



Alterations in heparan sulfate proteoglycan synthesis and sulfation and the impact on vascular endothelial function

Danielle Pretorius^{a1}, Robert P. Richter^{b,c1}, Tanya Anand^d,
Jessica C. Cardenas^{e,f} and Jillian R. Richter^{a,c*}

a - Division of Trauma & Acute Care Surgery, Department of Surgery, University of Alabama at Birmingham, Birmingham, AL, United States

b - Division of Pediatric Critical Care Medicine, Department of Pediatrics, University of Alabama at Birmingham, Birmingham, AL, United States

c - Center for Injury Science, University of Alabama at Birmingham, Birmingham, AL, United States

d - Division of Trauma, Critical Care, Burn & Emergency Surgery, Department of Surgery, University of Arizona, Tucson, AZ, United States

e - Division of Acute Care Surgery, Department of Surgery, McGovern Medical School, University of Texas Health Science Center at Houston, Houston, TX, United States

f - Center for Translational Injury Research, University of Texas Health Science Center at Houston, Houston, TX, United States

Correspondence to Jillian R. Richter: Tinsley Harrison Tower 1042, 1900 University Boulevard, Birmingham, AL 35233, United States. jillianrichter@uabmc.edu (J.R. Richter)
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Abstract

The glycocalyx attached to the apical surface of vascular endothelial cells is a rich network of proteoglycans, glycosaminoglycans, and glycoproteins with instrumental roles in vascular homeostasis. Given their molecular complexity and ability to interact with the intra- and extracellular environment, heparan sulfate proteoglycans uniquely contribute to the glycocalyx's role in regulating endothelial permeability, mechanosignaling, and ligand recognition by cognate cell surface receptors. Much attention has recently been devoted to the enzymatic shedding of heparan sulfate proteoglycans from the endothelial glycocalyx and its impact on vascular function. However, other molecular modifications to heparan sulfate proteoglycans are possible and may have equal or complementary clinical significance. In this narrative review, we focus on putative mechanisms driving non-proteolytic changes in heparan sulfate proteoglycan expression and alterations in the sulfation of heparan sulfate side chains within the endothelial glycocalyx. We then discuss how these specific changes to the endothelial glycocalyx impact endothelial cell function and highlight therapeutic strategies to target or potentially reverse these pathologic changes.

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Introduction

The endothelial glycocalyx (eGCX) is a highly dynamic, gel-like coating anchored to the apical surface of vascular endothelial cells with myriad functions in maintaining blood vessel homeostasis. The delicate eGCX is comprised of heparan sulfate (HS) proteoglycans (HSPG),

hyaluronan, and sialic acid-capped glycoproteins that contribute uniquely to the regulation of vascular permeability, immune cell trafficking, hemostasis, and vascular tone [1,2]. HSPGs, in particular, have critical roles in homeostatic endothelial cell signaling given their unique structures and interactions with both the intra- and extracellular environments [3]. Therefore, it is not surprising that

pathological modifications to eGCX HSPGs promote endothelial dysfunction and vascular disease.

Research into the structure and function of HSPGs in the context of normal endothelial cell biology burgeoned in the 1980s and 1990s as summarized in reviews by Rosenberg *et al.* [4] and Weinbaum *et al.* [5]. Over the last 20 years, advances in our understanding of vascular biology have brought about greater clarity around the roles of eGCX-specific HSPGs in cardiovascular homeostasis and how HSPG modifications impact endothelial function. The purpose of this narrative review is to provide a modern perspective on the role of endothelial apical surface HSPGs in vascular disease by highlighting the latest research into mechanisms that alter the synthesis and sulfation status of eGCX HSPGs and the untoward effects of these alterations on endothelial functions. In addition, practical and theoretical treatment strategies to improve HSPG integrity are discussed and remaining knowledge gaps identified to help inform future research efforts.

Overview of eGCX HSPG structure, synthesis, and function

The major HSPGs identified within the eGCX are syndecans (SDC) (subtypes 1–4) and glypican (GPC) (subtype 1 only) [4,6–8]. Membrane-spanning SDCs are primarily differentiated from GPCs by their single-pass, highly conserved transmembrane domains and their cytoplasmic endodomains that anchor to cytoskeletal proteins, allowing direct interaction with signaling molecules such as protein kinase C, synectin/syntenin, and calcium/calmodulin-dependent serine protein kinase [4,9]. GPCs, on the other hand, are anchored by glycosylphosphatidylinositol linkages to lipid microdomains in the cell membrane, including caveolae [10,11]. GPCs indirectly participate in intracellular signaling through affiliation with lipid domain- or caveolae-linked effector molecules such as Src family kinases, endothelial nitric oxide synthase (eNOS), and G protein-coupled receptors [12,13]. SDCs are also distinct from GPCs in the location of glycosaminoglycan (GAG) linkages (e.g., heparan sulfate (HS), chondroitin sulfate) within the proteoglycan ectodomain. SDCs express HS attachment sites (potentially 2–5 chains per proteoglycan) near the amino-terminus distal to the cell membrane with some SDC subfamilies (e.g., SDC-1 and –3) expressing chondroitin sulfate side chains near the carboxyl-terminus proximal to the cell membrane [3]. GPCs express only HS chains with 2–3 putative GAG attachments near the ectodomain carboxyl-terminus [14,15].

Endothelial cell synthesis of HSPGs begins in the rough endoplasmic reticulum with the formation of

the core proteoglycan [16]. A xylose (Xyl) molecule is then added to an available serine residue within the proteoglycan HS attachment region by xylosyltransferase (XYLT) in either the endoplasmic reticulum or Golgi [17]. Next, within the Golgi two galactose (Gal) molecules and a D-glucuronic acid (GlcA) molecule are sequentially attached to xylose to form a Xyl-Gal-Gal-GlcA tetrasaccharide linkage [18]. The exostosin (EXT)-like family of glycosyltransferases (EXTL) then transfer an N-acetyl glucosamine (GlcNAc) residue to the linkage tetrasaccharide, committing the molecule to become HS [19]. Then, multiple repeating disaccharide sequences of a hexuronic acid (UA) linked to GlcNAc are attached by sequential [UA(1 → 4) GlcNAc] linkages predominantly by EXT1 with support by EXT2 (generally estimated to be 50–150 disaccharide repeats at an approximate length of 40–125 nm per HS chain) [14,18–20]. The hexuronic acid residue within each disaccharide unit typically begins as GlcA; however, during HS chain lengthening, GlcA may undergo epimerization by D-glucuronyl C5-epimerase to form L-iduronic acid. The unbranched HS chain may undergo further modification by sulfation of varying disaccharide units at the N-, 3-O or 6-O positions on GlcNAc or the 2-O position on the hexuronic acid (Fig. 1). While not all disaccharide units undergo the same pattern of sulfation, addition of sulfate residues typically begins with N-deacetylation and N-sulfation of GlcNAc by N-deacetylase/N-sulfotransferase (NDST). Next, 2-O sulfation of the hexuronic acid may occur by HS 2-O-sulfotransferase (HS2ST). Then, HS 3-O-sulfotransferase (HS3ST) and HS 6-O-sulfotransferase (HS6ST) may attach sulfate residues to GlcNAc at the 3-O and 6-O positions, respectively. There are four isoforms of NDST, one isoform of HS2ST, seven isoforms of HS3ST, and three isoforms of HS6ST that have been identified in humans [21,22]. Once formed, HSPGs are transported from the Golgi to the endothelial cell membrane by secretory vesicle exocytosis [18]. After HSPGs are expressed in the eGCX, endothelial- or leukocyte-derived endosulfatases (Sulf) 1 and 2 may further modify HS side chains on HSPGs through the selective cleavage of 6-O-sulfo groups. The vast permutations in number of disaccharide repeats, hexuronic acid expression, and sulfation patterns within attached HS side chains give rise to extraordinary diversity in HSPG structure at the vascular endothelial surface.

The physicochemical properties (e.g., size, composition, and charge) of each individual HSPG shape its function within the eGCX. The molecular size, complexity, and negative fixed charge density of HSPGs contribute to the eGCX's function as a sentry for circulating cell interactions with luminal surface adhesion molecules and selective permeability to circulating proteins through steric hindrance and electrostatic repulsion [23–25]. Distinct sulfation patterns within

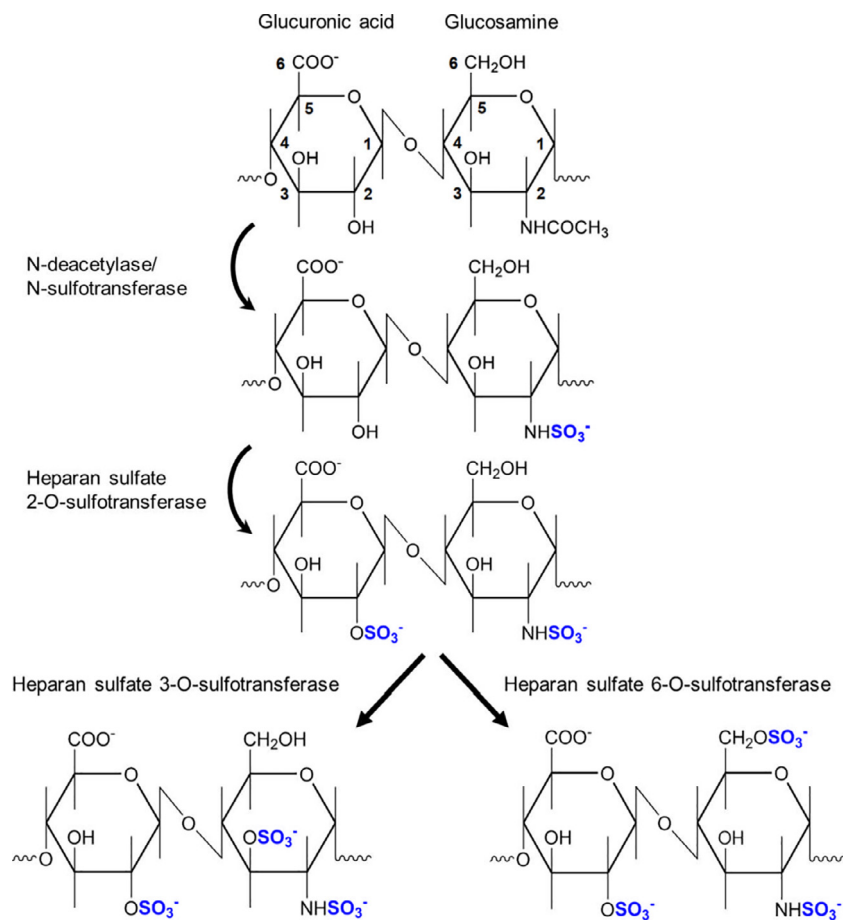


Fig. 1. Heparan sulfate (HS) disaccharide structural modification/sulfation process. Polymerized HS chains attached to core proteoglycans destined for the endothelial surface may undergo several sulfation modifications within the endothelial cell Golgi apparatus prior to apical surface expression. First, *N*-deacetylase/*N*-sulfotransferase replaces the acetyl group for a sulfate at the amino linkage to the second carbon of a glucosamine residue. Next, HS 2-O-sulfotransferase may attach a sulfate group to the hexuronic acid residue (either *D*-glucuronic acid or *L*-iduronic acid). Glucosamine residues within the various disaccharides may undergo further sulfation by HS 3-O-sulfotransferase and/or 6-O-sulfotransferase.

the HS moieties of HSPGs dictate their affinity to bind specific proteins, enabling the eGCX to have a tremendous capacity for interacting with and storing a broad range of circulating and locally derived proteins. Moreover, particular ligands such as fibroblast growth factor (FGF) [26–28] and vascular endothelial growth factor (VEGF) [29–31] engage the HS side chains of eGCX HSPGs as coreceptors to synergize ligand-cognate receptor interactions at the endothelial cell surface to affect putative endothelial signaling (see more specific detail below).

Endothelial surface HSPGs regulate transmural fluid movement in the microcirculation through what is considered the revised Starling principle [32]. In this update to the traditional understanding of the physiologic principles driving microvascular transmural fluid movement, Hu and Weinbaum [1] uncovered that the eGCX adds an additional layer of complexity to transvascular fluid flux by creating an oncotic pressure gradient between the vascular

lumen and the sub-eGCX space through its reflection of plasma macromolecules. Therefore, transvascular fluid flux is not simply dictated by hydrostatic and oncotic pressure gradients between the intra- and extravascular spaces by instead involves a more complex relationship between eGCX integrity, endothelial cell–cell junctional integrity, and changes in intraluminal hydrostatic and oncotic pressure [33–35].

HSPGs are also integral in the transduction of circulatory shear stress to the endothelial intracellular environment, activating signaling mechanisms that support competent endothelial focal adhesions and intercellular junction integrity [36,37] while providing a feed-forward mechanism to support robust eGCX expression [38]. Shear stress transduction through HSPGs also activates eNOS, generating nitric oxide critical to the regulation of surrounding vascular smooth muscle tone [39,40]. Additionally, HSPG binding and activation of anti-coagulant mediators such as antithrombin

helps prevent baseline levels of coagulation and clot formation [41]. In summary, an intact eGCX promotes a local anti-inflammatory, anti-coagulant/anti-thrombotic environment that maintains vascular patency and barrier integrity.

Modifications to eGCX HSPGs

A number of important mechanisms promoting structural and biochemical changes to HSPGs within the vascular eGCX have been uncovered that contribute to aberrant vascular biology. Cleavage of HSPGs and GAGs from the eGCX is a hallmark of vascular damage associated with many pathologic conditions, and plasma levels of shed HS and HSPG ectodomains have been investigated as prognostic biomarkers and measures of injury/illness severity [42–44]. As several recent review manuscripts extensively cover the mechanisms driving eGCX degradation resulting in vasculopathy [45–47], herein we focus on structural eGCX remodeling that arises from alterations in endothelial HSPG biosynthesis and post-translational sulfation modifications to HS.

Mechanisms of impaired eGCX HSPG synthesis

Endothelial GCX integrity is impacted by inflammatory insults, hemodynamic perturbations, and comorbidities that promote variable HSPG synthesis and expression. The local and systemic inflammatory responses to acute insults such as trauma, hemorrhagic shock, burns, or sepsis, for example, generate a cytokine response that augments HSPG synthesis within vascular endothelial cells. In isolated cell culture, tumor necrosis factor alpha (TNF α) has been shown to increase *SDC2* mRNA expression in human primary umbilical vein endothelial cells (HUVEC) but suppress *SDC1* in Ea.hy926 HUVEC-A549 epithelial hybridoma cells [7,48]. Interleukin-1 β , on the other hand, appears to suppress *SDC1* and *SDC2* expression while upregulating *SDC4* in HUVECs [49]. FGF2 and transforming growth factor β 1 have been demonstrated to upregulate *SDC4* mRNA and SDC-4 proteoglycan expression in bovine aortic endothelial cells [50,51]. Similarly, the proline-rich peptide PR-39 released during soft tissue injury has been demonstrated to upregulate both SDC-1 and -4 proteoglycan expression on the surface of bovine adrenal microvascular endothelial cells [52]. In light of the fact that acute inflammation promotes increased HSPG degradation by eGCX-targeting sheddases [53–56], results from these *in vitro* studies may point to potential compensatory mechanisms upregulating HSPG synthesis in the face of increased HSPG turnover. Furthermore, there may also be an interdependence between the expression of specific

HSPGs. Work by Hara *et al.* [57], complemented by studies performed by Vuong *et al.* [49], suggests that endothelial SDC-4 expression may be inversely related to SDC-1 expression. Thus, loss of homeostatic SDC-1 expression within the eGCX may lead to the compensatory upregulation of SDC-4 at the endothelial surface, though the precise mechanism and purpose of this compensatory response remains unclear.

MicroRNA (miRNA) may also contribute to the regulation of endothelial HSPG synthesis. Using a mouse model of acute ischemia/reperfusion, Wu *et al.* [58] discovered that upregulation of miR-19b contributes to lung injury observed following hemorrhage and resuscitation, corresponding with decreased *SDC1* gene expression within lung tissue. The authors corroborated *in vivo* findings by demonstrating a miR-19b-mediated decrease in SDC-1 protein expression in human primary lung microvascular endothelial cells (HLMVEC). However, the mechanisms responsible for miR-19b upregulation following hemorrhagic shock were unresolved. Other work suggests that miR-126, an endothelial-derived microRNA shown to preserve endothelial monolayer integrity [59,60], may increase eGCX SDC-4 expression in an autocrine/paracrine manner [61]. On the other hand, activity by the more ubiquitous miR-149 was shown to diminish eGCX *GPC1* gene and GPC-1 protein expression [62]. However, at present it is unclear how miR-126 or miR-149 are regulated during inflammation and to what degree these microRNAs contribute to HSPG expression during inflammation *in vivo*.

Systemic inflammation may also attenuate HS expression in the eGCX. Yang *et al.* [63] observed that recovery of eGCX thickness within mouse pulmonary microcirculation required 24 h following sterile enzymatic erosion of HS from the eGCX; however, mouse pulmonary microvascular eGCX thinning in a cecal ligation and puncture (CLP) model of sepsis required at least 72 h for recovery. The authors found that delayed eGCX recovery in mice with sepsis was unrelated to ongoing HS shedding but instead occurred in response to impaired FGF receptor-1 (FGFR1) signaling, resulting in decreased *EXT1* expression and decreased HS synthesis. Though further research is needed to clarify how FGFR1 signaling is depressed during sepsis, this work is an important step forward in understanding the mechanisms driving eGCX HS expression and suggests a cell signaling pathway regulating HS synthesis as a potential therapeutic target during systemic inflammation.

Disturbances in shear stress at the endothelial surface likely contribute to variations in eGCX HSPG synthesis and expression. Zeng *et al.* [64] observed that rat fat pad endothelial cells exposed to laminar shear stress express higher eGCX levels of SDC-1, GPC-1, and HS relative to static culture. When exposed to turbulent flow, Harding *et al.* [65]

found that rat fat pad endothelial cells express significantly lower levels of HS within the eGCX relative to laminar flow. Conversely, Russo *et al.* [66] reported that SDC-4 expression and whole cell expression of sulfated GAGs decreased in immortalized rabbit aortic endothelial cells in response to laminar shear stress compared to static culture. Koo *et al.* [67] observed increased HS and SDC-1 expression on the surface of HUVECs conditioned in higher shear stress (~ 12 dynes/cm² [68]) relative to HUVECs maintained in either static culture or near-static flow conditions (~ 1 dyne/cm² [68]). However, these authors found that *GPC1* mRNA and GPC-1 protein expression decreased with higher shear. We similarly observed increased HS expression over HLMVECs conditioned with laminar shear stress (Fig. 2, A and B), concurrent with increased *XYLT1*, *XYLT2*, and *EXT2* gene expression and variably increased expression for *EXTL1-3* (Fig. 2C). Intriguingly, we also found that flow conditioning HLMVECs increased *GPC1* gene expression while relatively uniformly suppressing *SDC1-4* (Fig. 2, C and D). Taken together, these studies suggest that surface shear stress may impact HSPG expression in a manner dependent upon both the magnitude of change in shear stress and the endothelial cell type. Moreover, these studies suggest that changes in the expression of individual HSPG subtypes may not be uniform in response to shear stress alterations. Further investigation is required to clarify how variable flow profiles and shear stress magnitude impact the expression of HSPG subtypes within the eGCX of different circulation beds in humans both during physiologic and pathophysiologic conditions.

Advancing age and development of cardiovascular comorbidities also appear to impact baseline HSPG expression. As blood vessels age, they accrue fibrotic changes and calcifications that gradually diminish vascular compliance [69]. Groundbreaking work by Mahmoud *et al.* [70] revealed that these biomechanical changes in the subendothelial architecture may be an important driver of reduced eGCX thickness by means of reduced GPC-1 expression. However, it remains unknown how vessel stiffness impacts the expression of other HSPGs within the eGCX. Hyperlipidemia may also contribute to changes in endothelial surface HSPG expression as suggested by Kang *et al.* [71] in a study showing that a 12-week high-cholesterol diet caused eGCX thinning and reduction in sulfated GAG content of the eGCX of carotid arteries. Moreover, *GPC1* and *EXT1* gene expression was reduced within the carotid arteries (not endothelial-specific) of high-cholesterol-fed rats while *SDC4* gene expression was unchanged (*SDC1* was not reported). These studies provide important insights into the co-morbidities and pathobiology that may be driving age-related changes to HSPG synthesis and expression within the eGCX.

Mechanisms driving sulfation modifications to HS

Localized and systemic inflammation alters the expression level and activity of sulfotransferases and endosulfatases regulating HS sulfation, which leads to distinct changes in sulfation patterns and bioactivity of HSPGs within the eGCX. While these changes would be predicted to significantly alter putative cell and molecular interactions with eGCX HSPGs, at present relatively little is known about the impact of inflammation on the sulfation of eGCX HSPGs and downstream effects. Here, we present what evidence has more recently come to light about these changes and speculate on potential mechanisms that may be at play.

Rops *et al.* [72] observed that TNF α and interleukin-1 β treatment of mouse glomerular endothelial cells increased expression of *NDST1*, *NDST2*, *HS6ST1* and *HS6ST2* and decreased expression of *SULF2* through the activation of canonical nuclear factor kappa-light-chain-enhancer of activated B cells signaling. These findings corresponded with a higher presence of N- and 6-O-sulfo groups within eGCX HS molecules and were confirmed within the cortices of kidneys from two separate murine models of local nephritic inflammation [72]. Similar sulfation changes to HS were observed in the eGCX of HUVECs in response to TNF α treatment. Carava *et al.* [73] found that the presence of disulfated [UA-GlcNS(6S)] disaccharides increased within the eGCX of HUVECs following a 24-hour treatment with TNF α corresponding with reductions in the amount of monosulfated [UA-GlcNAc(6S)] and [UA-GlcNS] disaccharides and unsulfated disaccharides, suggesting significant increases in NDST and HS6ST activities. Smits *et al.* [74] took these findings a step further, observing that TNF α promoted the expression of the unique [IdoA(2S)-GlcNS(6S)]₃ hexasaccharide motif within eGCX HS molecules on HUVECs (rarely observed in healthy human tissue) that is instrumental for leukocyte adhesion to the endothelial cell surface. In a murine CLP model of sepsis, Oshima *et al.* [75] observed that the pulmonary vascular eGCX reconstituted within 72 h of the initial systemic inflammatory insult and eGCX injury and was largely enriched in [UA-GlcNAc(6S)] disaccharides with only a slight increase in [UA-GlcNS(6S)] expression, a finding that correlated with abrogation of *SULF1* expression within lung tissue of septic mice. Taken together, these findings point to an important interplay between cytokine-induced modifications to the eGCX that may feed-forward to support leukocyte-endothelial interactions. However, more work is needed to determine the impact of these and other sulfation changes to the eGCX on the function of various vascular beds in vital organs.

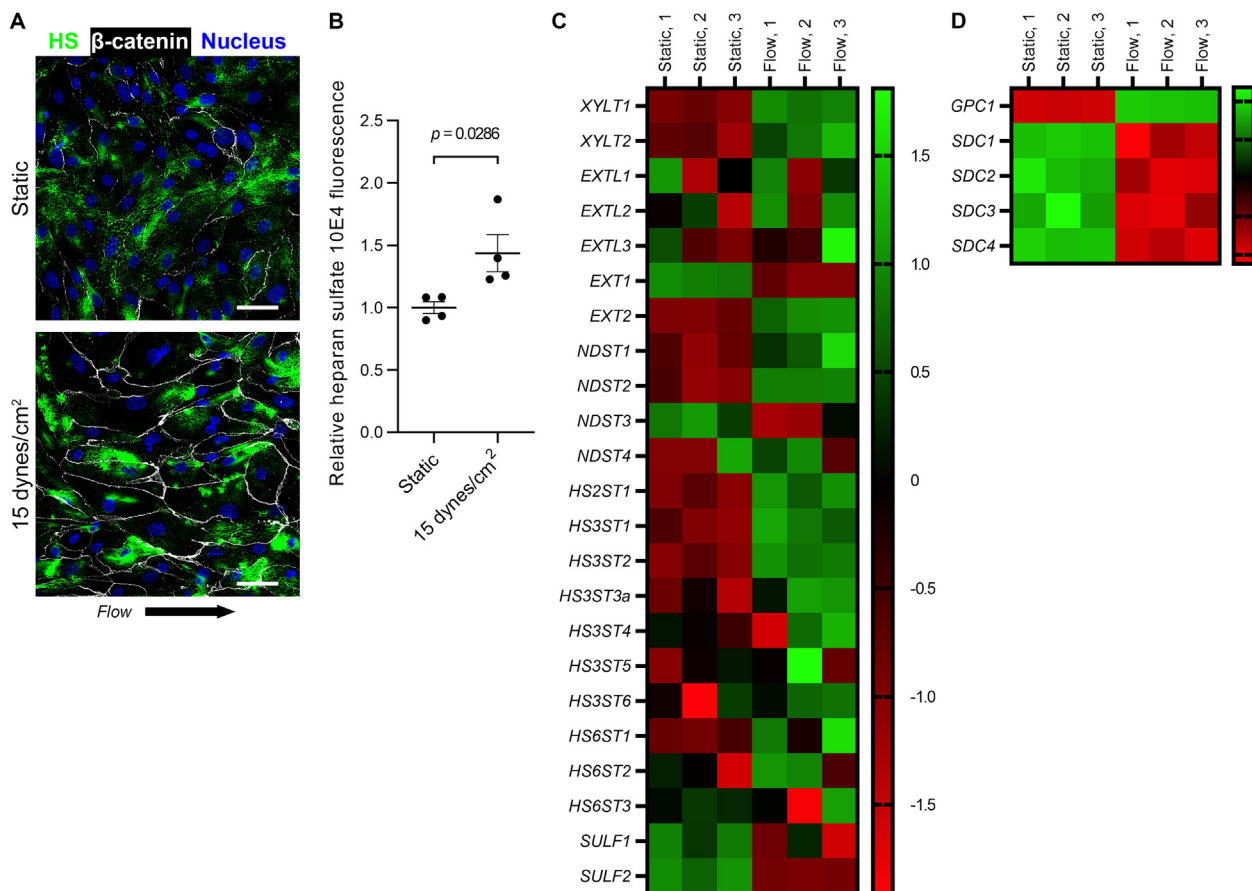


Fig. 2. Shear stress increases heparan sulfate (HS) expression within the glycocalyx on human primary lung microvascular endothelial cells (HLMVEC), concurrent with increased gene expression of HS-synthesizing enzymes. A) Stacked confocal micrographs of HLMVECs incubated for 48 h in either static conditions (top panel) or 15 dynes/cm² of laminar shear stress (bottom panel) demonstrating staining for HS 10E4 epitope (cells demarcated by β-catenin with DAPI overlay). Scale bar represents 20 μm. B) Relative staining intensity for HS 10E4 epitope on HLMVECs following 48 h of static culture or laminar flow (from 4 representative 20x images). Data are presented as mean ± SEM and analyzed by Mann-Whitney *U* test. C) Heat map displaying the relative fold change in gene expression for select HS-synthesizing enzymes measured in HLMVECs incubated in static or flow conditions (*n* = 3 per group) by an nCounter (NanoString, Seattle, WA) digital multiplexed gene expression array. Overall, shear stress promoted increased mRNA expression for xylotransferases, exostosin-like glycosyltransferases, and exostosin 2 while also increasing mRNA expression for most sulfotransferases. Flow conditioning decreased mRNA expression for sulfatases. D) Interestingly, flow conditioning promoted higher expression of *GPC1* mRNA while uniformly reducing *SDC1-4* mRNA in HLMVECs.

Hypoxia and tissue ischemia may similarly initiate sulfation modifications to eGCX HSPGs by augmenting the activity of various sulfotransferases and/or both endosulfatases. Li *et al.* [76] found that incubating HUVECs and mouse primary cardiac microvascular endothelial cells in a hypoxic environment significantly increased expression of *NDST1*, *NDST2*, and *HS2ST* in both cell types without substantial changes in *HS6ST1* or *HS6ST2*. Similar findings were observed following adenoviral transduction-mediated overexpression of hypoxia inducible factor-1α, suggesting a putative transcriptional regulator of these sulfotransferases. However, the authors did not investigate the impact of

hypoxia on specific changes in the sulfation of eGCX HS side chains. Using a mouse model of coronary ischemia, Korf-Klingebiel *et al.* [77] found that tissue hypoxia increased expression for *SULF1* and *SULF2* in tissue samples from infarcted myocardia. More granular analysis of gene expression within individual cell types from infarcted mouse myocardia revealed that endothelial cells and fibroblasts largely accounted for increased *SULF1* while monocytes were the primary source of increased *SULF2*.

Perturbations in exostosin expression may also impact HS sulfation via regulation of *NDST1* activity. Current evidence suggests that *NDST1* competes with *EXT1* for binding to *EXT2* and

subsequent transport to the Golgi where it is available to deacetylate and sulfate GlcNAc residues as the initiating step for HS sulfation [78]. Thus, changes in the relative amounts of EXT1 and EXT2 may influence shuttling of NDST enzymes and their subsequent availability to modify HS residues as evidenced by increased NDST1 activity in cardiac tissue of transgenic mice overexpressing EXT2 protein [78]. However, the translational applicability of exostosin-mediated HS sulfation modifications and the relevance to eGCX-specific sulfation patterns has not been explored. Modulations in exostosin expression are typically associated with overall abrogation of HS expression as observed in hereditary multiple exostoses, and the relationship between EXT and sulfation modifications has been less studied. However, as discussed above, Yang *et al.* discovered that *EXT1* gene expression is attenuated in lung tissue from septic mice, corresponding with elevated circulating levels of *N*-sulfated HS fragments [63]. Although the authors did not specifically interrogate the role of EXT1 in regulating HS sulfation modifications, their findings support the possibility that decreased EXT1 expression could allow for increased interactions between EXT2 and NDST to promote NDST shuttling and HS sulfation in sepsis. Further studies are warranted to validate these speculations and to more clearly establish the role for exostosins in regulating HS remodeling in sepsis and other disease processes in which vascular dysfunction is relevant.

In summary, existing literature, though sparse, supports an important role for inflammatory processes in structural remodeling of eGCX HSPGs. However, sulfation patterning of HS disaccharides involves complex and multifaceted interactions of many enzymes, and our understanding of how inflammation and other disease factors impact these orchestrated interactions is only in its infancy.

Impact of eGCX HSPG expression and sulfation modifications on vascular function

Alterations in eGCX HSPG expression and modifications to HS side chains have important ramifications on endothelial homeostasis that may contribute to vascular pathobiology. Techniques such as high-performance liquid chromatography-tandem mass spectrometry and surface plasmon resonance have aided in characterizing how the finely tuned sulfation properties of HS may impact various endothelial cell signaling axis *in vitro*. Moreover, development of endothelial-specific murine knockout models permit researchers in the fields of glycobiology

and vascular biology to challenge/validate *in vitro* findings and elucidate their physiologic relevance to vascular health and disease. Here we describe the latest available research into the implications of impaired eGCX HSPG expression and/or sulfation modifications to eGCX HSPGs in endothelial pathobiology (summarized in Table 1).

Due to the strong anionic charges imparted to the HSPG by HS side chains, the level of HS and/or HSPG expression at the endothelial apical surface is a critical determinant of eGCX, and thereby endothelial, macromolecular permeability (see revised Starling principle above). Thus, factors that interfere with HS and/or HSPG expression within the eGCX may contribute to increased vascular permeability [97]. This principle has been generally demonstrated in models of enzymatic eGCX injury whereby cleavage of endothelial surface HS or HSPG increases paracellular passage of albumin or reduces transendothelial electrical resistance [98–100]. Although, to our knowledge, the impact of a selective reduction in the expression levels of specific eGCX HSPGs on endothelial permeability remains to be clarified. Additionally, intracellular domains of SDC molecules, particularly SDC-1 [79], are integral in transducing apical shear stress to the actin cytoskeleton and downstream intracellular signaling that promotes competent cell–cell adhesions during homeostatic flow conditions [101]. Xu *et al.* [102] found that increased albumin movement across monolayers of TNF α -treated human glomerular or mouse renal endothelial cells depended upon activation of RhoA/Rho-associated kinase and myosin light chain kinase signaling that resulted in downstream actin stress fiber formation, increased number of intercellular fenestrae, and greater transendothelial albumin leakage. In light of the fact that TNF α may downregulate SDC-1 expression over the endothelial apical surface [48], it is plausible that TNF α -related endothelial permeability may involve, at least in part, diminished SDC-1 expression that impairs homeostatic endothelial signaling governing the formation of actin stress fibers. This hypothesis is supported by *in vivo* data in which Voyvodic *et al.* [79] observed greater vascular permeability in *SDC1*^{-/-} knockout mice to the inflammatory stimulus by mustard oil relative to wild type mice. However, further investigation is needed to more firmly link alterations in eGCX HSPG expression and the mechanisms regulating vascular permeability.

Reduced HS/HSPG expression and altered HS sulfation within the eGCX may propagate vascular inflammation by increasing endothelial cell-leukocyte interactions and strengthening the adhesion of leukocytes to the endothelial surface. *In vitro* and *in vivo* work suggests that reduction in eGCX HS/HSPG expression may expose surface adhesion molecules (e.g., intercellular adhesion molecule-1, vascular cell adhesion molecule-1,

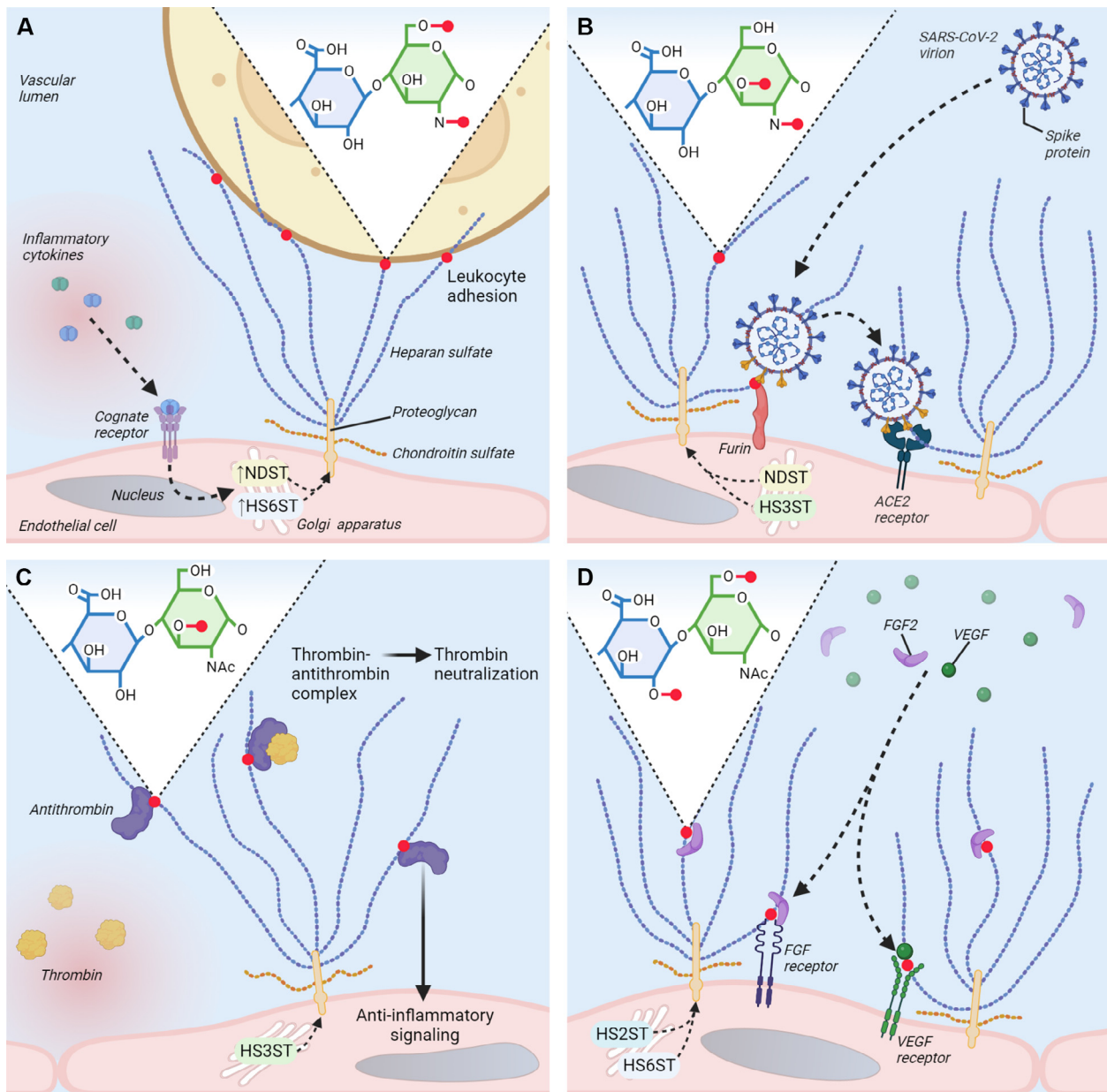
Table 1 Impact of altered heparan sulfate proteoglycan expression and heparan sulfate side chain sulfation modifications within the endothelial apical glycocalyx on endothelial cell biology.

HSPG Modification	Mechanism	Effect on Endothelium	Reference
↓ SDC-1 expression	↓ <i>SDC1</i> expression	↓ PI3K/protein kinase B pathway activation ↑ Endothelial monolayer permeability ↑ <i>ICAM1</i> gene expression; ↑ monocyte adhesion	[79]
↓ GPC-1 expression ↑ GlcNAc <i>N</i> -sulfation	↓ <i>GPC1</i> expression ↑ <i>NDST</i> expression	↓ eNOS activity ↑ Leukocyte adhesion and trafficking Furin attachment for SARS-CoV-2 spike protein preactivation	[6,80,81] [68,72,82–8788]
↑ GlcNAc 2- <i>O</i> -sulfation	↑ <i>HS2ST</i> expression	FGF2-induced angiogenesis Inhibit neutrophil-derived cathelin-related antimicrobial peptide	[7689]
↑ UA 3- <i>O</i> -sulfation	↑ <i>HS3ST</i> expression	↑ Antithrombin binding ↑ Antithrombin-mediated anti-inflammation ↑ Herpes simplex viral entry/infectivity Furin attachment for SARS-CoV-2 spike protein preactivation	[90–92939488]
↑ GlcNAc 6- <i>O</i> -sulfation	↑ <i>HS6ST1-3</i> expression	FGFR1-mediated angiogenesis VEGF-induced angiogenesis ↑ Leukocyte adhesion	[95,969572]
↑ GlcNAc 6- <i>O</i> -sulfation	↓ <i>SULF1</i> expression	↓ Post-sepsis leukocyte adhesion	[75]
↓ GlcNAc 6- <i>O</i> -sulfation	↑ <i>SULF1,2</i> expression	↑ Post-myocardial infarction angiogenesis	[77]

Abbreviations: eNOS, endothelial nitric oxide synthase; FGF, fibroblast growth factor; FGFR, fibroblast growth factor receptor; GlcNAc, glucosamine residue; GPC, glypican; HS2ST, heparan sulfate 2-*O*-sulfotransferase; HS3ST, heparan sulfate 3-*O*-sulfotransferase; HS6ST, heparan sulfate 6-*O*-sulfotransferase; HSPG, heparan sulfate proteoglycan; ICAM, intercellular adhesion molecule; NDST, *N*-deacetylase/*N*-sulfotransferase; PI3K, Phosphatidylinositol 3-kinase; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; SDC, syndecan; SULF, endosulfatase; UA, hexuronic acid.

platelet endothelial cell adhesion molecule-1, P- and E-selectins) to circulating leukocytes and platelets, thereby immobilizing and activating these cells. Enzymatic heparan sulfate cleavage reduces leukocyte rolling while enhancing leukocyte adhesion at the surface of both cultured human primary abdominal aortic endothelial cells and murine cremasteric venular endothelial cells [2,103]. Similarly, broad-spectrum inhibition of matrix metalloproteinases reduced leukocyte adhesion to the surface of mesenteric venules in male Wistar rats [104]. Interestingly, Voyvodic *et al.* [79] demonstrated that loss of *SDC1* expression from mouse lung endothelial cells increased *ICAM1* mRNA expression that coincided with increased monocyte adhesion to a monolayer of lung endothelial cells from *SDC1*^{-/-} knockout mice. Together these data suggest that impairment in HS and/or HSPG expression in the eGCX may promote a phenotypic change in the endothelial cell toward proinflammatory signaling. Furthermore, N- and 6-*O*-sulfation of glucosamine residues in HS have been shown to play a key role in inflammation propagation by mediating leukocyte adhesion to the vascular endothelium (Fig. 3A). Upregulation of *NDST1* and *HS6ST* is observed in a variety of inflammatory conditions [82,83], and increased expression of N- and 6-*O*-sulfated HS domains on endothelial cells *in vitro* results in firmer adhesion of leukocytes to the endothelial monolayer [72,83]. Similarly, myriad experiments using mice with endothelial-specific *NDST1*^{-/-} knockout (*Ndst1*^{flox/flox}*Tie2Cre*⁺) consistently demonstrate substantial reduction in leukocyte adhesion to the vascular endothelium [82,84–

87,105], supporting a proinflammatory role by N-sulfated HS in the eGCX. The role of 6-*O*-sulfated HS, however, may be more contextually nuanced. Oshima *et al.* observed that wild type mice recovering from CLP-induced sepsis developed less leukocyte infiltration following intratracheal instillation of lipopolysaccharide (LPS), concurrent with both a reduction in *SULF1* expression within isolated pulmonary endothelial cells and an increase in pulmonary levels of HS with 6-*O*-sulfation [75]. Further, these authors found that pretreating mice recovering from CLP-induced sepsis with recombinant Sulf1 intravenously before intratracheal LPS administration significantly increased pulmonary inflammation, confirming the role of decreased Sulf1 expression in regulating the immunosuppressive phenotype observed in mice following CLP-induced sepsis. Interestingly, the authors did not observe any difference in pulmonary leukocyte recruitment following intratracheal LPS administration to mice with endothelial-specific, conditional *SULF1/2*^{-/-} knockout (*Sulf1*^{flox/flox}*Sulf2*^{flox/flox}*VEcadCreERT2*⁺), suggesting that the 6-*O*-sulfation enrichment appears to promote an anti-inflammatory vascular phenotype specifically following sepsis. Moreover, they found that *ICAM1* gene expression is attenuated in HLMVECs following *SULF1* knockdown with small interfering RNA. Together, these data point to a complex interaction between the inflammatory milieu and the vascular endothelium by which inflammatory mediators influence the expression of specific enzymes regulating HSPG sulfation that, in turn, direct the endothelial cell's interaction with circulating leukocytes.



Endothelial surface HSPGs may contribute to the pathogenesis and systemic manifestations of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the virus responsible for the coronavirus disease 2019 (COVID-19) pandemic (Fig. 3B). It is now well-established that SARS-CoV-2 infectivity occurs through virion spike protein binding to angiotensin-converting enzyme 2 (ACE2) receptors over the surface of host cells with spike protein activation occurring through the protease activity of the host enzyme furin [106]. HS appears to be integrally involved in each of these steps of host cell infection. Targosz-Korecka *et al.* [107] demonstrated that SARS-CoV-2 spike proteins bind tightly to the surface of human primary pulmonary artery endothelial cells through interac-

tions with eGCX HS. Clausen *et al.* [108] corroborated this finding, demonstrating that binding of SARS-CoV-2 virion spike protein to eGCX HS promotes a conformational change in the spike protein receptor binding domain that enhances spike protein interactions with and anchoring to the endothelial surface ACE2 receptor. Moreover, furin availability for SARS-CoV-2 spike protein preactivation may depend on the presence of eGCX HS, specifically HS molecules rich in N- and 3-O-sulfation [88]. Notably, advanced age and underlying co-morbidities (e.g., obesity, hypertension) are considerable risk factors for severe SARS-CoV-2 infection [109]. However, these risk factors for worse SARS-CoV-2 are also associated with thinning of the eGCX [110,111], which would be

predicted to result in reduced furin and HS availability to support spike protein interactions with ACE2 receptors and, therefore, less viral infectivity. Though it is possible that a less robust eGCX may permit greater interactions between the SARS-CoV-2 virion and host endothelium and epithelium, making those with advanced age and cardiovascular co-morbidities more vulnerable to SARS-CoV-2 infection with worse disease severity, further research is needed to resolve the more granular mechanisms (including the role of eGCX HSPGs) that make these particular populations more susceptible to COVID-19.

HSPGs within the eGCX have traditionally been considered a key contributor to the antithrombotic phenotype of the vascular endothelial luminal surface by harboring and accelerating the activity of hemostatic regulators such as antithrombin, heparin cofactor II, thrombomodulin, and tissue factor pathway inhibitor [112–114]. Antithrombin activation, in particular, is historically thought to rely on 3-O-sulfation of HS glucosamine residues for eGCX binding and activation [41,90] (Fig. 3C). In support of this notion, HajMohammadi *et al.* [115] observed that *HS3ST1* overexpression in CHO4B cells, which lack constitutive *HS3ST1* expression and basal thrombin inhibition, significantly elevated the level of thrombin inhibition to that of mouse cardiac microvascular endothelial cells. Moreover, Weiss *et al.* [116] demonstrated that knockdown of zinc finger transcriptional protein ZNF263, a repressor of *HS3ST1* expression, from HeLa cells resulted in a significant increase in cell surface antithrombin binding and accelerated factor Xa inhibition. However, further investigations and endothelial-specific studies have challenged the

anti-coagulation paradigm of antithrombin-HS interactions. Richard *et al.* [117] demonstrated that, though removal of 3-O-sulfo groups from heparin (a highly sulfated heparan sulfate analog) substantially reduced the affinity of antithrombin for heparin, antithrombin bound to 3-O-desulfated heparin retained the same degree of reactivity toward factor Xa. HajMohammadi *et al.* similarly observed that although *HS3ST1*^{-/-} knockout mice exhibit reduced antithrombin binding to the vascular endothelial surface, they do not have increased vascular fibrin deposition at baseline or following global hypoxia (to incite tissue factor release) nor do they exhibit a propensity for thrombosis after local endothelial surface injury by ferric chloride [115]. Further, the authors found that *HS3ST1* overexpression in mouse primary cardiac microvascular endothelial cells did not result in higher levels of thrombin inhibition. Interestingly, they also observed that HS molecules isolated from tissues in *HS3ST1*^{-/-} mice retained 2–25 % residual anti-Xa activity in the presence of antithrombin, which may suggest that other 3-O-sulfotransferase isoforms contribute to HS 3-O-sulfation that supports antithrombin activity at the endothelial surface. Taken together, these data do not conclusively demonstrate a role for GAG 3-O-sulfation in antithrombin activation but instead support a need for further investigation into this elusive area of endothelial biology.

Though the impact of eGCX HSPG on the anticoagulant property of antithrombin remains an area of controversy, studies consistently demonstrate that antithrombin interactions with 3-O-sulfated HS of eGCX HSPGs play a central role in suppressing vascular inflammation [118] (Fig. 3C). Shworak and colleagues [93] observed



Fig. 3. Cartoon thematically depicting the impact of sulfation on heparan sulfate (HS)-mediated leukocyte trafficking, severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) infectivity, antithrombin activity, and growth factor signaling at the luminal surface of the vascular endothelium. A) Inflammatory cytokines promote upregulation of *N*-deacetylase/*N*-sulfotransferase (NDST) and HS 6-O-sulfotransferase (HS6ST) activity within the Golgi of endothelial cells. This results in increased endothelial glycocalyx (eGCX) expression of proteoglycan HS side chains with *N*- and 6-O-sulfation of *N*-acetyl glucosamine residues (represented by a green ring in inset; blue ring represents the hexuronic acid), respectively, that support leukocyte adhesion and transmigration. B) Furin preactivation of SARS-CoV-2 virion spike proteins (represented by the change in spike protein color to yellow) is enhanced by interaction with eGCX HS molecules that are enriched in *N*- and 3-O-sulfation by NDST and HS 3-O-sulfotransferases (HS3ST). Following furin preactivation, HS molecules further support virion spike protein adherence to surface angiotensin-converting enzyme 2 (ACE2) receptors that is essential for cell infectivity. C) Antithrombin binding to 3-O-sulfated HS moieties within the eGCX may promote a conformational change that increases its efficiency to inactivate thrombin, thereby contributing to coagulation regulation at the endothelial surface. Further, antithrombin binding to 3-O-sulfated HS side chains in the endothelial glycocalyx promotes homeostatic, anti-inflammatory endothelial cell signaling. D) The eGCX harbors fibroblast growth factor 2 (FGF2) near HS regions enriched in 2-O-sulfation by HS 2-O-sulfotransferase (HS2ST) [155]. Moreover, eGCX HS side chains enriched in 6-O-sulfation by HS 6-O-sulfotransferase (HS6ST) synergize cognate receptor binding with FGF2 (and other FGF variants) and vascular endothelial growth factor (VEGF) at the endothelial surface with putative effects on angiogenesis and vascular permeability. For simplicity, the proteoglycan depicted in all panels is syndecan; however, it is currently unknown how sulfation varies within the HS side chains of specific proteoglycans within the eGCX. Figure generated using Biorender. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

that antithrombin administration significantly reduced mortality in LPS-treated wild type mice but had no impact on mortality in LPS-treated *HS3ST1*^{-/-} mice. Moreover, antithrombin treatment reduced leukocyte adhesion to the endothelium of cremasteric venules in LPS-treated wild type mice but surprisingly increased leukocyte adhesion in LPS-treated *HS3ST1*^{-/-} mice [119]. Finally, in a large cohort of 2,144 adult cardiology patients, Shworak *et al.* discovered that patients with worse coronary artery disease demonstrated a greater preponderance of homozygosity for the rs16881446^G recessive allele, a single nucleotide polymorphism found within the human *HS3ST1* intron [119]. Work by Ma *et al.* [120] corroborates the essential role that 3-O-sulfated HS plays in promoting antithrombin's endothelial anti-inflammatory activity. Using Ea.hy926 endothelial cells, these authors found that *HS3ST1* knockdown significantly diminished antithrombin's ability to preserve cell-cell junctional integrity in the face of LPS treatment by reducing antithrombin-HS-mediated 5' adenosine monophosphate-activated protein kinase activation that, in turn, promoted c-Jun N-terminal kinase proinflammatory signaling. Interestingly, Smits *et al.* demonstrated that 3-O-sulfated HS is most highly expressed *in vivo* at arterial branch points and vascular regions exposed to high shear stress [93], areas of the vascular tree with known susceptibility to atherosclerotic plaque formation. As endothelial expression of 3-O-sulfated HS motifs may decline with advancing age [121], these data implicate a potential contribution of waning antithrombin-HS-mediated protective endothelial cell signaling in age-associated cardiovascular disease.

eGCX HSPGs are instrumental in transducing luminal shear stress to endothelial cell signaling axes that govern vascular smooth muscle tonicity, in particular through eNOS activation and downstream nitric oxide production [39,40]. Therefore, reduced expression of HSPGs at the endothelial apical surface may impair homeostatic vasoregulation, culminating in tissue hypoperfusion. Loss of SDC-1 expression in statically cultured murine lung endothelial cells was shown to reduce phosphatidylinositol-3 kinase/protein kinase B signaling activity [79], a commonly accepted upstream promoter of eNOS activation [122]. Conversely, Ebong *et al.* [6] observed no reduction in eNOS phosphorylation in bovine aortic endothelial cells conditioned with shear stress (15 dynes/cm², 24 h) following SDC-1 knockdown. Arguably, this *in vitro* work under flow conditions more convincingly suggests that SDC-1 does not have a role in shear stress activation of eNOS; however, differences in endothelial cell type must be considered and systematically evaluated. Ebong *et al.* also found that GPC-1 knockdown in bovine aortic endothelial cells returned eNOS phosphorylation to levels of statically cultured wild type cells [6]. Similar findings were demonstrated in murine aortic

endothelial cells and HUVECs [70], pointing to a more central role for GPC-1 in the mechanism driving flow-induced eNOS phosphorylation. In light of prior work implicating that platelet endothelial cell adhesion molecule-1 (PECAM-1) is involved in the process of shear stress-induced eNOS activation [123,124], Bartosch *et al.* [81] advanced our understanding of the role GPC-1 has in shear stress-induced eNOS signaling by investigating the interaction between GPC-1 and PECAM-1 as a cooperative linchpin in this complex cascade. Through a series of elegant experiments combining *in vitro* work with atomic force microscopy and *in vivo* studies with *GPC1*^{-/-} knockout mice, the authors demonstrated that PECAM-1 serves as an essential linkage between GPC-1-mediated shear sensing and intracellular eNOS activation [81]. While the specific molecular interactions between GPC-1 and PECAM-1 remain to be clarified, this important study sheds insight into the complexity by which HSPGs contribute to endothelial mechanosignaling. Further, it is logical to conjecture that any disease or biological process that impairs endothelial cell expression of GPC-1 may attenuate eNOS activity and thus compromise homeostatic vasoregulation. In fact, the same laboratory that linked GPC-1 to PECAM-1-mediated eNOS activation demonstrated that HUVECs cultured on stiffer substrates (thereby mimicking arteriosclerosis) attenuated *GPC1* mRNA and GPC-1 protein expression while simultaneously suppressing eNOS phosphorylation to levels found in HUVECs cultured on softer substrate after *GPC1* knockdown [70]. Thus, processes such as arteriosclerosis or advancing age may suppress GPC-1 expression within the eGCX that, in turn, may culminate in impaired vasoregulation by reducing nitric oxide signaling.

Finally, sulfation modifications to eGCX HSPG are instrumental in regulating growth factor interactions with endothelial apical surface cognate receptors that direct vascular angiogenesis (Fig. 3D). Li *et al.* observed that incubation of HUVECs or mouse primary cardiac microvascular endothelial cells in a hypoxic environment increased *HS2ST* expression and total HS GAG expression in both cell types [76]. They also observed that co-treatment with FGF2, a potent instigator of angiogenesis [125], in the setting of hypoxia resulted in substantially greater cell replication of both cell types as compared to FGF2 treatment alone. Together, these findings suggest that hypoxia increases expression of 2-O sulfation within eGCX HSPGs, an HS motif to which FGF2 has known affinity [126,127], thus priming the endothelial surface for proangiogenic FGF signaling. Furthermore, the presence of 6-O sulfation within eGCX HS side chains appears to be essential for FGFR1-mediated angiogenesis by strengthening the formation of the endothelial surface FGF2-HS-FGFR1 ternary complex [95,96]. Prior work using surface plasmon resonance suggests that HS 6-O-sulfation significantly enhances

recombinant human VEGF₁₆₅ binding to its endothelial surface cognate VEGF receptor 2 [128]. Ferreras *et al.* [95] observed that loss of 6-O sulfation in HS in HUVECs through *HS6ST1* and *HS6ST2* silencing resulted in substantial reduction of endothelial cell sprouting and tube formation in response to VEGF₁₆₅. However, surprisingly Korf-Klingebiel *et al.* found that reduced *SULF1* expression in HUVECs, which would presumably increase 6-O-sulfo group expression in eGCX HS, abrogated endothelial recovery from scratch injury in response to VEGF₁₆₅ [77]. Taken together, these data suggest that HS interactions with VEGF signaling are perhaps more complicated than initially thought, prompting a call for further investigation into other potential signaling contributors (e.g., transglutaminase 2 [129]).

Therapeutic strategies and future directions

In light of recent discoveries that have identified the eGCX as a valuable therapeutic target in ameliorating aberrant vascular biology, investigators have begun to explore strategies to promote HSPG synthesis or remodeling within the eGCX. Many of these strategies utilize mimetics of HS or other eGCX components to reconstitute the eGCX following erosion, while others involve targeting pathways of HSPG biosynthesis. Moreover, approaches to fine-tune the sulfation properties of HSPGs have also emerged as potential strategies for controlling eGCX interactions with circulating cells and proteins that have important influence over mechanisms regulating endothelial cell function. In this section, we provide an overview of the most recent and promising strategies that bolster the expression HSPGs to reform a robust, healthy eGCX.

Promotion of HSPG expression

Many preclinical studies have demonstrated therapeutic benefits for abrogating endothelial dysfunction following eGCX erosion using treatments that promote HSPG expression at the endothelial cell surface. Recently, the HS mimetic fucoidan was shown to restore eGCX expression in HLMVECs following treatment with plasma from critically ill COVID19 patients. Fucoidan treatment and eGCX recovery corresponded with a reduction in endothelial activation, improvement in barrier function and restoration of an anticoagulant cell surface [130]. Sulodexide is a commercially-available, purified mixture of natural GAGs consisting of 80 % HS and 20 % dermatan sulfate that has shown promise for aiding eGCX reconstitution in preclinical models of sepsis [131] and vascular injury [132] and in patients with type 2 diabetes mellitus [133] and peripheral vascular disease

[134,135]. Notably, sulodexide inhibits the activity of heparanase-1, the only known mammalian endoglucuronidase with activity on eGCX HS [136], and is itself resistant to enzymatic degradation, indicating that sulodexide may both reconstitute the eGCX and prevent ongoing enzymatic HS degradation. Sulodexide has also been shown to prevent LPS-induced eGCX damage in brain microvascular endothelial cells and to improve eGCX integrity, vascular permeability and mortality following induction of sepsis in mice [131]. Recently, sulodexide treatment in patients with early stage COVID-19 was associated with attenuated levels of C-reactive protein (i.e., less systemic inflammation) and D-dimer (i.e., less thrombus burden), decreased need for supplemental oxygen, and reduced the need for hospitalization [137]. EC-SEAL is another GAG mimetic that consists of selectin-binding peptides attached to a dermatan sulfate backbone that is designed to bind selectins on injured endothelium to prevent leukocyte and platelet binding. EC-SEAL has been shown to prevent platelet [138] and leukocyte [139] binding to activated endothelial cells *in vitro* and to reduce thrombus formation *in vivo* following ligation of the inferior vena cava in mice [138].

Administration of fresh frozen plasma (FFP) is gaining attention as a first-line therapeutic strategy against eGCX damage caused by trauma-hemorrhagic shock [140,141] and burn injuries [142]. In rodent models of severe hemorrhage, administration of FFP results in significantly less eGCX damage compared to administration of crystalloid solutions like lactated Ringer's solution [143,144]. Partial restoration of SDC-1 expression was demonstrated following hemodynamic stabilization with FFP via mechanisms related to both the antithrombin [145] and fibrinogen [146] components of plasma. Furthermore, albumin, a key component of FFP, has exceptional binding to the eGCX and has been shown to reinforce eGCX barrier function by contributing to steric hindrance and electrostatic repulsion of circulating proteins and cells [147]. Moreover, albumin's restorative effects on the eGCX may also be related to its role in delivering sphingosine-1-phosphate to the endothelium, which stabilizes the eGCX by both inhibiting SDC-1 shedding and promoting SDC-1 synthesis [148–150].

Targeting cellular pathways that regulate HSPG biosynthesis also holds promise for enhancing eGCX reconstitution following insult. For example, *EXT1* expression and eGCX repair after heparinase-mediated cleavage is mediated by FGFR1 signaling in the pulmonary endothelium of mice, and impairments in FGFR1 signaling are associated with delayed eGCX recovery [63]. Thus, FGFR1-promoting therapies (e.g., FGF2, sphingosine-1-phosphate) are likely candidates to facilitate *EXT1* expression and HS biosynthesis to promote eGCX reconstitution following pathologic damage.

Nutraceuticals have also been employed by researchers as an alternative therapeutic approach for promoting eGCX expression. Arterosil[®] and Endocalyx[™] are two commercially available nutraceutical products marketed to enhance the integrity of the vascular eGCX. The major component of Arterosil[®], rhamnan sulfate extracted from algae, has been shown to enhance HS expression on endothelial cell monolayers and prevent the permeability of low density lipoprotein [151]. Endocalyx[™] contains a unique blend of HS-mimicking polysaccharides and GlcNAc (for HS synthesis) along with hyaluronan and antioxidants. Endocalyx[™] was shown to improve age-related deficits in eGCX integrity, restore vascular barrier function and increase nitric oxide bioavailability in aged mice [152]. A clinical trial is currently underway to establish the efficacy of Endocalyx[™] in patients with type 2 diabetes mellitus complicated by albuminuria [153]. Though additional therapeutic applications for nutraceuticals remain to be tested, their use in the treatment approach for acute manifestations of eGCX injury, as seen in traumatic injury or sepsis [37,154], is rational.

Tailoring HSPG sulfation

Given the importance of HSPG structural modifications in regulating pathologic endothelial functions, therapies that control HS sulfation could be used to fine-tune the functional properties of a damaged or freshly reconstituted eGCX layer. As a proof of concept to support this approach, Korf-Klingebiel *et al.* used adenoviral transfections to overexpress *SULF1* and *SULF2* in cardiac tissue prior to myocardial infarction [77]. Endosulfatase overexpression corresponded with decreased HS sulfation within the infarcted tissue leading to less VEGF binding to HS. Functionally, the attenuated interactions between VEGF and HS resulted in enhanced capillarization of the infarct border zone, reduced scarring and improved left ventricular systolic function. In a second approach, the small molecule and HS-antagonist surfen was used to block HS interactions with protein ligands following myocardial infarction. Similar to overexpressing sulfatases, surfen-treated mice showed decreased VEGF binding to HS resulting in reduced scar formation and enhanced cardiac function [77]. Although these strategies did not specifically target the vascular endothelium, this work does establish a premise for modulating HSPG sulfation patterns as a therapeutic strategy to improve pathologic outcomes.

Conclusions

Our understanding of the vital role of the eGCX in vascular health and disease has grown immensely in recent years. The eGCX not only acts as a physical barrier to protect the underlying

endothelial surface, but it also actively participates in the homeostatic mechanisms that regulate vascular patency, inflammation, and coagulation. Disruption of the eGCX by activated sheddases, reduced HSPG expression and modulation of HS sulfation can have local and systemic consequences on endothelial mechanisms that drive pathologic processes for a wide variety of conditions. Although there is compelling evidence from *in vitro* studies and animal work to suggest that strategies to promote eGCX synthesis or remodeling will improve or tailor vascular functions, for the most part, the efficacy of these therapies for treating vasculopathy in humans remains elusive. Considering the progress in the field, we anticipate significant translational developments of therapies aimed at eGCX restoration and remodeling to rapidly evolve.

CRedit authorship contribution statement

Danielle Pretorius: Writing – original draft. **Robert P. Richter:** Conceptualization, Writing – review & editing. **Tanya Anand:** Writing – review & editing. **Jessica C. Cardenas:** Writing – review & editing. **Jillian R. Richter:** Conceptualization, Writing – review & editing.

DATA AVAILABILITY

Data will be made available on request.

DECLARATION OF COMPETING INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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1 Co-first authors.

Abbreviations:

ACE2, Angiotensin-converting enzyme 2; CLP, cecal ligation and puncture; COVID-19, Coronavirus disease 2019; eGCX, Endothelial glycocalyx; eNOS, Endothelial nitric oxide synthase; EXT, Exostosin; EXTL, Exostosin-like glycosyltransferase; FFP, Fresh frozen plasma; FGF, Fibroblast growth factor; FGFR1, Fibroblast growth factor receptor 1; GAG, Glycosaminoglycan; Gal, Galactose; GlcNAc, *N*-acetyl glucosamine; GlcA, Glucuronic acid; GPC, Glypican; HLMVEC, Human lung microvascular endothelial cell; HS, Heparan sulfate; HS2ST, Heparan sulfate 2-O-sulfotransferase; HS3ST, Heparan sulfate 3-O-sulfotransferase; HS6ST, Heparan sulfate 6-O-sulfotransferase; HSPG, Heparan sulfate proteoglycan; HUVEC, Human umbilical vein endothelial cell; LPS, lipopolysaccharide; NDST, *N*-deacetylase/*N*-sulfotransferase; SARS-CoV-2, Severe acute respiratory syndrome coronavirus 2; SDC, Syndecan; Sulf, Endosulfatase; TNF α , Tumor necrosis factor alpha; UA, Hexuronic acid; VEGF, Vascular endothelial growth factor; Xyl, Xylose; XYLT, Xylosyltransferase

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