danger-associated molecular patterns; MMP, metalloproteinase; IL-1β, interleukin-1β; TNF-α, tumor necrosis factor-α. Scissors symbol means "degradation".

both the luminal side and opposite side facing the perisinusoidal space (Okada et al., 2017).

In organs like the brain and heart, where the capillary endothelium is categorized as continuous (non-fenestrated), the endothelial eGCX appears to be denser compared to that in the lung, whose capillaries are also covered by continuous endothelium (Ando et al., 2018). These differences might be explained by the mechanotransduction properties of the eGCX in sensing fluid shear stress, which alters GAGs synthesis (Arisaka et al., 1995; Gouverneur et al., 2006; Zeng and Tarbell, 2014). Since the pulmonary circulation is a low fluid shear stress system (because of its low resistance), a lower rate of GAGs synthesis renders a thinner eGCX on the pulmonary capillaries compared to other organs like the heart or the kidney. However, experimental evidence shows discrepancies in eGCX depth between pulmonary eGC (>1.5 micrometers) exceeding that of systemic vessels such as the eGCX in cremaster muscle capillaries (Schmidt et al., 2012; Han et al., 2016). The same principle can be applied to the macro vs. microvascular network, where arteries receiving higher shear stress exhibit greater eGCX depths compared to venules and capillaries with lower shear stress (Lipowsky et al., 1978, 1980; van den Berg et al., 2003). In light of recent discoveries, differences in capillary EC structure and shear stress might not be sufficient to explain eGCX heterogeneity. Gene expression profiling and single–cell RNA-sequencing might yield a more comprehensive picture of the distinct EC subsets and associated eGCX structures (Jambusaria et al., 2020; Gao and Galis, 2021).

Severe Inflammation as a Cause of eGCX Dysfunction

Recently, the eGCX integrity has emerged as an important determinant of cardiovascular health and disease. Given the fundamental role of the eGCX in maintaining vascular homeostasis, one would predict that when components of the eGCX are lost or degraded, the endothelial function could be impaired, which has indeed been demonstrated. eGCX degradation is triggered by inflammatory mechanisms through the activation of specific enzymes such as metalloproteinases, heparanase, and hyaluronidase. These enzymes are activated by reactive oxygen species (ROS) and pro-inflammatory cytokines such as tumor necrosis factor alpha (TNF-α) and interleukin-1 beta (IL-1β) (**Figure 2**) (Chappell et al., 2008; Schmidt et al., 2012; Lipowsky and Lescanic, 2013; Manon-Jensen et al., 2013; Becker et al., 2015).

The lack of an intact eGCX has been observed in several pathological conditions, the best characterized being sepsis. In the broad scheme of sepsis, systemic inflammatory injury of the eGCX leads to capillary leak, adverse immune response, and impaired vasodilation. Following septic challenge, enzymes such as ADAM15 (a disintegrin and metalloproteinase 15) and heparanase can shed glycoproteins (CD44) and heparan sulfate, respectively, leading to eGCX disruption (Schmidt et al., 2012; Yang et al., 2018). As a result of eGCX damage, the eGCX layer becomes thinner and more sparse while its degradation products are released into the bloodstream, a phenomenon that has been observed in animal models of sepsis as well as in human patients with sepsis, trauma or shock (Nelson et al., 2008; Haywood-Watson et al., 2011; Sallisalmi et al., 2012; Luker et al., 2018; Uchimido et al., 2019).

Similar to sepsis, sterile inflammation following trauma or tissue injury also causes shedding of proteoglycans, hyaluronan and heparan sulfate chains. The eGCX fragments function as Danger-Associated Molecules Patterns (DAMPs) that activate toll–like receptor or/and RAGE receptor-dependent pathways (Johnson et al., 2002) RAGE (Xu et al., 2011, 2013). High levels of circulating eGCX elements, which propagate sterile inflammation and drive trauma induced coagulopathy (TIC), are highly correlated with the severity of injury and clinical outcomes (Johansson et al., 2011a,b).

Oxidative stress also plays an important role in eGCX degradation during inflammation. The eGCX along with vascular ECs are vulnerable to circulating ROS produced during oxidative stress. *In vitro* exposure of ROS (superoxide and hydroxyl radicals) to the eGCX promotes fragmentation of GAGs and loss of some of its components. Previous studies have demonstrated that hyaluronan and chondroitin sulfate are the most susceptible to depolymerization and chemical modifications by ROS (Halliwell, 1978; Greenwald and Moy, 1980; Bartold et al., 1984; Moseley et al., 1995, 1997; Lipowsky and Lescanic, 2013; Singh et al., 2013). Intact eGCX has the capability to quench free radicals by having binding sites for anti-oxidant enzymes like xanthine oxidoreductase (Adachi et al., 1993) and endothelial superoxide dismutase (eSOD) (Becker et al., 1994).

Viral infections, such as those caused by dengue, hanta and the novel severe acute respiratory syndrome (SARS)-CoV-2 (COVID-19), are also accompanied by eGCX disruption. In the case of the dengue virus, in particular, the secreted dengue virus (DENV) non-structural protein 1 (NS1) disrupts the eGCX on human pulmonary capillaries by increasing the expression of sialidases, heparanase and metalloproteinases. All these events cause systemic microvascular leakage leading to hypovolemic shock and potentially fatal complications in severe dengue infections (Luplertlop and Misse, 2008; Puerta-Guardo et al., 2016; Glasner et al., 2017; Suwarto et al., 2017; Tang et al., 2017; Chen et al., 2018; Wang et al., 2019). Hantavirus infection is also associated with endothelial dysfunction and elevated circulating levels of syndecan-1, allowing a clinical association of disease severity with eGCX damage (Marsac et al., 2011; Connolly-Andersen et al., 2014). In contrast, other viruses do not seem to cause eGCX shedding, but they exploit eGCX components on the host cell surface as a binding site to infect target cells. For example, Influenza A uses sialic acid as a receptor (Weis et al., 1988; Matrosovich et al., 1993; Suzuki, 2003; Russell et al., 2008) while HIV lentivirus (Saphire et al., 2001; Bobardt et al., 2003; Gallay, 2004) and SARS-CoV-2 (Clausen et al., 2020) interact with heparan sulfate. Also, several recent studies have emphasized the implications of eGCX damage and endothelial dysfunction in the pathogenesis of COVID-19 (Jung et al., 2020; Kaur et al., 2020; Libby and Luscher, 2020; Teuwen et al., 2020; Yamaoka-Tojo, 2020).

Previous research on fluid resuscitation for critical illness management has shown mixed results, some show attenuating eGCX degradation while others show inducing eGCX disruption (Hippensteel et al., 2019b). However, there is consensus that colloids (e.g., albumin), or fresh frozen plasma, reduce eGCX damage following sepsis, hemorrhagic shock and traumatic brain injury (Zehtabchi and Nishijima, 2009; Haywood-Watson et al., 2011; Kozar et al., 2011; Peng et al., 2013; Mica et al., 2016; Nikolian et al., 2018).

Endothelial GCX in Blood–Brain Barrier (BBB) Injury

The diagnostic utility of eGCX degradation products as a biomarker of disease is supported by the correlation between circulating eGCX fragments and clinical outcomes [reviewed by Uchimido et al. (2019)]. Compared to the cardiac and pulmonary capillaries, cerebral capillaries have a thicker eGCX layer which is better preserved following lipopolysaccharide (LPS) administration (Ando et al., 2018). Additionally, the eGCX joins astrocyte endfeet and basement membrane in reinforcing BBB properties as a part of a newly defined "tripartite" BBB layered structure (Kutuzov et al., 2018). During sepsis, heparan sulfate fragments released from the injured eGCX can circulate in the bloodstream for days and penetrate into the hippocampal area, interfering with long-term potentiation (LTP) and contributing to sepsis–associated encephalopathy (SAE), a common neurological complication of sepsis in the absence of direct brain infection (Hippensteel et al., 2019a). Circulating eGCX fragments predicted cognitive impairment in septic patients, however, whether they have potential diagnostic utility as biomarkers to predict cognitive dysfunction in sepsis survivors, still remains to be confirmed.

CONCLUSION

The eGCX, a complex and fragile structure that protects endothelial barrier integrity, plays a crucial role in maintaining microcirculatory homeostasis and blood-tissue exchange. Disruption of eGCX is a consequence as well as cause of microvascular injury, as eGCX degradation products act as pathogenic factors capable of inducing endothelial hyperpermeability and microvascular leakage during inflammation. Further studies are required to understand eGCX structure and function in order to maximize its protective contribution to endothelial cell stability while minimizing its pathological role in vascular disease and injury.

AUTHOR CONTRIBUTIONS

NV performed literature search, drafted the manuscript, and prepared the figures. SB and SY participated in manuscript editing. SY initiated, directed, and sponsored the work throughout all levels of development. All authors approved the final version for publication.

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