Minireview

The neurovascular extracellular matrix in health and disease

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Impact statement

The extracellular matrix (ECM) enmeshes endothelial cells (ECs) in brain to maintain neurovascular homeostasis. The lumen, or blood-facing side, of the EC exhibits a thin gel-like layer of specialized proteins and carbohydrates known as the endothelial glycocalyx layer (EGL). The abundance and composition of the EGL can dictate neurovascular functions, including cerebral blood flow, immune cell interactions, and blood–brain barrier (BBB) integrity. For decades, researchers have measured how genetic and environmental factors influence the peripheral EGL constituents; however, much less is known about the neurovascular EGL. Recent technological advancements in two-photon microscopy (TPM), electron microscopy (EM), and mass spectrometry (MS) have improved our understanding of how the EGL contributes to neurovascular function. Future studies should employ these techniques to measure neurovascular EGL changes and its relationship to CNS disease.

Abstract

The blood–brain barrier (BBB) is a vital interface that supports normal brain functions. Endothelial cells (ECs) are the main component of the BBB and are highly specialized to govern the transfer of substances into brain. The EC lumen is enmeshed with an extracellular matrix (ECM), known as the endothelial glycocalyx layer (EGL). The lumen-facing EGL is primarily comprised of proteoglycans (PGs) and glycosaminoglycans (GAGs), which function as the first line of defense for blood-to-brain transfer of substances. Circulating factors must first penetrate the EGL before interacting with the EC. The abundance and composition of the PG and GAGs can dictate EGL function, and determine which circulating substances communicate with the ECs. The EGL can interact with circulating factors through physio-chemical interactions with the EC. Some disease states reveal a "thinning" of the EGL that may increase EC interactions with components of the systemic circulation and alter BBB function. EGL changes may also contribute to the cognitive complications of systemic diseases, such as sepsis and diabetes. For decades, researchers have measured how genetic and environmental factors influence the peripheral EGL constituents; however, much less is known about the neurovascular EGL. In this mini-review, we introduce components of the EGL and innovative ways to measure their abundance and composition that may contribute to BBB dysfunction.

Keywords: Glycocalyx, blood–brain barrier, extracellular matrix, proteoglycans, glycosaminoglycans

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Introduction

The blood–brain barrier (BBB) is a natural defense system for the central nervous system (CNS) and regulates the exchange of substances between the blood and the brain. Endothelial cells (ECs) are the major component of the BBB and are joined together by regulatory tight junction proteins that control the paracellular transfer of relatively small substances.[1,2] The BBB can also transfer larger substances via transcellular mechanisms.[3]

The EC lumen is enmeshed with an exquisite extracellular matrix (ECM), known as the endothelial glycocalyx layer (EGL). The EGL is a thin gel-like layer that is predominantly comprised of proteoglycans (PGs), including syndecans, biglycan, and glypicans[4–6] and glycosaminoglycans (GAGs) including heparan sulfate (HS), chondroitin sulfate (CS), and hyaluronan (HA).[5,7] The abundance and composition of each EGL constituent can regulate vascular structure and function, as well as EC interactions with circulating factors.[8] Recent studies suggest that the BBB EGL is a distinct interface between the blood and brain, and perhaps serves as the brain's first line of defense against potentially harmful circulating substances.[8–11]

Little is known about how the EGL influences BBB function or how it is involved in CNS disorders. In this mini-review we will introduce the constituents of the EGL, existing technologies to study EGL structure and function, and

novel aspects of the BBB EGL that have been recently described.

History of the endothelial glycocalyx layer

Virchow first discovered the vascular ECM in 1846, which he described as "mucosubstances" appearing in human atheromatous lesions at autopsy. Nearly a century later, Danielli proposed that a continuous protein layer existed along the lumen of blood vessels.[12] In 1963, Bennett coined the term "glyco-calyx", greek for "sweet-husk", based on electron micrographs proposing that sugar structures lined peripheral blood vessels.[13] The existence of mucopolysaccharides within the EGL was first described by Luft in 1966 with ruthenium red staining of the endocapillary layer that was suggested to serve as an interface that could regulate vascular function.[14]

Much of what we know about EGL function comes from pioneering research performed in peripheral blood vessels. Elegant studies performed in frog mesenteric vessels found that circulating plasma proteins were essential to EGL integrity,[15] and that EGL integrity prevents unregulated vascular permeability.[16,17] Subsequent studies determined that the EGL senses circulating factors, and maintains vascular homeostasis. More specifically, the EGL functions to appropriate blood flow distribution,[6,18–20] regulate immune–endothelial interactions,[21] and to respond to shear-stress.[22] Very few studies have investigated the EGL in the context of the brain vasculature,[23–25] and only recently has the neurovascular EGL been visualized within the living rodent,[11,26] and human brain.[27] The ability to monitor the EGL in a dynamic environment will allow novel mechanistic insights into the role of the EGL in CNS disease.

Proteoglycans of the endothelial glycocalyx layer

PGs are essential components of the EGL that can affect EC function. Within this section we will briefly highlight the mechanistic role of three major PGs that associate with the endothelial glycocalyx, including syndecans, biglycan, and glypicans (Figure 1). We will also discuss how each PG can affect EC function in the context of vascular inflammation.

Syndecans are PGs that are decorated with CS- and HS-GAGs that play an important role in neurovascular structure and function.[28] Syndecans span the EC membrane to interact with luminal circulating factors[29] and transduce signals to the cytoskeleton.[30] A variety of circulating factors including cytokines, chemokines, and growth factors communicate with syndecans to alter EC function.[31–33] Syndecans are upregulated by inflammation and associated conditions such as hypoxia,[34–36] promote survival under severe inflammatory conditions,[37] and protect against inflammatory diseases such as fibrosis.[38] Immune–endothelial interactions are regulated by syndecans through their ability to sequester cytokines and chemokines and thus contribute to the onset and resolution of localized inflammatory responses.[34,35] The relationship between syndecans and the EC presents an interesting therapeutic target for vascular disease.

Biglycan is another PG comprised of a small (42 kDa), leucine-rich core protein that is decorated exclusively by CS-GAG chains and is ubiquitously expressed by multiple cell types, including ECs.[39] Numerous immune functions have been attributed to biglycan, and it can function as an endogenous danger signal when proteolytically cleaved from the ECM.[36] Cleaved forms of biglycan can activate innate immune responses through toll-like receptors 2 and 4,[40,41] P2X receptors, and the NLRP3 inflammasome.[42] Soluble biglycan can also contribute to the adaptive immune response, and was shown to exacerbate both B-cell and T-cell mediated autoimmune disease progression in mice.[36] In contrast, biglycan protects against the progression of atherosclerosis in mice through its antithrombin activity.[39] Overall, biglycan plays a vital role in the immune response to inflammatory signals and serves as a viable therapeutic target for vascular disease.[5]

Glypican-1 is a brain-specific PG that is exclusively decorated with HS-GAG chains.[28] Glypicans are bound to the luminal membrane of the EC by a glycosylphosphatidylinositol anchor and is predominantly localized to the cell junctions.[43] Shear stress and the activation of endothelial nitric oxide production can subsequently nitrosylate cysteine residues of glypican-1 and cleave their associated HS-GAG chains.[44,45] It has also been reported that a high-cholesterol diet reduces EGL dimensions and impairs normal EC function with an associated downregulation in glypican-1 expression.[46] The neurovascular EGL response to changes in shear stress warrants further investigation.

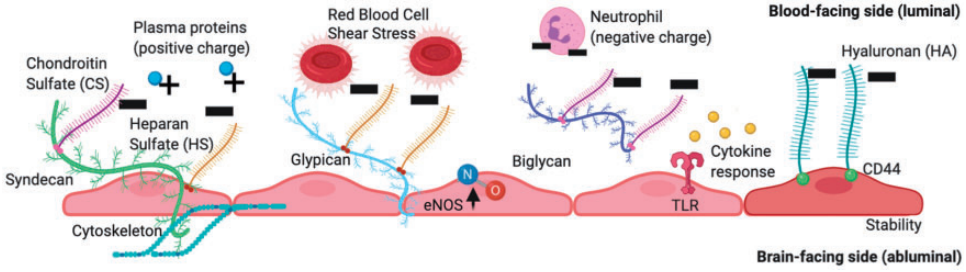

Figure 1. Structure and function of the neurovascular glycocalyx. Prominent components of the luminal (blood-facing) side of the endothelial glycocalyx layer (EGL) include: proteoglycans (PGs; syndecan, glypican, and biglycan), and glycosaminoglycans (GAGs; chondroitin sulfate, heparan sulfate and hyaluronan). EGL functions are demonstrated including, physiochemical and mechanical interactions. Created with BioRender.com. (A color version of this figure is available in the online journal.)

Glycosaminoglycans of the endothelial glycocalyx layer

Within the EGL milieu exists a plethora of polyanionic sugar GAG chains comprised of HS and CS that are sulfated and covalently bound to various PGs, as well as the 'free' non-sulfated GAG chain HA that associates with the membrane-bound protein CD44.

GAG chains are covalently associated to PG to create a polyanionic environment within the EGL. ECs regulate the abundance and composition of surface GAGs through high turnover rates in order to adapt to blood flow. Elegant experiments performed in rodent mesenteric vessels by Duling and co-workers (2000) first characterized the importance of EGL-associated GAG chains on vascular function. Specifically, this group used bacterial enzymes to degrade GAG chains and measure vascular permeability.[16,17] Recent work in a similar model system, confirmed by intravital imaging, found that certain GAG chain degradation strategies created a graded effect on vascular function.[8] These studies suggest that the rodent EGL contains a higher abundance of HS-GAG chains compared to CS- and HA-GAG chains, and the GAG composition of the EGL may be different between mice and men.

Early studies showed that the human iliac artery exhibited a higher composition of CS-GAGs relative to HS-GAGs and HA.[47] It is important to note that the vascular GAG composition also differed between species depending on their susceptibility to diet-induced atherosclerosis (reviewed in Wight[5]). Humans, like chickens and rabbits, are susceptible to diet-induced atherosclerosis and their aortas were enriched in CS-GAGs, whereas the aortas of diet-induced atherosclerosis-resistant species (rats and mice) were enriched in HS-GAGs.[48,49] Moreover, recent studies revealed a negative association between HS-GAGs and tissue cholesterol in diabetic monkeys.[50] From the literature above, it is important to note that species-specific GAG discrepancies are found within peripheral vascular beds. It remains to be seen whether neurovascular beds are species-specific and future studies should venture to confirm these discrepancies and advance the field for translational success.

HA is a GAG chain comprised of repeating disaccharide units of non-sulfated glucuronic acids and N-acetyl glucosamines. HA is a ubiquitous component of the EGL that was initially underappreciated due to losses during tissue processing.[51] HA is synthesized and secreted in large forms up to 20,000 kDa, but is often rapidly degraded into smaller forms that have size-dependent downstream effects (recently reviewed in[52,53]). It has long been appreciated that HA serves a key role in regulating EGL permeability.[54] However, determining the precise mechanisms by which HA affects EGL function is complicated by local induction of HA synthesis in the vasculature, rapid turnover of nascent and newly synthesized HA, and deposition of circulatory HA during periods of stress such as inflammation.[55] HA effects on cellular responses are usually regulated by the CD44 receptor, but HA also binds to receptor for HA-mediated motility[56] and toll-like receptors in pathways that are both dependent and independent of HA size.[57] The effect of HA on the EGL is also determined by its binding to sulfated GAGs and to classic hyaladherins, such as tumor necrosis factor α-stimulated-gene-6 (TSG-6). TSG-6 exclusively mediates the covalent linkage of the heavy chain of serum protein inter alpha inhibitor onto HA.[58,59] The subsequent effects on stabilizing HA and modulating inflammation, in either a detrimental or beneficial manner, are tissue specific and have been recently reviewed.[60]

New technology to assess the neurovascular glycocalyx

It is difficult to study the brain EGL using the techniques that have helped define the peripheral EGL. In this section, we will highlight recent innovations that have shed light on the unique structure and function of the brain EGL (Figure 2). A majority of the technologic advances are based on imaging modalities that allow for intravital recordings of EC function in a living organism. Some techniques presented are older techniques that have been adapted in order to better visualize the often-elusive glycocalyx. We emphasize recent research that has applied these methods to animal models of severe inflammation in order to detect a robust change in the EGL.

Limitations of traditional immunohistochemistry to assess the endothelial glycocalyx

Transcardial perfusion fixation is commonly used to remove auto-fluorescing red blood cells to effectively visualize neuropathology. Unfortunately, traditional exsanguination techniques variably remove sensitive EGL structures, and subsequent fixation can result in differential loss of specific EGL molecules.[10,51] Neurovascular perfusion has been adapted with low-pressure physiologic buffers (often containing albumin), followed by perfusion with a lectin-binding preparation to enhance the appearance of the post-mortem EGL.[52]

No single fixation technique is optimal to visualize all epitopes. For instance, lectin-binding compounds and alcohol-based fixatives generally maintain fragile strands of GAGs to a greater degree than aldehyde-based solutions.[51,61] Histological examination is best achieved through use of fluorescence-based antibodies followed by confocal microscopy; however, EGL quantification is difficult to perform with current restraints on optical resolution. Stochastic optical reconstruction microscopy (STORM) enhances the optical resolution to better distinguish structural EGL changes at the cellular level of histological preparations. STORM has recently been employed to visualize the ultrastructural spatio-chemical organization of the EGL in cultured ECs.[62] STORM imaging should also be conducted on fixed brain tissue to visualize structural EGL changes under a variety of physiological conditions. Unfortunately, when GAG chains are assessed via histology, conclusions are limited by the inherent cross-reactivity of GAG-specific antibodies[63,64] and confounds in tissue processing, as mentioned above. Future studies should validate antibodies and adapt from standard perfusion

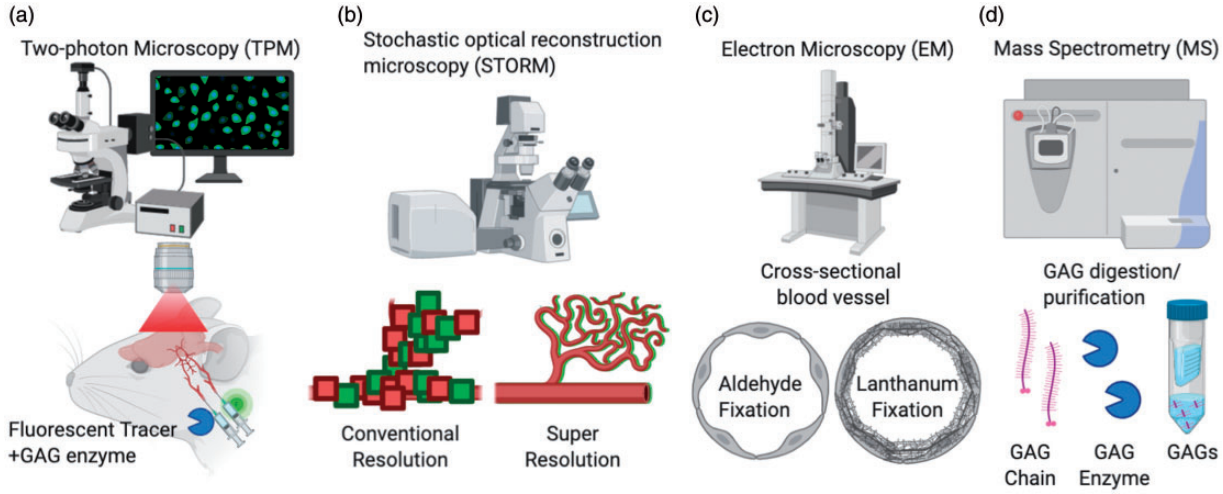

Figure 2. Methods to assess the neurovascular glycocalyx. Technological advancements including: (a) two-photon microscopy (TPM), (b) stochastic optical reconstruction microscopy (STORM), (c) electron microscopy (EM), and (d) mass spectroscopy (MS) have enhanced our understanding of the endothelial glycocalyx layer (EGL). Each illustration represents the basic concept and model system for each technology. Created with BioRender.com. (A color version of this figure is available in the online journal.)

fixation techniques, combined with STORM-based super-resolution imaging, in order to effectively visualize the neurovascular ECM.

Detection of endothelial glycocalyx by electron microscopy

Specialized methods of tissue preparation are needed to visualize the EGL, and Luft[14] was among the first to demonstrate the presence and fine structure of the EGL by transmission electron microscopy (EM). Luft's method implemented staining with ruthenium red, an 860 Da cationic dye with affinity for acidic mucopolysaccharides, which binds components of the glycocalyx and generates a detectable electron density with osmium tetroxide.[14] Since the findings of Luft, other dyes such as alcian blue and lanthanum have also been used to visualize the EGL for EM.[65,66] It has been acknowledged that methods for visualization of the EGL by EM likely underestimate EGL thickness since loss of the more fluid or gel-like components of the EGL can occur during tissue processing. Fixation techniques may also introduce dehydration artifacts or otherwise alter structure through molecular interactions with EGL stains,[28] although improvements in structural preservation and visualization of finer details have been made possible with non-aqueous fixatives and novel contrast agents, such as cationic thorium dioxide colloids.[67,68] More recently, a lanthanum staining method has been used to visualize the EGL in both transmission and scanning EM.[69] Most EM studies have estimated a capillary EGL thickness ranging from 20 to 200 nm,[28,68] whereas other *in vivo* imaging-based methods (described below) estimate a larger thickness of 400–500 nm.[28]

It is also appreciated that structural features of the EGL vary based on the size of the vessel and the tissue vascular bed. For example, a recent study compared capillary EGL structural features in continuous ECs from the heart, fenestrated capillaries of renal glomeruli, and sinusoidal capillaries of the liver using the lanthanum perfusion method. Differences in EGL structure in each tissue bed were noted, with EGL comprising about 13% and 16% of the capillary area in the heart and kidney, respectively, and only 3% of the area in the liver.[70] The same study evaluated a model of septic vasculitis that is induced by giving a very high dose of bacterial lipopolysaccharide (LPS) that induces about 84% lethality after 48 h. Of the mice that survived, a significant loss of glycocalyx was observed in all tissues. A similarly designed study evaluated differences in the capillary glycocalyx of brain, heart, and lung, and found that the EGL comprises about 40% of capillary area in the brain, whereas EGL from heart and lung capillaries were only about 15% and 4%, respectively.[10] Following high dose LPS, there was a substantial degradation of the EGL in heart and lung, but not in brain. However, the percent EGL area in the brain was lowered to about 15%.[10] In the next section, we will discuss new techniques used to quantify circulating EGL components as a surrogate for EGL loss.

Quantification of endothelial glycocalyx with liquid chromatography mass spectrometry

Quantification of GAG chains can be achieved using high performance liquid chromatography paired with mass spectrometry (LC-MS).[71,72] Recently, LC-MS has been employed to determine the relative abundance and composition of CS- and HS-GAG chains within a variety of biological tissues.[72–74] The sensitivity and precision of LC-MS allows for the compositional analysis of GAGs from both rodent[74] and human brain regions.[73] Future research should pair single-cell isolation with LC-MS to explore cell-specific GAG changes.

Under severe inflammatory conditions, constituents of the EGL can be fragmented and released into the bloodstream;[75,76] however, the initiating vascular bed would be unknown due to the ubiquitous nature of the EGL. LC-MS quantification of the GAGs that are shed from the EC lumen may serve as surrogate markers for EGL abundance.[75–78]

In addition to measuring HS-GAG levels in blood after LPS, Hippensteel *et al.* discovered HS fragments to cross the BBB and contribute to cognitive impairment.[78] These findings present a new biological target for the treatment of cognitive decline.

Intravital imaging of the neurovascular glycocalyx

Fluorescent-labeled dextrans can be used to determine the size of the glycocalyx as well as the partition coefficient of a particular plasma substrate in various sized capillaries. Fluorescent dextrans were first used *in vivo* to analyze the glycocalyx in mouse muscle capillaries using intravital microscopy.[9] Large fluorescent molecules (>70 kDa) are restricted to the center of capillaries, suggesting that these inert molecules have limited access to a 0.4- to 0.5-μm-thick region on the capillary surface.[9] This is approximately 15–20% of the smallest capillaries in the microcirculation.[79] Erythrocytes labeled with a 40 kDa dextran can also be used to reproducibly estimate the glycocalyx in humans.[80]

The advent of multiphoton microscopy has allowed researchers to tease apart BBB properties at the single cell level *in vivo*.[11,81] These technological advancements have highlighted the functional contribution of subcellular structures within the BBB, including those within the luminal membrane, or the EGL. Two-photon microscopy (TPM) permits the visualization of the cerebrovascular system through a cranial window made within the skull. An elegant study performed by Kutuzev *et al.* used TPM and various sized fluorescent dextrans to measure the size of the brain EGL present within capillaries perpendicular to the focal plane in a living mouse.[11] This technique allows one to quantify the glycocalyx in the absence of red blood cells, which are known to disturb the structure of the glycocalyx. A partition coefficient, defined by the fluorescence intensity of a dextran marker overlayed on a fluorescent glycocalyx marker (i.e. WGA), can be calculated. No difference in partition coefficients for markers under 643 Da was observed. However, markers of size 40 and 150 kDa did differ in their diffusion into the mouse brain EGL, suggesting larger sized plasma substrates have limited diffusion through the glycocalyx. This work suggests that the EGL serves as an additional size-selective barrier that defends the EC from blood-borne factors, which has important implications for CNS diseases and CNS drug delivery strategies.

In addition to using different sized dextrans to estimate partition coefficients, a combination of fluorescent markers (to distinguish between the glycocalyx and vessel lumen) can be used to visualize the cerebral glycocalyx *in vivo* using TPM.[26] Recent work by Yoon *et al.* used this technique to find that the thickness of the glycocalyx layer in brain varies between different types of arteries and capillaries but does not correlate with vessel diameter. In addition, the authors found that *ex vivo* preparations of the glycocalyx are much thinner compared to the EGL observed with TPM;[26] providing additional evidence for the confounds of traditional histology when assessing the EGL as mentioned above.

The human neurovascular EGL has also been visualized using a technique known as sidestream dark field (SDF) imaging[27] paired with a custom software specific to EGL assessment.[25] SDF imaging is often employed during cranial surgery to assess vascular parameters such as vessel density and blood flow velocity. Only a few subjects were enrolled in this study and therefore this study was underpowered to make any conclusions based on disease state. Clinicians should consider SDF imaging of the glycocalyx during cranial surgery to advance the field. For now, intravital imaging of the living rodent brain will continue to be employed to better understand the role of the EGL in CNS inflammation and other disease processes.

Diseases linked to the neurovascular endothelial glycocalyx

Given the recent evidence that the brain EGL forms a size-selective diffusion barrier,[11] it is plausible to consider that the brain EGL, when intact, contributes to the barrier properties of the BBB and may actually be the first line of defense against solute leakage into the brain. Conversely, loss or increased permeability of the brain EGL may contribute to many aspects of BBB dysfunction that include disruption, damage from shear stress, and pathological changes in brain EC signaling and/or transport. Below, we will discuss the aspects of brain EGL dysfunction that are known to occur with CNS diseases.

Septic encephalopathy and CNS infections

Septic encephalopathy (SE) is a frequent neurological complication of sepsis. Although SE is considered a reversible syndrome, there can be long-lasting cognitive symptoms after the sepsis resolves.[82] BBB dysfunction is thought to contribute to SE, and aspects of BBB dysfunction in SE have been recently reviewed elsewhere.[83] It is worth noting that systemic inflammation and asphyxia can strongly reduce EGL integrity.[84,85] As was discussed in a previous section, administration of LPS appears to contribute to EGL loss in capillaries of diverse vascular beds,[70] including brain capillaries,[10] as visualized by EM. However, these studies were limited to a single 20 mg/kg LPS dose that produces high lethality within 48 h. It is unclear to what extent EGL loss occurs/is detectable at lower LPS doses that induce neuroinflammation, sickness behaviors, and BBB disruption,[86] or to what extent brain EGL changes occur and persist in infectious models of sepsis that capture the resolution of inflammation and associated cognitive sequalae.[87]

Rodent models of CNS infections have also elucidated a role for the brain EGL in disease pathogenesis. For example, in a rodent model of experimental cerebral malaria (CM), an almost complete loss of brain EGL was evident from EM in mice with severe, terminal disease.[24] It was further shown quantitatively using an *in vivo* biotinylation assay that brain EGL-derived syndecan-1 levels decreased with terminal CM.[88] Notably, this model of CM caused 100% lethality in mice, and EGL loss was not apparent in experimental malaria infection with equivalent systemic parasitic burden but no CM.[24,88] Future research should

seek to investigate milder forms of systemic inflammation to investigate the involvement of the EGL.

Metabolic disease

Much work has been done to investigate the impact of diabetes and cardiovascular disease on the glycocalyx. While most of this work has investigated the peripheral EGL, a shift to the neurovascular glycocalyx is occurring due to the impact diabetes has on BBB structure[89] and function.[90] Patients with type 1 and 2 diabetes have damage to the EGL.[91,92] Specifically, the glycocalyx is reduced in sublingual capillaries in type-1 diabetes. Additionally, markers of glycocalyx damage, plasma HA and hyaluronidase levels, are increased.

Glucotoxicity, oxidative stress and neuroinflammation, as occurs in the diabetic *db/db* model, is known to induce shedding of the neurovascular EGL.[69] Indeed, *db/db* mice up to five months of age have damage to the EGL in cerebral microvessels, exhibited by decreased cationic ferritin binding to the luminal surface.[93] This damage was not directly related to oxidative microvascular changes. In another genetic model of type 2 diabetes and obesity, BTBR *ob/ob* mice display an altered glycocalyx using EM (unpublished observations by our group). A recent review has shown how diabetic effects on the EGL can render vascular beds vulnerable to viral invasions such as SARS-CoV-2.[94]

EGL loss in diabetes contributes to vascular complications including decreased arterial vasodilation,[95] loss of pericytes in retinas,[96] increased leukocyte adhesion,[97] and deterioration of the BBB.[93] Hyperglycemia leads to EGL shedding through several mechanisms some of which include reactive oxygen species, advanced glycation end products, and hyaluronidase activity.[98] Shrinkage, alteration, or degradation of the EGL is associated with development of cardiovascular diseases.[99]

In addition to diabetes, atherosclerosis is also associated with EGL degradation.[100] EGL thickness is correlated with cardiovascular risk factors including fasting plasma glucose levels, HDL and LDL levels, and body mass index.[80] Moreover, LDL interactions with the EGL are required for triglyceride transport into cells.[101] Diabetes and atherosclerosis are intimately associated with the EGL, and this relationship suggests the EGL as a novel therapeutic target for the treatment and/or prevention of these diseases.

Normative aging and Alzheimer's disease

ECM changes are generally accepted as associated with Alzheimer's disease (AD) and vascular dementia.[102–104] Vascular dementia is associated with a gradual loss in neurovascular density and deregulated cerebral blood flow.[105] Changes in cerebral blood flow have also been observed in normative aging,[106] human AD,[107] and animal models of AD.[108] Limited work has been done on the effects of normative aging, vascular dementia or AD on EGL structure and function in the brain. Within peripheral blood vessels, Machin *et al.* showed that aged mice, and humans, have a thinner EGL compared to young controls.[109] It remains to be determined whether the neurovascular EGL exhibits a similar age-related change.

Age-dependent deterioration of the BBB is associated with cognitive impairment in humans,[110] and as discussed above, the EGL plays a vital role in BBB function.[11] As such, it becomes important for us to understand how the brain EGL contributes to disease, in order to better treat brain disease. The EGL is affected by a variety of diseases, especially those that are age related, such as stroke and diabetes.[23,92,111] Moreover, recent reports indicate that plasma EGL constituents are increased in humans with stroke,[112] and animal models of multiple sclerosis.[113] The effects of normative aging on EGL abundance and composition have been difficult to study, as not all concurrent pathologies and exposures can be accounted for, and there is no consensus on what is 'aged'. Determining values for normal EGL thickness and composition may help us to better understand the pathophysiology of neurovascular aging.

Stroke, subarachnoid hemorrhage, and epilepsy

BBB dysfunction has been associated with both stroke, subarachnoid hemorrhage and epilepsy, and EGL health may play a vital role in the pathophysiology associated with these neurological disorders. Evidence suggests that the human neurovascular EGL changes after stroke.[23,112] EGL thickness can be measured clinically using non-invasive SDF imaging of accessible vasculature. Martens *et al.* discovered that human subjects with lacunar infarcts combined with white matter lesions exhibited an increased perfusion boundary region (PBR) in sublingual blood vessels.[23] An increased PBR suggests that white matter lesions from stroke may cause thinning of the neurovascular EGL and provides a novel target to potentially improve stroke outcome.

Subarachnoid hemorrhage (SAH) is a severe neurovascular disorder that associates with delayed cerebral ischemia (DCI) and neurological deficits. In a mouse model of SAH, a dramatic reduction in capillary blood flow was measured and subsequent neuronal loss was observed.[114] Hyaluronidase treatment in these SAH mice rescued blood flow and mitigated hypoxic brain injury.[114] These findings indicate that SAH may alter the neurovascular EGL, thereby impacting capillary blood flow and thus contributing to the onset of DCI. Increased blood levels of syndecan-1 and CD44 were also measured in human subjects with SAH and DCI,[115] suggesting that components of the EGL may serve as novel SAH biomarkers and potential therapeutic targets for neurovascular disorders.

Neurovascular dysfunction has also been associated with the pathophysiology of epilepsy.[116–118] Recently Haeren *et al.* reported a 5- to 15-μm increase in PBR within the cortical vasculature of humans with epilepsy, which did not correlate with sublingual PBR values.[27] These findings suggest that epilepsy may cause thinning of the neurovascular EGL and that further studies are warranted to directly measure the contribution of EGL health to BBB dysfunction.

Conclusions

The EGL serves as an active interface for communication between the CNS and periphery. Evidence suggests that neurovascular function may be dependent upon EGL abundance and composition. Alterations in the EGL may also be a causal factor for a wide range of CNS diseases. In this mini-review, we discussed the aspects of structure and function of the neurovascular EGL that have been investigated, and which may be predicted based on characterization of non-brain vasculature. We featured innovative approaches that have been used to study the neurovascular EGL within the human and in rodents. We also highlight potential mechanisms of EGL alteration that could inform novel therapeutic strategies for CNS-related disease.

Communication between the EGL and the immune system is crucial for proper EC function. Systemic inflammation strongly reduces the neurovascular EGL in mice,[10] which may promote adverse CNS effects[78] involving interactions between the EGL and immune cells at the EC surface.[34] BBB integrity is also affected by changes to the EGL,[11] which could contribute to the development of neurovascular-related diseases, including AD, stroke, and multiple sclerosis.

Reports indicate that the abundance and composition of the EGL play a role in neurovascular functions, including cerebral blood flow, immune-cell interactions, and even BBB integrity. Much is known about how genetic and environmental factors influence the peripheral EGL; however, little is known about the neurovascular EGL. Recent technological advancements, including TPM, have sophisticated our understanding of how the EGL contributes to neurovascular function. Future studies should employ these techniques to measure neurovascular EGL changes in the context of CNS disease in order to facilitate novel therapeutic design strategies.

AUTHORS' CONTRIBUTIONS

All the authors collected information, wrote the manuscript, and revised it critically.

DECLARATION OF CONFLICTING INTERESTS

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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