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The endothelial glycocalyx: Structure and function in health and critical illness

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Abstract

Objective: To conduct a narrative review of the current literature in reference to the structure and function of the endothelial glycocalyx (EG) and its contribution to the pathophysiology of conditions relevant to the veterinary emergency and critical care clinician. Novel therapies for restoring or preserving the EG will also be discussed.

Data Sources: Online databases (PubMed, CAB abstracts, Scopus) were searched between January 1st 2017 and May 1st 2017 for English language articles without publication date restriction. Keywords included EG, endothelial surface layer, degradation, syndecan-1, heparan sulfate, critical illness, sepsis, trauma, and therapeutics.

Data Synthesis: The EG is a complex and important structure located on the luminal surface of all blood vessels throughout the body. It plays an important role in normal vascular homeostasis including control of fluid exchange across the vascular barrier. Loss or degradation of the EG has an impact on inflammation, coagulation, and vascular permeability and tone. These changes are essential components in the pathophysiology of many conditions including sepsis and trauma. A substantial body of experimental animal and human clinical research over the last decade has demonstrated increased circulating concentrations of EG degradation products in these conditions. However, veterinary-specific research into the EG and critical illness is currently lacking. The utility of EG degradation products as diagnostic and prognostic tools continues to be investigated and new therapies to preserve or improve EG structure and function are under development.

Conclusions: The recognition of the presence of the EG has changed our understanding of transvascular fluid flux and the pathophysiology of many conditions of critical illness. The EG is an exciting target for novel therapeutics to improve morbidity and mortality in conditions such as sepsis and trauma.

KEYWORDS
critical care, hypotension, peripheral edema, septic shock, vascular leak

Abbreviations: ANP, atrial natriuretic peptide; APC, activated protein C; ALI, acute lung injury; AT, antithrombin; COP, colloid osmotic pressure; CRP, C-reactive protein; CS, chondroitin sulfate; DS, dermatan sulfate; EC, endothelial cell; EG, endothelial glycocalyx; eNOS, endothelial nitric oxide synthase; ESL, endothelial surface layer; FFP, fresh frozen plasma; GAG, glycosaminoglycan; GP, glycoprotein; HA, hyaluronan, hyaluronic acid; HES, hydroxyethyl starch; HP, hydrostatic pressure; HS, heparan sulfate; IM, intravital microscopy; I-R, ischemia-reperfusion; LMWH, low molecular weight heparin; LPS, lipopolysaccharide; MMP, matrix metalloproteinase; NO, nitric oxide; PBR, perfused boundary region; PCA, postcardiac arrest; PG, proteoglycan; ROS, reactive oxygen species; SGS, subglyceal space; SIRS, systemic inflammatory response syndrome; SDC1, syndecan-1; TFPI, tissue factor pathway inhibitor; TM, thrombomodulin; TNF-α, tumor necrosis factor-α.

1 | INTRODUCTION

In 1940, biologist James Danielli first hypothesized that there was a protein layer covering the inner walls of the vasculature.[1] Subsequent research has confirmed the presence of a fragile, mesh-like layer covering the luminal surface of all blood vessels throughout the body, termed the endothelial glycocalyx (EG).[2] In 1963, Stanley Bennett noted that all cells are covered with an extracellular layer rich in polysaccharides and coined the term glycocalyx, which is Greek for sweet husk.[2] Over the last decade, with the advent of new technology, a large volume of research has highlighted the critical physiological role of the EG. The EG is essential in the regulation of vascular permeability, inflammation, and coagulation and is integral in the pathophysiology of conditions such as sepsis and shock. This review covers the structure and function of the EG in health, EG dysfunction in critical illness, including emerging biomarkers, and experimental treatments to preserve or repair the EG.

2 | NORMAL STRUCTURE AND FUNCTION OF THE ENDOTHELIAL GLYCOCALYX

The EG is a mesh-like gel matrix layer that, under normal conditions, covers the luminal aspect of all blood vessels. It is a complex, filamentous coating composed of a scaffolding of proteoglycans (PGs), glycoproteins (GPs), and glycosaminoglycans (GAGs) associated with the underlying endothelial cells (ECs) (Figures 1 and 2). The EG and its associated molecules and fluid comprise the endothelial surface layer (ESL) (Figure 3). In health, the EG is a highly dynamic but stable structure that is maintained via a fine balance between normal degradation and new biosynthesis.[3,4] Nevertheless, it is highly fragile and easily disrupted so visualization of the EG to elucidate both its composition and thickness has proven challenging.[5]

2.1 | Composition

2.1.1 | Proteoglycans

Proteoglycans form the main scaffolding of the EG and are composed of a core protein and covalently attached GAG chains (Figure 2). The EG contains 2 main PGs: syndecans and glypicans. Syndecans were named based on the Greek word "syndein" which means, "to bind together."[6] Syndecans, found on the surface of most cells in the body, are transmembrane proteins comprised of extracellular, transmembrane, and cytosolic domains.[6,7] The extracellular domain binds GAGs and detects extracellular signals, such as shear stress, which are transduced to the intracellular environment via the transmembrane portion and the cytoplasmic tail.[8,9] There are 4 known syndecans in vertebrates: syndecans 1, 2, 3, and 4; however, the EG primarily contains syndecan-1 (SDC1).[7]

In contrast to syndecans, glypicans are not transmembrane proteins but are attached to the EC luminal membrane via a glycosyl phosphatidylinositol anchor.[8,10] There are 6 known glypicans present in mammals[11] with glypican-1 being the only glypican expressed on the endothelium. Its ectodomain binds only the GAG heparan sulfate (HS)[8,10] and its anchor molecule is thought to localize it around lipid rafts and caveolae. Caveolae are membrane structures rich in signaling molecules that serve as communication hubs in the cell membrane.[8] This location allows glypicans to partake in various signaling cascades with cytokines and other molecules such as the vasodilator nitric oxide (NO).[8]

2.1.2 | Glycosaminoglycans (GAGs)

Glycosaminoglycans are nonbranching linear polysaccharides composed of 20 to 200 repeating disaccharide units.[7] They are the only type of PG side chains and extend off the core protein into the vascular lumen creating the mesh-like structure characteristic of the EG. One study reported that GAGs form up to 95% of the PG composition suggesting that GAGs are the most abundant component of the EG.[7] There are 5 main GAGs: HS, chondroitin sulfate (CS), dermatan sulfate (DS), keratin sulfate, and hyaluronan (HA).[8,12] Although all 5 GAGs are found within the EG, they are not evenly represented. HS is the most abundant GAG (50–90%) in a ratio of 4:1 with CS, the second most prevalent GAG.[3,13] Generally, SDC1 binds 3 HS and 1–3 CS molecules, whereas, glypican has 3–4 attachment sites that exclusively bind HS.[3] Sulfate groups attached to the GAG disaccharide units impart a net negative charge to the EG.[7]

The location and functional role differ among the main GAGs within the EG. HS is found primarily at the luminal surface of the EG, whereas HA and CS are located deeper within the EG closer to the EC surface.[14] This distribution is likely linked to their function, as HS is thought to play a more dominant role in structural integrity and cellular interactions of the EG, whereas HA and CS are considered more important in vascular permeability.[14] HA is larger than the other GAGs and is not covalently attached to a core protein; instead, it is anchored directly to the EC membrane via the transmembrane receptor CD44.[8,15] Additionally, HA is nonsulfated and acquires its net negative charge via the presence of carboxyl groups.[8,16]

Normal endothelial biosynthesis of the PG core proteins syndecan and glypican occurs on membrane bound ribosomes.[7] After synthesis, the core protein is transferred to the lumen of the endoplasmic reticulum followed by the Golgi apparatus where attachment, polymerization, and sulfation of GAG side chains occur. The core protein along with the attached GAGs is then transferred to the cell surface where they are either incorporated into the cell membrane (syndecans) or attached to the cell surface with an anchor molecule (glypican). Unlike the other GAGs, HA is not attached to a core protein and is synthesized on the cell membrane rather than in the Golgi apparatus.[15] Overall the composition of the EG is controlled via a fine balance between normal biosynthesis of the EG constituents and the rate of shedding or loss. Shedding of EG constituents occurs adaptively under normal physiological conditions as well as maladaptively during pathological processes.[3,4,17,18]

2.1.3 | Glycoproteins

Glycoproteins are located on the EC surface and are covered by the EG in health. Unlike PGs, GPs do not bind to long-chained GAGs but instead carry short, covalently bound, branched oligosaccharide

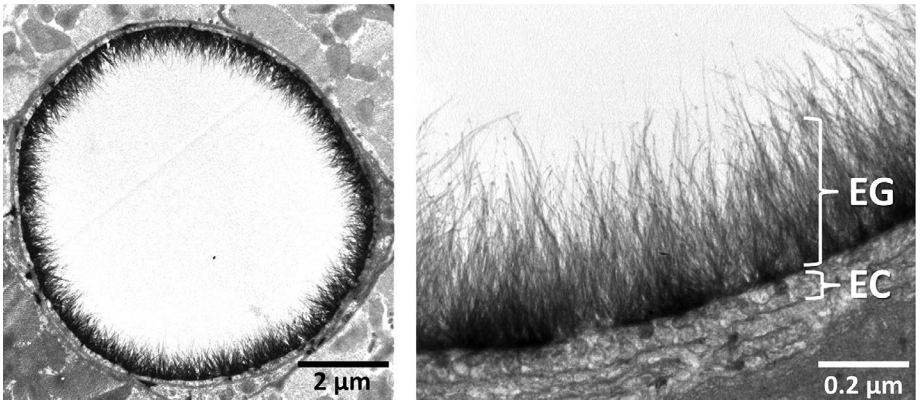

FIGURE 1 Electron micrograph of a goat coronary capillary. The brush-like structure of the EG components covers the entire luminal aspect of the vessel. The EG occupies a considerable volume and is multiple times as thick as the ECs. EC, endothelial cell; EG, endothelial glycocalyx. Reproduced with permission from Van den Berg BM et al. Endothelial luminal glycocalyx. In: Aird WC, ed. *Endothelial Biomedicine.* New York, NY: Cambridge University Press;2007:689-695

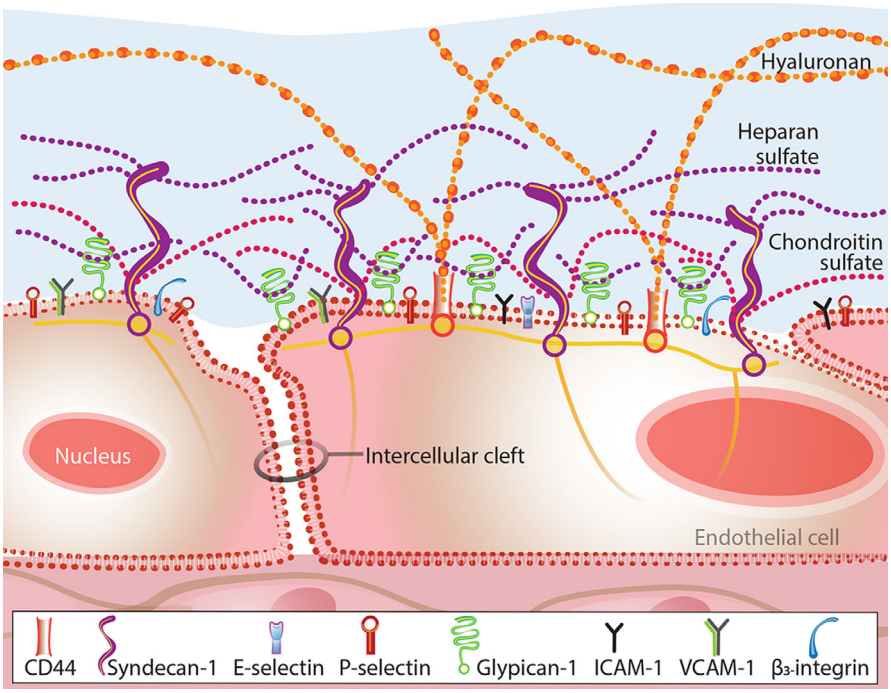

FIGURE 2 Major components of the endothelial glycocalyx. ICAM-1, intercellular cell adhesion molecule-1; VCAM-1, vascular cell adhesion molecule-1

units.[19] Endothelial GPs are membrane-bound cell adhesion molecules that are separated into 3 different families based on structural and functional characteristics: selectins, immunoglobulins, and integrins.[20] The presence of these endothelial GPs is required for the functioning of the normal leukocyte recruitment cascade, which consists of a regulated series of events: rolling, adhesion, and transmigration.[21,22] Ultimately, diapedesis of neutrophils, monocytes, eosinophils, and some lymphocytes is achieved when these adhesion molecules bind to their respective leukocyte integrins.

The main selectins found within the EG are P-selectin and E-selectin, both of which are involved in the initial contact and adhesion of leukocytes and platelets to the activated endothelium.[20] P-selectin is constitutively expressed and stored within granules of Weibel-Palade bodies of the EC and platelets; it is preformed and stored in a relatively constant amount regardless of local environmental factors. Upon stimulation by complement components, thrombin, histamine, or fibrin,[23] translocation of P-selectin to the cell surface occurs within minutes.[24] E-selectin is found exclusively on the endothelium, whereas P-selectin is found on both the endothelium and platelets. Unlike P-selectin, E-selectin is inducible rather than stored, thus, it requires transcription, translation, and translocation to the cell surface.[22,25] Upon cytokine or antigenic stimulation, E-selectin synthesis is upregulated and reaches maximal levels within 4–6 hours before returning to baseline within 24–48 hours.[20]

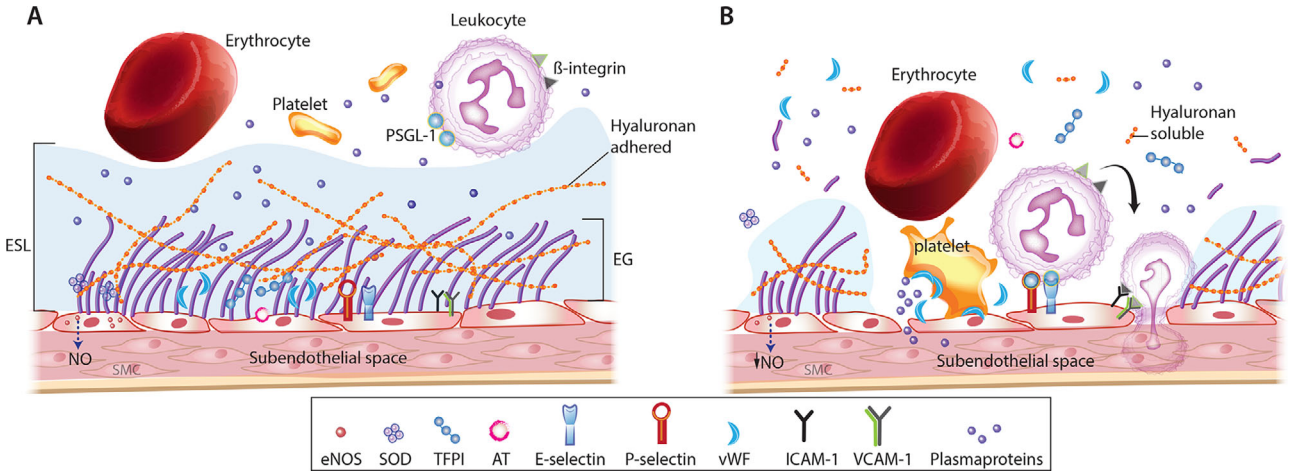

FIGURE 3 A-B Composition of the ESL in health (A) and in conditions where significant shedding of its components occurred (B). In health, the ESL is composed of the core EG proteins, bound or adhered glycosaminoglycans, plasma proteins and various dissolved or adhered proteins that modulate coagulation and inflammation. The ESL generates an exclusion zone for cellular blood components, erythrocytes, platelets, and leukocytes and large molecules, such as plasma proteins, are largely reflected on the EG surface. With reduction of the ESL, platelets and leukocytes (eg, monocytes, neutrophils) are allowed to interact with adhesion molecules expressed on the endothelial surface while modulators of coagulation and inflammation are displaced from the endothelial surface. Reduction of the EG may lead to increased transvascular protein flux. AT, antithrombin; EG, endothelial glycocalyx; ESL, endothelial surface layer; ECSOD, endothelial cell superoxide dismutase; eNOS, endothelial nitric oxide synthase; ICAM-1, intercellular cell adhesion molecule-1; PSGL-1, P-selectin glycoprotein ligand-1; TFPI, tissue factor pathway inhibitor; vWF, von Willebrand factor; VCAM-1, vascular cell adhesion molecule-1. Modified with permission from Nieuwdorp M et al. The endothelial glycocalyx: a potential barrier between health and vascular disease. *Curr Opin Lipidol*. 2005;16:507-511

The endothelial GPs of the immunoglobulin superfamily support the adhesion and transmigration of leukocytes between ECs. Members of the immunoglobulin superfamily include intercellular adhesion molecule-1, vascular cell adhesion molecule-1, and platelet EC adhesion molecule-1.[26] These adhesion molecules are either constitutively expressed on the EC or are upregulated upon EC activation by various agonists. The immunoglobulin adhesion molecules are not found exclusively on ECs but are also associated with other cell types such as fibroblasts and epithelial cells.[20]

Integrins are heterodimeric transmembrane molecules containing alpha- and beta-subunits linked together by disulfide bonds. Currently at least 19 alpha-chains and 9 beta-chains have been characterized, the dimeric combination of which allows a multitude of different integrins.[27] The classification scheme is based on either the beta-subunit or the molecule to which the integrin binds. Integrins are expressed on a number of cells including leukocytes, platelets, and ECs. They are important for the firm attachment of leukocytes and platelets to the EC as well as transduction of mechanical or chemical signals from the extracellular to the intracellular microenvironment.[27] Integrins associated with the endothelium are primarily in the beta-3 subgroup, such as $\alpha_v\beta_3$ integrin, that facilitates platelet binding to the EC.[27,28]

2.1.4 | Soluble components

Soluble plasma components are incorporated into the EG and together they form the ESL.[29] These various soluble plasma components further enhance the EG by altering its physical properties such as its thickness and permeability.[30] Albumin, the main plasma protein, is one of the key soluble components within the EG and appears to be required to impart normal barrier function to the ESL. Evidence suggests, however, that albumin alone is not sufficient to maintain the barrier function of the ESL and that other components of plasma must also play a role.[30-32] In 2 experimental studies using perfused frog[33] or rat[34] microvessels, perfusion of the vessel with plasma resulted in restoration of the normal permeability barrier and reduced fluid extravasation compared to perfusion with albumin alone. Orosomucoid, for example, is another plasma protein that has been demonstrated to be part of the ESL and to participate in maintaining normal permeability.[35] In an experimental study involving hamster cremaster muscles, tracer molecules were used to investigate the properties of the ESL.[36] In addition to charge and size affecting the inclusion of molecules into the ESL, the shape of the molecule also appears to be important. Fibrinogen (340 kDa), despite being a much larger molecule than albumin (67 kDa), was incorporated into the ESL, whereas dextran 70 (70 kDa) was excluded.[36] Both the tertiary structure of the large fibrinogen molecule and its charge, which is similar to albumin, were thought to be important properties in its ability to be included into the ESL.[36]

Lastly, different GAGs also bind to other molecules, which facilitate their integration into the ESL. Multiple anticoagulants are incorporated into the ESL as discussed in detail below. Incorporation of the antioxidants superoxide dismutase[37] and xanthine oxidoreductase[38] shields the EC from damaging reactive oxygen species (ROS).[39,40]

2.2 | Thickness and visualization

Due to the labile nature of the EG, in vivo visualization has been proven challenging and much of the original research relied on in vitro[41,42] or ex vivo[29,31] studies. The EG was first visualized in 1966 in an ex vivo

study using transmission electron microscopy.[43] Despite technological advances, experimentally determined EG dimensions in normal microvessels vary substantially between studies, a fact that is likely caused by methodological heterogeneity. In vitro and ex vivo investigative methods have included different types of microscopy techniques, which require extensive tissue fixation; indirect measurements using tracer molecules and cell exclusion zones; and constructed computer models.[29,31,41,42,44-47] In addition, the estimated thickness depends upon whether the EG alone or the entire ESL (as occurs in vivo) is measured. Lastly, EG thickness likely varies among different vessel segments and organ systems, and among species and individuals.[31,48] For example, the ESL of pulmonary microvessels in mice as measured by intravital microscopy (IM) was 3 to 5 times thicker than the ESL of cremasteric microvessels.[49] In a study of ex vivo human umbilical veins, the EG thickness varied by up to 0.85 μm among individuals.[41] Reported measurements of the EG dimension range from 0.2 to 0.5 μm in microvessels, such as coronary capillaries, from 0.6 to 0.8 μm in veins, and up to 4.5 μm in larger arteries such as the carotid artery.[41,44,47]

Intravital microscopy allows for the visualization and measurement of the ESL in vivo[5] with minimal manipulation of the vessels and associated tissue.[50] Unfortunately, an inherent limitation is that IM can only be used in superficial microvascular beds.[47] This technique has been used to examine the thickness of sublingual microvessels in conscious healthy human volunteers,[51,52] anesthetized cats,[53] and in people with cardiovascular disease[52] and critical illness[54] with the measurement taking only minutes. In these studies, side-stream dark field microscopy is used in conjunction with glycocalyx measurement software to assess the dimensions of the EG. Dimensions of the EG are determined based on the perfused boundary region (PBR), which is the area accessible to RBCs within the microvasculature. In health, the negatively charged EG repels RBCs from its surface to facilitate laminar flow. Consequently, an increase in the PBR has been shown to be associated with a loss of EG thickness. In critically ill people, the PBR was significantly increased compared to that in healthy controls, and among the critically ill patients, septic patients had the largest PBR.[54] This method allows for rapid, serial bedside assessment of the EG and may in the future aid in distinguishing septic from nonseptic critically ill patients and/or in determining the effect of treatments aimed at restoring the EG.[54] However, how the dimensions of the EG in sublingual microvessels compare to the EG in other parts of the body and to what extent this technique serves as a source of valid identification of EG alterations at distant sites requires further investigation.[54]

2.3 | Function of the endothelial glycocalyx in health

The EG is a dynamic and adaptive structure rather than an inert surface layer. Given its active nature and ubiquitous presence on the surface of all ECs it likely plays a pivotal role in many aspects of normal homeostasis. The EG appears crucial in maintaining normal vascular permeability[55] and transvascular fluid flux,[56] in cell-to-cell interactions (inflammation and coagulation),[57,58] and in vascular mechanotransduction.[59,60]

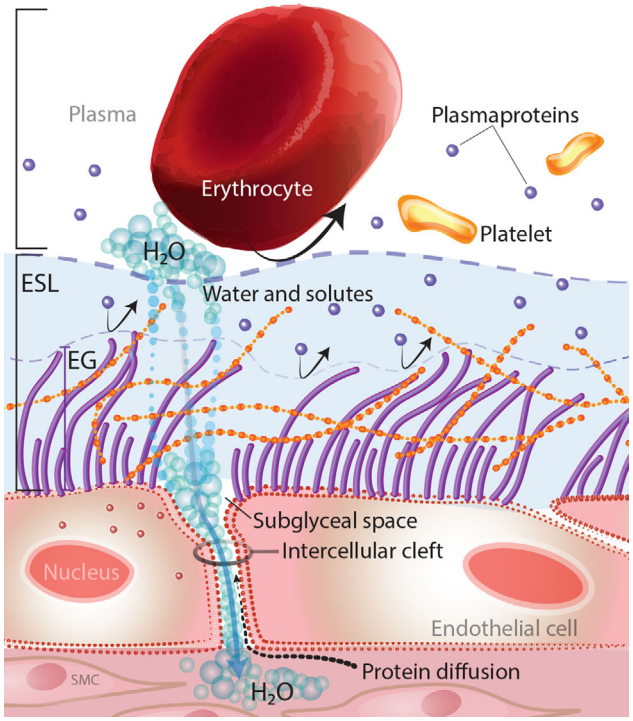

FIGURE 4 Functional compartments involved in transvascular fluid flux. Plasma proteins are largely reflected on the surface of the endothelial glycocalyx, while water and small solutes are allowed to passage according to the transendothelial hydrostatic pressure gradient. This leads to the generation of a space immediately underlying the endothelial glycocalyx (ie, subglyceal space) that contains fluid of very low colloid osmotic pressure. Protein diffusion from the interstitium into the subglyceal space is mitigated by the filtrate convection in the opposite direction. EG, endothelial glycocalyx; ESL, endothelial surface layer; SMC, smooth muscle cell

2.3.1 | Modulation of vascular permeability and transvascular fluid flux

More than 120 years ago, Starling developed a hypothesis to explain the absorption and filtration of fluid within capillaries and the formation of lymph within the body.[61] He described that the movement of fluid between the intracapillary and interstitial spaces depended on the osmotic pressure generated by plasma proteins and the hydrostatic pressure (HP) differences between these 2 compartments.[61] According to the Starling hypothesis, intravascular fluid is filtered out of the vessel at the arterial end of the capillary and reabsorbed at the venous end.[61]

With the discovery of the EG, the classic Starling hypothesis has been modified.[56,62-64] Studies have shown that the colloid osmotic pressure (COP) within the interstitium contributes much less to the transendothelial fluid flux than previously thought.[65] The revised hypothesis suggests that the main COP gradient is not the difference of forces between the plasma and the interstitium but rather between the plasma and the space immediately below the EG, the subglyceal space (SGS) (Figure 4). Within the SGS, the COP is very low for 2 main reasons. First, albumin incorporation into the ESL increases its filter function by effectively excluding entry of larger macromolecules.[31]

Second, any back diffusion of proteins from the interstitium into the SGS is prevented by the high velocity of filtered fluid funneled through the interendothelial clefts directionally toward the interstitium.[65,66] These clefts are the main sites of fluid movement from the vascular lumen to the interstitium.[55,66] The consequence of the very low COP within the SGS is that the net COP difference between the SGS and the capillary lumen is much larger than the COP difference between the interstitium and the lumen. It is also possible that the most physiologically relevant COP gradient exists between the ESL (where albumin is concentrated) and the SGS, which is an even greater gradient than that between the capillary lumen and the SGS. Hence, under steady state conditions (when capillary HP is constant and relatively low) the rate of fluid extravasation is low, but occurs along the entire length of the capillary and without net absorption of fluid at the venous end of the capillary as previously thought.[65] The COP gradient, which is entirely intravascular, opposes but does not reverse fluid filtration.[65,66]

The EG plays a major role in the maintenance of normal vascular permeability. The negatively charged GAGs along with the EG's complex ultrastructure controls solute exchange, restricting the passage of solutes based on size, shape, and charge.[55,67-69] Studies have been conducted to investigate the ultrastructure of the EG and its contribution to permeability. Curry and Michel proposed the fiber-matrix theory of the EG microstructure, hypothesizing that a fibrous mesh (the EG) covers the entire endothelial surface and confers the molecular sieving properties to the vessel.[70] The fiber-matrix theory has been experimentally confirmed: enzymatic degradation of the EG resulted in a 60% increase in permeability.[55,71]

The fiber-matrix theory explains the selective permeability of the EG, excluding large molecules while maintaining a relative permeability to smaller molecules, specifically those with radii less than 4–5 nm. An electron microscopy study of the EG of frog mesenteric vessels was consistent with this model.[72] The EG formed a regular, 3-dimensional meshwork with a fiber diameter of 10–12 nm and regular spacing of 20 nm in both the parallel and perpendicular planes surrounding a larger framework built around tufts or units arrayed approximately 100 nm apart (Figure 5).[72] The resulting gaps of <10 nm significantly restrict the permeability of this matrix for larger molecules.

Albumin, concentrated within the EG, appears necessary to maintain normal vascular permeability characteristics.[31,65,73] Albumin alters the EG structure to a regular, lattice-like structure which, in conjunction with its net negative charge, enhances the EGs selectivity to macromolecules.[74,75] Interestingly, albumin's effect of reducing vascular permeability is likely not solely due to an increase in COP but also its ability to become incorporated into the EG.[31,73] One study using isolated guinea pig hearts measured fluid extravasation after perfusion with either albumin or hydroxyethyl starch (HES). Although HES led to a higher COP compared to albumin, it did not reduce the capillary permeability to the same extent that albumin did. These findings suggest that different colloids have different effects on transvascular fluid flux and that the ability of albumin to afford reduced vascular permeability is due at least partially to its incorporation into the EG.[31] Albumin's incorporation into the negatively charged EG is facilitated by its amphiphilic nature (ie, having both positively and negatively charged regions).[29,31,75] Other plasma macromolecules, such as fibrinogen[36] and orosomucoid,[35] are also important to maintain permeability characteristics owing to their incorporation into the ESL.[30,33,76]

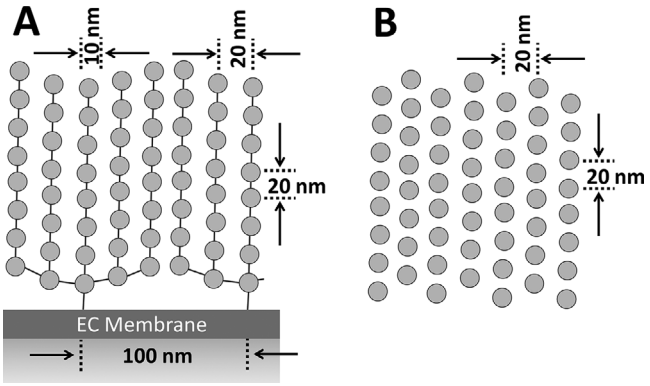

FIGURE 5 A-B Model of the electron microscopy-derived ultrastructure of the glycocalyx, viewed from the side (A) and from the vascular lumen (B). The glycocalyx is formed by a matrix of fibers each 10–12 nm in diameter and 20 nm apart from one another in all dimensions. The small size of the resulting gaps in the fiber lattice contributes to the filter function of the glycocalyx that selectively excludes large molecules (eg, more than 5 nm). The glycocalyx fibres form tufts that are implanted into endothelial cell membrane at a regular distance of 100 nm and may link the extracellular glycocalyx components to the intracellular cytoskeleton (eg, actin filaments). EC, endothelial cell. Modified with permission from Squire JM, Chew M, Nneji G, Neal C, Barry J, Michel C. Quasi-periodic substructure in the microvessel endothelial glycocalyx: a possible explanation for molecular filtering? *J Struct Biol*. 2001;136(3):239-255

All 3 of the major GAGs (HS, CS, and HA) also contribute to EG permeability qualities, and permeability increases after their enzymatic removal.[14,15] However, HA and CS may play a more important role as their enzymatic removal results in greater permeation of larger molecular weight molecules into the EG compared to HS.[14,15] Loss of HA may lead to the development of a more "open" or porous meshwork of the EG.[15] In conclusion, the EG and in extension the ESL are essential in the maintenance of normal vascular permeability, which is achieved by multiple key components acting synergistically.

2.3.2 | Regulation of endothelial cell and circulating cell interactions

The strategic location of the EG on the luminal side of the EC allows it to influence interactions between ECs and RBCs, WBCs, and platelets. The thickness of the EG helps maintain normal RBC movement while simultaneously modulating the amount of fluid sheer stress on the EC.[77] Microvascular flow occurs in a classic laminar pattern with an inner region of RBCs surrounded by an outer layer of plasma and platelets. The EG aids in maintaining this flow pattern by preventing RBC from getting attached to the EC.[52,78-80] Like ECs, RBCs have their own negatively charged glycocalyx and they are repelled by the negatively charged EG to maintain laminar flow. The presence of an intact EG may also improve microcirculatory perfusion by causing RBCs to develop a more elongated conformation, which improves both the

efficiency and speed of RBC transit.[52,81] Ultimately, these changes lead to an increase in the oxygen exchange capacity and reduction in friction as blood moves through the microcirculation.[52,79] Additionally, the glycocalyces of the RBC and EC appear to influence each other's composition with changes in the EG leading to subsequent changes in the RBC glycocalyx and vice versa.[79] This interaction could aid in the understanding of cardiovascular diseases, such as hypertension,[82] malaria,[83] sickle cell anemia,[84] as well as the effects of conditions like chronic kidney disease[85] and sepsis[86] on the endothelium. Furthermore, the influence of the EG on the RBC glycocalyx may provide a future diagnostic target, in that assessment of the RBC glycocalyx from a blood sample could provide insight into the state of EG.[79]

In health, the various GPs responsible for WBC recruitment and initiation of coagulation are hidden beneath the EG such that the ESL is essentially anti-inflammatory and anticoagulant. Both the physical thickness of the EG and its charge prevent circulating WBCs, which have their own glycocalyces, from accessing ECs.[22,87,88] Endothelial adhesion molecules responsible for WBC diapedesis are <50 nm in length while the EG, even in the microcirculation, is approximately 500 nm thick; thus, the EG completely submerges the WBC adhesion molecules, which prevents WBC-EC interaction at rest.[78,79,89,90] Due to this "protective covering" the EG must be partially shed under inflammatory conditions to allow exposure of EC adhesion molecules and subsequent WBC diapedesis.[58,91] Multiple studies have demonstrated that experimental enzymatic degradation of the EG elicits a marked increase in WBC adhesion.[49,57,87,92,93]

The EG also concentrates various WBC activators, including chemokines, near the EC surface, which prevents these molecules from interacting with circulating WBCs or being washed away in flowing blood.[87,94] Shedding of the EG exposes these chemoattractants, which upregulates WBC integrin expression and potentiates binding to their respective EC adhesion molecules.[87] The GAG HS is thought to play an important role in several aspects of WBC transmigration. For example, HS binds chemokines via their positively charged domains, thereby, facilitating a chemokine concentration gradient to guide WBC migration during inflammation.[94,95]

In health, the EG regulates coagulation by acting as a physical barrier to prevent EC and platelet adhesion molecule interaction as well as concentrating anticoagulant molecules within its structure.[58] On the surface of ECs, von Willebrand factor is constitutively expressed and hidden beneath the EG, which shields von Willebrand factor from platelets, thus, preventing unwanted platelet adhesion and activation.[96] The EG also binds many anticoagulant molecules including antithrombin (AT),[97] thrombomodulin (TM),[98] protein C,[99] and tissue factor pathway inhibitor (TFPI).[100] AT is a potent anticoagulant deactivating thrombin and activated factors IX and X. Within the EG, AT binds to regions of HS, which greatly enhances its anticoagulant activity on the EC surface.[97] TM, an integral membrane protein containing CS, is also constitutively expressed on ECs beneath the EG. The association with CS is essential to both TM's anticoagulant ability and its inclusion into the EG.[98] Binding of TM to thrombin and further complex formation with the endothelial protein C receptor expressed on the EC surface potentiates activation of the protein C anticoagulant pathway.[99] Lastly, TFPI is bound to HS within the EG and inhibits the formation of the tissue factor-factor VII complex, which is crucial in initiating thrombosis.[100,101]

2.3.3 | Mechanotransduction

The EG plays a key role in mechanotransduction (the transformation of a mechanical force into a biochemical response) but the mechanism is complex and incompletely understood.[102] The EG core proteins sense shear stress and transmit this signal to the actin cytoskeleton via their transmembrane domain.[59,102] The classic example of mechanotransduction is that, under shear stress, ECs produce NO. Production of NO occurs via the activation of the enzyme endothelial NO synthase (eNOS), which leads to relaxation of subendothelial smooth muscle cells and thus vasodilation.[8,103]

Degradation of the EG is associated with the loss of shear-induced NO release.[8,60,102-104] In vitro studies using cultured ECs suggest that HS is responsible for sensing vascular shear stress because the enzymatic removal of HS eliminates shear-induced NO release.[8,103] Other studies have demonstrated that removal of HA leads to a similar loss of NO release secondary to shear stress.[8,105] The important role that both HS and HA play in mechanotransduction is consistent with the information known about the EG structure. Glypicans that contain HS are preferentially colocated with specific EC surface structures, caveolae and lipid rafts, where eNOS resides.[8,60,102-104] Similarly, HA is known to bind to the CD44 receptor, which is located within caveolae and therefore near eNOS.[8] The precise mechanisms responsible for shear-induced NO release are not fully understood but appear to require multiple components of the EG. Without an intact EG, the vasculature cannot appropriately respond to hemodynamic forces, which could lead to direct mechanical damage to the EC and the inability to regulate vascular tone. The EG is also capable of adaptively reorganizing its structure under conditions of high flow. During short-term exposure to shear stress, for example, glypican-1 with attached HS side chains moves from the central region of the EC to the intercellular junctions.[102,104] This reorganization of the EG under shear stress conditions may benefit the ECs as a method to attenuate injury due to shearing forces.[102,104,106] Mechanotransduction is yet another key role the EG plays in health allowing for a dynamic vasculature system able to respond and adapt to local conditions.

2.4 | Effects of an altered or damaged EG

Given the robust and diverse role the EG plays in health, damage during disease states leads to serious sequelae such as increased vascular permeability and interstitial edema, development of a pro-inflammatory state, alterations in coagulation, and an inability to regulate vascular tone. Each of these consequences of EG degradation can have a profound impact on the critically ill patient (Figure 3B).

2.4.1 | Alteration of vascular permeability and transvascular fluid flux

Damage to the EG can lead to alterations in transvascular fluid movement, capillary leak, and the development of edema. As previously

discussed, the large COP gradient across the EG supports retention of fluid within the vascular lumen. When the EG is damaged, this COP gradient is reduced or lost and fluid movement becomes more dependent on intravascular HP and the transendothelial HP gradient. Edema can be detrimental in any tissue; however, it can have more serious and potentially fatal consequences when it occurs in the myocardium, lungs,[107] or brain[108,109]; coronary EG loss has been shown to result in myocardial edema.[44]

The destruction of the EG also results in the loss of its molecular sieving properties. This leads to the extravasation of large macromolecules into the interstitium, which decreases intravascular COP and increases interstitial COP. In severe vasculitis, the relevant COP gradient is then between the plasma and the perivascular (interstitial) fluid (ie, the old Starling hypothesis) rather than between the plasma and the SGS.[64] Vascular inflammation is associated with the loss of the normally uniform, 3-dimensional structure of the EG.[72] This loss means that inflamed vessels develop larger gaps in the EG leading to increased permeability. An increase in the extravasation of fluid and macromolecules into the interstitium occurs after EG removal by various agonists.[49,91,110] Syndecan-1 knockout mice exhibit increased vascular permeability and fluid extravasation compared to wild-type mice.[9] Taken together, these studies highlight the important role of the EG in maintaining transvascular fluid movement and the effect of EG degradation on development of capillary leak and tissue edema.

2.4.2 | Regulation of endothelial cell and circulating cell interactions

In health, the EG shields numerous WBC adhesion molecules from circulating cells and creates an anti-inflammatory phenotype. During inflammation, loss or thinning of the EG is physiologically advantageous by exposing the adhesion molecules required for WBC transmigration and, ultimately, the resolution of infection or tissue injury.[3] With the loss of the EG, the endothelium, therefore, changes from an anti-inflammatory phenotype to a proinflammatory phenotype.[88] With widespread loss of the EG or with persistent activation of the endothelium, this adaptive response can become detrimental.[111] The endothelium in SDC1 knockout mice has been demonstrated to be proinflammatory in nature, characterized by increased cytokine expression and WBC adhesion.[9] Excessive WBC adhesion can become detrimental as it physically obstructs the vascular lumen, particularly within microvessels.[112] This obstruction increases resistance to blood flow and, if widespread, microvascular dysfunction can occur.[113]

Freely circulating GAGs shed from the EG may also directly affect inflammation in critical illness.[3,114,115] Circulating GAGs can bind and impede the action of locally released antimicrobial peptides and activated complement fragments, thus, impairing the body's innate defense mechanisms. An in vitro study demonstrated that GAG concentrations >10 μg/mL, a concentration commonly seen in sepsis, impaired the normal antibacterial activity of plasma.[114] Therefore, freely circulating GAGs could be associated with a reduced ability to clear infection in sepsis. Additionally, circulating GAG fragments may act as local and distant inflammatory stimulators and activate both the innate and adaptive immune systems.[115] Circulating GAGs potentiate ongoing inflammation by binding to various molecules and, thus, triggering the release of more proinflammatory mediators, such as chemokines, from within the ESL.[3] In summary, EG reduction in disease states can play a significant role in altering the progression of inflammation, but this role is highly complex and paradoxically can be both proinflammatory and anti-inflammatory in nature. The specific mechanisms behind the EG and inflammation remain to be fully elucidated.

The loss of the EG is a key step in the transition of the endothelium from an antithrombotic to a prothrombotic state that is common in critical illness.[46,58] As previously discussed, the EG physically shields the circulating blood from platelet adhesion molecules on the EC surface. Loss of the EG results in an increase in platelet adhesion and markers of hypercoagulability.[46,58] Two possible hypotheses for the increased adhesion are the loss of antiplatelet substances (eg, NO) or more importantly the unmasking of platelet-endothelial adhesion molecules.[46] Additionally, increased platelet-endothelial binding triggers EC activation resulting in activation of WBCs and complement.[58] With EG loss the anticoagulant molecules, including AT and TFPI, which are concentrated within the EG, are shed into the systemic circulation. These shed anticoagulants may then act at distant sites and contribute to a generalized hypocoagulable state as described in both sepsis and severe trauma.[116-118] The destruction of the EG is an important factor in the transition of the EC to a procoagulant phenotype; however, the interplay between EG and coagulation remains to be fully understood, as it is further complicated by concurrent inflammation and by variability among tissues and over time.

2.4.3 | Mechanotransduction

Loss of the EG leads to a reduction in normal vascular reactivity preventing required alterations in vessel tone. Syndecan-1 knockout mice had a reduced ability to regulate vascular tone.[9] Additionally, animal septic shock models demonstrate that EG shedding is associated with reduced vascular reactivity and an increased requirement for vasopressor use.[119,120] However, loss of vasoreactivity in disease states is multifaceted and the EG is likely not solely responsible for this pathology.

The loss of the EG in critical illness can have a dramatic effect on the vascular, immune, and coagulation systems. The shedding of the EG plays a key role in the pathophysiology of many conditions affecting the critical care patient. Given this link, there is increasing research into how EG dysfunction contributes to critical illness and how damage to the EG can be determined in an individual patient.

2.5 | Biomarkers of endothelial glycocalyx degradation

Currently, direct visualization of the EG within a living patient is challenging and limited to IM of sublingual vessels. Circulating EG degradation products as objective biomarkers to identify EG alterations or dysfunction are therefore of particular interest.

Shedding of EG, implied by increased concentrations of non-EC associated EG components in cell culture supernatant or in circulating blood, occurs due to the activation of various intracellular and membrane bound enzymes. Matrix metalloproteinase (MMPs) are a family of cell surface proteases that are responsible for degrading extracellular matrices.[121] MMPs are thought to be some of the key enzymes responsible for the shedding of the EG in a variety of diseases ranging from diabetes to inflammatory conditions.[49,93,113,122,123] Syndecan-1, HS, CS, and HA are all considered valid markers of EG integrity and applied in both experimental and clinical research to identify EG shedding.[18,120,124-126]

Continuous shedding and subsequent biosynthesis of new EG components occurs as part of normal EG physiology.[127] However, shedding is exaggerated under pathological conditions with multiple processes being investigated as triggers of EG shedding and dysfunction. These conditions include systemic inflammatory response syndrome (SIRS) and sepsis,[33,87,94,97,99,128] trauma,[116,129–131] ischemia and reperfusion (I-R) injury,[3,64,132] hyperglycemia,[133] hypervolemia,[98,134] and major surgery.[94,18,135]

3 | DISEASE STATES LEADING TO GLYCOCALYX DYSFUNCTION

3.1 | Systemic inflammatory response syndrome and sepsis

An increase in circulating biomarkers of EG degradation, such as SDC1, HS, and HA, occurs in animals and people with SIRS or sepsis.[18,117,126,128,136-139] IM has allowed for the direct visualization of EG reduction in people with sepsis.[5,54] Unfortunately, there are currently no clinical studies investigating the presence of biomarkers of EG degradation in dogs or cats suffering from sepsis or SIRS. Dogs with experimental, endotoxin-induced sepsis had an increase in both plasma SDC1 and HS, which suggests a similar link between sepsis and EG degradation in the species.[138] Because that study had some limitations, including experimental design and small sample size, it remains unclear how the results relate to naturally occurring sepsis and prospective clinical veterinary studies are warranted.

Multiple molecules have been identified as possible instigators of EG degradation during sepsis, including tumor necrosis factor-α (TNF-α), ROS, MMPs, C-reactive protein (CRP), endogenous catecholamines, and heparanases. Given the highly heterogeneous nature of sepsis and its complicated pathophysiology, the underlying mechanism for EG degradation likely includes multiple pathways.

The proinflammatory cytokine TNF-α has been linked to the degradation of the EG in sepsis.[45,49,140,141] In a hamster model, TNF-α administration led to EG shedding, which resulted in the penetration of normally excluded macromolecules, such as dextran 70, into the ESL and an increase in the intraluminal volume occupied by RBCs.[91] In people, experimental endotoxin infusion resulted in a 50% reduction in the thickness of the EG as determined by sublingual IM and concurrent activation of the inflammatory and coagulation systems.[140] Inhibition of TNF-α attenuated EG loss leading to the conclusion that TNF-α plays at least a partial role in EG degradation during inflammation.[140] Similarly, in a porcine endotoxemia model, endotoxin administration led to a marked increase in the concentration of the EG degradation product HS. In this study, there was also a concurrent increase in the concentration of TNF-α and it was speculated that TNF-α may be ultimately responsible for the degradation of the EG.[119] MMPs are important in tissue remodeling in many disease processes and their role in EG shedding has been studied in inflammatory states.[93,113,122] During inflammation TNF-α release may activate MMPs and lead to degradation of the EG.[122] In a rat endotoxemia model, lipopolysaccharide (LPS) induced the expression of MMPs, which directly led to the degradation of the EG.[123] Irrespective of whether TNF-α or MMPs are directly or indirectly responsible for EG degradation in inflammatory conditions, they both appear to play an important role.

CRP is a commonly used marker of inflammation, but has also been implicated in directly contributing to EG degradation in sepsis.[125] Both in vivo and in vitro exposure of EC to increased CRP concentrations was associated with shedding of both HS and HA.[125] This shedding resulted in a greater than 50% reduction in EG thickness and altered EC vasoreactivity, suggesting that CRP may not only be a marker of inflammation but an active contributor to the associated evolution of vascular dysfunction.[125]

Increased sympathoadrenal activation in patients with sepsis may also be responsible for EG degradation. In people with sepsis, concentrations of circulating EG degradation products were associated with increased plasma catecholamines.[117,136,137] Hence, a large catecholamine surge may be partly responsible for EG degradation in sepsis. The association between catecholamines and EG degradation is supported by a study comparing naturally acquired sepsis to experimentally induced endotoxemia in people.[117] Subjects with LPS injection-induced endotoxemia did not have detectable sympathoadrenal activation (measured by an unchanged concentration of plasma catecholamine concentrations from baseline) or evidence of EG degradation (unchanged level of SDC1) compared to patients with naturally acquired sepsis, who had significant elevations in both. Furthermore, in people with naturally occurring sepsis, the level of increase in both catecholamine and SDC1 concentrations was correlated with disease severity.[117] This study highlights the complex role of catecholamines in sepsis and the likely multifactorial mechanism of EG loss in this condition.[117]

Heparanase, an enzyme specific for the cleavage of HS, is implicated in the development of acute lung injury (ALI) in septic patients. In an experimental murine model of ALI, LPS administration led to the loss of pulmonary HS with concurrent increase in the expression of heparanase on the pulmonary endothelial surface.[49] These changes coincided with an increase in pulmonary capillary permeability and neutrophil extravasation consistent with ALI. Similarly, in the same study, alveolar biopsies from people with ALI were evaluated and revealed an 8-fold higher heparanase concentration in diseased lung tissue compared to controls.[49] It is speculated that heparanase expression is induced by ECs after their stimulation by circulating molecules termed “danger signals.” Heparanases then partially degrade the EG

allowing the exposure of EC leukocyte adhesion molecules to facilitate WBC diapedesis, which ultimately clears the insult.[49] The term "danger signals" refers to either endogenous molecules or molecular structures produced or released from cells that are damaged or undergoing cell death, or to exogenous molecules from pathogens, both of which, activate the immune system.[142,143] During sepsis, where there is a large concentration of circulating danger signals, there may be diffuse and persistent stimulation of pulmonary heparanase expression, which may lead to widespread pulmonary EG shedding.[49]

Loss of the EG in sepsis has serious systemic effects: development of tissue edema, excessive inflammation, alterations in coagulation, and reduced vasomotor function. A porcine endotoxemia model demonstrated that animals with increased concentrations of circulating EG degradation products required a 3-fold increase in IV fluid volume and administration of norepinephrine to maintain appropriate hemodynamics.[119] During sepsis, shedding of the EG led to reduced vasoreactivity and an increase in the expression of WBC adhesion molecules contributing to a proinflammatory state.[54,125] In a rodent ALI model, the widespread loss of the pulmonary EG was followed by a large increase in endothelial WBC adhesion with a resultant increase in pulmonary endothelial permeability and pulmonary edema.[49] Similarly, a prospective study of sepsis in people found that of patients with a high SDC1 concentration at presentation, 61% required intubation after receiving >4 L of IV fluids compared to 30% in those who received <4 L of IV fluids. This study suggests a possible clinical link between loss of the EG in sepsis and increased fluid extravasation, in this case the development of pulmonary edema.[144] Based on these findings, the identification of high EG biomarkers at presentation to the ICU may be useful to help identify patients at risk of harm from large volume fluid resuscitation and who may benefit from early vasopressor support.

Coagulation during sepsis is complex. Initial EC activation has been associated with an initial period of hypercoagulability which can later progress to hypocoagulability.[145] In 2 prospective studies of people with sepsis there was an association between increased EG biomarkers and a hypocoagulable state as identified by thromoboelastography.[117,128] These studies identify an association but do not determine causality between increased EG degradation products and the development of hypocoagulability in sepsis. Further studies are needed to better understand coagulation changes in sepsis and possible association with EG dysfunction.

Biomarkers of EG dysfunction may also be useful to aid prognostication in patients with sepsis. People with respiratory failure due to various causes requiring mechanical ventilation had increased plasma concentrations of HS, which were correlated with length of ICU stay. Furthermore, the type of circulating GAG was associated with the underlying cause of respiratory failure, with high HS concentrations found in patients with indirect lung injury and high HA concentrations in those with direct lung injury compared to control samples.[107] Multiple studies in people with sepsis have demonstrated an association between increased levels of plasma EG biomarkers and an increase in mortality.[114,126,136,144] A study of people with severe sepsis and septic shock demonstrated that the concentrations of HA and SDC1 were 1,000 times greater in the severe sepsis group compared to a control group and up to 3 times greater than the sepsis group. In the same study, HA and SDC1 concentrations were able to differentiate between survivors and nonsurvivors, with a specificity of 90% and 86%, respectively.[126] The use of GAG biomarkers in the urine, rather than plasma, has also been investigated. In a prospective study of human patients with septic shock and acute respiratory distress syndrome, increased urinary GAG concentrations were associated with higher mortality. Furthermore, the presence of urinary GAGs in patients with acute respiratory distress syndrome and normal baseline renal function predicted the later development of acute kidney injury.[146] It should be noted that there is conflicting evidence in the literature with some studies failing to identify an association between biomarker level and mortality.[18,147] Further research will refine the potential of biomarkers as a prognostic tool.

3.2 | Ischemia reperfusion injury

There is considerable overlap in the pathomechanisms of EG shedding in cases of inflammation and during I-R injury. It was originally demonstrated in a murine model of I-R that this injury was associated with the loss of EG components.[3] Major vascular surgeries, such as coronary artery bypass surgery, have been associated with the development of I-R injury and a concurrent increase in EG degradation products.[124,135,148] In companion animals, I-R is clinically relevant in the setting of postcardiac arrest (PCA) care and cases of thromboembolic disease, particularly feline aortic thromboembolism.

Postcardiac arrest syndrome is associated with the exposure of the body to widespread I-R injury[149] and an increase in EG degradation biomarkers has been demonstrated in this condition.[132,150,151] In comatose PCA human patients, higher admission lactate concentrations were associated with increased SDC1 concentrations.[132] Similarly, in a study of people suffering out-of-hospital cardiac arrest, the authors found that a longer time from cardiac arrest to return of spontaneous circulation and a lower arterial pH were associated with increased SDC1 levels.[151] Both of these findings suggest that global I-R may be the cause for EG degradation following cardiac arrest. Additionally, increased circulating catecholamine concentrations have also been associated with EG degradation in PCA human patients.[151] The loss of the EG likely contributes to the common sequelae seen in these cases, namely increased vascular permeability, hyperinflammation, coagulopathy, and reduced vascular responsivness.[152] Further research needs to be conducted to fully understand the roles of the EG that may play in these disease conditions and whether therapeutic measures to preserve the EG may lead to improved outcomes.

3.3 | Trauma and hemorrhage

There is increasing evidence to suggest that damage to the EG plays a role in the pathophysiology of trauma. In people, clinical studies have demonstrated an increase in EG biomarkers, including HS, HA, SDC1, and CS, after trauma or hemorrhagic shock.[130,153] Unfortunately, there are currently no clinical veterinary studies investigating the link between EG degradation and trauma; however, animal

hemorrhagic shock models have demonstrated EG loss after serious hemorrhage.[154,155]

Similar to sepsis, a suggested mechanism for EG degradation in trauma is related to the large catecholamine release and tissue hypoperfusion that occurs with shock; it is hypothesized that this leads to the direct loss of the EG.[129,156] The link between sympathetic stimulation and EG degradation in trauma was demonstrated experimentally in murine hemorrhagic shock models that used either chemical sympathectomy[157] or beta-blockade to investigate the catecholamine effect.[158] In both studies, a reduction in the concentration of SDC1 occurred in the treated group compared to the nontreated group.[157,158] In addition, the concentration of TNF-α increased from baseline 1 hour after hemorrhage was induced and this increase was abolished with chemical sympathectomy. This finding indicates that catecholamine release activates the inflammatory response in trauma and induction of TNF-α may be the mechanism for EG loss as previously hypothesized.[157] In experimental animal shock models and clinical human trials, a large catecholamine surge and tissue hypoperfusion that occur in shock appear to be associated with widespread loss of the EG.[129,155] Massive EG loss in trauma is associated with increased vascular permeability, increased systemic inflammation, hypocoagulability, and reduced vascular responsivess.[116,129,130]

Of particular interest is the relationship between EG dysfunction and development of coagulation abnormalities in trauma. Acute traumatic coagulopathy has a complex and incompletely understood pathophysiology that is beyond the scope of this review.[159] However, a correlation between increased EG biomarkers and hypocoagulability in human trauma patients has been demonstrated, which suggests that EG damage may play a role in acute traumatic coagulopathy.[129,155] One theory is that GAGs, particularly HS and CS, which are shed after a traumatic insult, act systemically as anticoagulants; this phenomenon has been termed "endogenous heparinization."[116,160] In 2 prospective studies of human trauma patients, increased SDC1, used as a marker of EG degradation, was associated with endogenous heparinization as evidenced by a hypocoagulable thromboelastography tracing.[116,118] Patients with endogenous heparinization had a higher transfusion requirement and higher mortality compared to patients with a normal thromboelastography tracing.[116] It is important to note that traumatic coagulopathy may represent an adaptive evolutionary response that in some cases becomes maladaptive when it is severe, unregulated, and widespread or when it is exacerbated by medical interventions such as fluid therapy.[116,161] It is hypothesized that after trauma creating an increasingly hypocoagulable state in the circulating blood through endogenous heparinization counterbalances the proinflammatory and procoagulant state of an activated endothelium, thereby reducing clot formation and maintaining perfusion through the microcirculation.[116,161] Furthermore, shedding of the EG may also be advantageous as it allows for increased vascular permeability and shifting of fluid from the intravascular to extravascular space. This loss of intravascular fluid reduces blood pressure, therefore, reducing ongoing hemorrhage and the shifting of fluid to the extravascular space allows for a pool of fluid for later mobilization should survival occur.[116,161] Given the importance of trauma, further research is required to better understand the underlying pathophysiology and to determine when this potentially adaptive response becomes maladaptive. In the future, therapies to preserve the EG may aid in reducing morbidity and mortality in trauma patients and irrespective of the mechanisms involved, increased EG biomarkers may prove useful as prognostic tools in patients suffering from trauma.[118,129,156]

3.4 | Hypervolemia

The detrimental effects of hypervolemia are becoming increasingly recognized in human and veterinary medicine. Hypervolemia leads to atrial distension and thus to the release of atrial natriuretic peptide (ANP) from atrial myocytes. ANP acts through multiple mechanisms to reduce intravascular volume including vasodilating individual vascular beds, increasing renal excretion of fluid, and increasing vascular permeability.[162] ANP release has been demonstrated to lead to EG shedding resulting in the extravasation of fluid and colloids from the vasculature.[110] In an experimental study using perfused guinea pig hearts, administration of ANP led to an 18-fold increase in SDC1 and an increase in the volume of fluid extravasation compared to the control group. In the same study, the EG of the coronary vessels was examined with electron microscopy: in the control group, a 0.2–0.3 μm EG was measured whereas after treatment with ANP nearly no measurable EG could be visualized.[163] Similarly, in a study in people undergoing volume loading prior to surgery, the resultant hypervolemia lead to ANP release and a subsequent increase in the concentration of circulating EG degradation products.[162] The loss of the EG as a result of hypervolemia and the resultant increase in fluid extravasation may lead to detrimental edema formation and reduced oxygenation. Multiple studies in human medicine[164-167] and veterinary medicine[168] have shown that fluid overload is associated with increased morbidity and mortality. These findings reemphasize the fact that resuscitative fluid therapy is not a benign intervention and call for a judicious and rational approach to such treatment.

3.5 | Hyperglycemia

Transient and chronic hyperglycemia also leads to EG degradation. An experimental study showed that hyperglycemia exposure of ECs in vitro resulted in HS loss from the EG.[169] This affected normal mechanotransduction with a reduced ability of ECs to sense and respond to changes in shear forces, which was in part due to lower eNOS activation. These findings suggest a link between hyperglycemia-induced EG degradation and an increased risk of cardiovascular disease in patients with diabetes mellitus.[169] Similar results were demonstrated in a clinical study of people with type 1 diabetes mellitus.[170] In this study, the EG of sublingual capillaries was visualized using orthogonal polarization spectral imaging and showed that the EG in patients with type 1 diabetes mellitus was approximately half as thick as that in healthy controls, and that this thinning coincided with increased circulating concentrations of HA in the diabetic group.[170] Acute hyperglycemia appears to result in similar changes, with the induction of hyperglycemia in human subjects leading to a

50% reduction in the volume of the EG and a concurrent increase in the concentration of HA in the plasma.[133] The authors of this study speculated that the loss of the EG in hyperglycemic patients occurred at least in part due to the production of ROS.[133]

Knowledge gaps remain in the current understanding of the EG in many pathological conditions affecting the critically ill patient. Much of the available research is either experimental utilizing animal models for specific conditions or is limited to early prospective, observational studies in people. Despite these gaps, it remains clear that the EG plays an important role in the pathophysiology of many critical illnesses. Further research needs to be conducted to evaluate dysfunction of the EG in the setting of critically ill companion animals. Such research may provide important insights into the development, progression, and treatment of many critical illnesses in veterinary species.

4 | THERAPEUTIC INTERVENTIONS TO PRESERVE OR RESTORE THE ENDOTHELIAL GLYCOCALYX

Given the emerging understanding of the importance of the EG in the pathophysiology of critical illness, it is a logical potential therapeutic target. Studies investigating how the EG can be modified, preserved, or repaired to aid in the treatment of various critical illnesses are ongoing, but most remain investigative in nature at this time.

The use of antioxidants has been examined as a method to reduce EG shedding. In a study of acute hyperglycemia leading to EG loss, pretreatment with the antioxidant N-acetylcysteine reduced EG shedding. However, the protective effects of N-acetylcysteine were only present when it was administered prior to hyperglycemia and before EG loss.[133]

In a rat endotoxemia model, the use of activated protein C (APC) was associated with a reduction in EG loss.[120] Furthermore, APC led to reduced markers of endothelial oxidative stress, improved microcirculatory function,[120] and an improved response to vasopressor therapy[171] in animal endotoxemia models. However, human clinical studies have failed to consistently find an overall mortality benefit with the use of recombinant APC in severe sepsis and septic shock. Septic people treated with APC had an increased risk of developing bleeding complications, and the drug is currently no longer commercially available.[172-175] With the improved understanding of the EG, revised treatment strategies with APC may prove promising.

The administration of exogenous GAGs to repair the EG is another area under investigation. Experimental IV infusion with HS reduced WBC adhesion to the EC surface after EG degradation induced by oxidizing agent injection. The reduced WBC adhesion was hypothesized to occur due to HS binding to the EC surface, thereby increasing the thickness of the EG, reinstating the EG's net negative charge and, thus, repelling circulating cells.[87] This experimental model suggests that exogenous GAGs may help reconstitute the EG after shedding; however, how this translates to a clinical population remains unknown. Diabetes mellitus in people is a condition known to be associated with EG thinning, and treatment with soludexide (glucuronylGAG sulfate) for 8 weeks resulted in an increase in the thickness of the EG in the treatment group compared to controls.[176] Soludexide is a commercially available oral medication composed of 4 different GAG components: CS, DS, "slow-moving heparin," and "fast-moving heparin."[177] Soludexide provides the precursor components of GAGs and therefore may aid in the reformation of the EG. Soludexide or administration of other exogenous GAGs would need to thicken the EG and improve its function more quickly than 8 weeks to be clinically relevant to the critical care population. A novel therapy (EC-SEAL) to reduce deep vein thrombosis formation is in the development phase. The drug is composed of a synthetic GAG, DS, and multiple selectin binding sites.[178] EC-SEAL utilizes its selectin binding sites to bind to activated or inflamed endothelium characterized by an increased expression of selectins on the luminal cell surface. Binding by EC-SEAL could restore the protective covering over platelet adhesion molecules and limit thrombus generation. In a mouse model of deep vein thrombosis, IV administration of EC-SEAL reduced thrombus formation as assessed by vessel ultrasonography.[178]

The use of heparin to reduce EG shedding has been investigated. In a canine septic shock model, treatment with unfractionated heparin attenuated shedding of SDC1 and HS and improved mean arterial pressure, cardiac index, and urine output.[138] The underlying pathophysiology of this "protective" mechanism remains unknown, but the authors speculate that unfractionated heparin mobilizes intracellular pools of SDC1, leading to reformation of the EG. Furthermore, unfractionated heparin may inhibit heparanase, which is known to cleave HS in sepsis.[49,138] The use of low molecular weight heparin (LMWH) has also been investigated. In an experimental study in rats with SIRS, a dose-dependent reduction in EG shedding and reduced endothelial leukocyte interactions resulted from infusion with LMWH. It is hypothesized that LMWH binds to components of the EG, such as HS, and inhibits or reduces the release of heparanase from the EC.[179]

It is well described that the ESL, including its incorporated plasma proteins, rather than the EG per se, is the in vivo structure responsible for normal vascular integrity. Treatment with plasma proteins may therefore aid in EG reconstitution. The infusion of 5% human albumin led to a reduction in the extravasation of fluid after I-R injury. Albumin appears to be able to penetrate and bind within the EG thereby reforming the ESL and restoring vascular integrity. In comparison, HES only partially improved the ESL barrier, being superior to crystalloids alone but inferior to albumin.[29] Conversely, in a randomized controlled trial of people undergoing coronary artery bypass graft placement, the administration of HES was associated with increased EG degradation, as measured by plasma SDC1 concentrations, compared to the use of crystalloids.[180] The balance of evidence suggests that synthetic colloids are not superior to crystalloids for the preservation of the EG, calling into question the clinical utility of HES. It is possible that providing albumin to reconstitute the EG in critical illness could help restore microvascular barrier function, but the appropriate albumin dose to achieve a beneficial effect remains unknown. Furthermore, it is also not possible to know, prior to administration of albumin, whether any EG scaffolding remains to absorb albumin.[31] Therefore, a risk remains that endogenous or synthetic colloid administration

could lead to the extravasation of these macromolecules into the interstitium and potentially worsen edema. There is limited availability of canine concentrated albumin solutions and human albumin may be harmful.[181-183]

Fresh frozen plasma (FFP) has been studied extensively as a treatment for severe hemorrhage, but less research has been done in regards to its efficacy in repairing the EG. Animal hemorrhagic shock models have demonstrated that fluid resuscitation with FFP compared to crystalloids successfully restores the EG.[184,185] The use of FFP leads to improved microhemodynamics, vascular hemostasis, and reduced leukocyte-endothelium interaction compared to crystalloids or synthetic colloids.[154,185] There was also a reduction in pulmonary hyperpermeability and secondary lung injury in animals resuscitated with FFP compared to those resuscitated with crystalloids.[154,185] One possible mechanism is that FFP may restore the structural scaffolding of the EG by replacing SDC1 and preventing its further loss.[185] Furthermore, FFP also contains albumin and other plasma proteins, such as fibrinogen, known to be important in maintaining the ESL.[154,185] Taken together, these studies indicate that the choice of fluids selected for intravascular volume expansion has an impact on the EG, either leading to improvements in the EG or conversely leading to additional injury. The use of FFP may be beneficial as a method to restore the ESL.

The use of protease inhibitors, such as AT, is also a promising therapeutic modality. Antithrombin, a serine protease inhibitor, is concentrated in the EG, inhibits coagulation, and has anti-inflammatory properties. In experimental I-R and sepsis models, treatment with AT reduced EG shedding and attenuated vascular permeability and tissue edema. There are multiple possible mechanisms for the protective effects of AT, including inhibition of cleaving enzymes such as heparanase at the site of inflammation, thrombin reduction (because thrombin has been shown to increase heparanase concentrations), and a reduction in the amount of heparanase released from mast cells during inflammation.[45,48,186] Antithrombin is found in FFP and therefore treatment with FFP may promote repair of the EG by multiple methods. However, the use of AT in critical illness remains controversial. A randomized controlled trial including people with severe sepsis and septic shock failed to demonstrate an overall mortality benefit with the use of AT, although a trend to increased survival was identified for a subpopulation not concurrently treated with heparin.[187] In a retrospective study, including people with sepsis and disseminated intravascular coagulation, the use of AT was associated with improved 28-day survival.[188] Given the lack of conclusive evidence for the benefit of AT and the documented risk for clinically significant hemorrhage, the 2017 Surviving Sepsis Campaign Guidelines recommend against the use of AT in sepsis and septic shock.[172]

The antimicrobial doxycycline has functions beyond its antimicrobial activity and has been studied as a treatment to preserve the EG. At subantimicrobial doses, doxycycline reduces EG shedding through inhibition of MMPs.[123,189] As discussed, MMPs have been implicated in EG degradation for various conditions. Therefore, inhibition of MMPs through the use of doxycycline may emerge as a clinically useful treatment to support EG preservation.[123,189]

Finally, glucocorticoid administration has reduced EG shedding in the face of various agonists known to damage the EG.[45,123] The mechanisms underlying these protective effects are incompletely understood and are likely multifactorial. In a rodent I-R injury model, treatment with hydrocortisone reduced EG shedding and protected against edema formation.[190] A suggested mechanism for this protective effect was stabilization of mast cells because their degranulation releases many proteases that can degrade the EG. Mast cell stabilization could also be a mechanism for the protective effects of glucocorticoids in sepsis since TNF-α, one of the primary cytokines implicated in sepsis, causes mast cell degranulation.[45] In a rodent sepsis model, treatment with dexamethasone reduced EG shedding and the authors postulated that the protective effects may be linked to the suppression of MMPs, a key protease involved in EG degradation in sepsis.[123] Further research is required to determine both how and at what dose glucocorticoids provide their protective effects and to determine their clinical utility. Currently, the Surviving Sepsis Campaign Guidelines do not advocate the use of corticosteroids in sepsis, but the potential benefits to the EG may reinvigorate this debate.[172] These therapeutic modalities all offer a potentially promising avenue for attenuation of EG dysfunction; however, further clinical studies need to be conducted to confirm these findings.

5 | CONCLUSIONS

The EG is a complex, ubiquitous, fragile structure lining the largest organ system in the body, the endothelium. Since the EG was first discovered, there has been mounting evidence to support its crucial role in health and in the pathophysiology of many important conditions affecting people and companion animals. Ongoing research into the EG will help to further elucidate the pathophysiology of conditions like sepsis and may also provide a promising area for novel therapeutic interventions to reduce the morbidity and mortality of many critical illnesses. Clinical veterinary research is currently lacking in regards to the EG; however, the EG should not be overlooked as an important system affecting the critically ill patient.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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