Like a Slippery Fish, a Little Slime Is a Good Thing: The Glycocalyx Revealed

Chuck Biddle, CRNA, PhD

The glycocalyx is a dynamic network of multiple membrane-bound complexes lining the vascular endothelium. Its role in maintaining vascular homeostasis includes regulating vascular permeability as well as a range of vital functions, such as mechanotransduction, hemostasis, modulation of inflammatory processes, and serving as an antiatherogenic. Revisionist thinking about the Starling principle is discussed in terms of the major influence of the glycocalyx on capillary and tissue fluid homeostasis. The clinical and pathophysiologic threats to the glycocalyx are reviewed as well as strategies to maintain its integrity.

Keywords: Diabetes, hypervolemia, intravenous fluids, vascular disease, vascular endothelial glycocalyx.

Objectives
At the completion of this course, the reader should be able to:

1. Describe what the glycocalyx is and review its constituent elements.
2. List the major functions of glycocalyx.
3. Identify the role of the glycocalyx in a revised view of the Starling principle.
4. Recognize the clinical and pathophysiologic threats that degrade the glycocalyx.
5. Develop a strategy that optimizes the function of the glycocalyx.

Introduction
If you have ever held—or attempted to hold—a fish, you have encountered what is termed the glycocalyx. This slippery, secreted substance provides the fish with a host of vital functions. It serves as a defense against parasites and disease, helps to regulate fluid balance within, contains both enzymes and antibodies, and serves to reduce friction, optimizing movement through the fluid milieu in which it lives. Damage to this slimy coat can result if the fish is physically traumatized, is exposed to toxins, encounters sudden ambient temperature fluctuations, or becomes stressed from a predator, or even if the ambient water pH or composition is altered.

More than 70 years ago a scientific article investigating the skins of frogs was published, which was to set the stage for modern-day considerations of how we view heart disease, immune function, blood coagulation, and the manner in which we hydrate our patients in the operating room. The paper proposed the existence of a protein-based material lining the endothelial tissues that played a vital role in how vascular fluid filtration took place. This lining, eventually termed the vascular endothelial glycocalyx, is now appreciated as a vital element of our physiology.

We have all been slimed in a manner of speaking. The slime being referred to exists not on the outside, but rather on the inside of our body, and its particular relevance to the anesthesia provider is the slimy, gel-like glycocalyx of the intravascular compartment.

The vascular endothelium is lined by a carbohydrate-rich layer of molecules largely made up of proteoglycans and glycoproteins. The proteoglycans are proteins with long, straight, molecular chains attached; hairlike projections extend into the vessel lumen. The overall makeup
is one of soluble plasma components that are interlinked in a very dynamic way. That is, there is an ongoing, continually replenishing process between constituents being lost and constituents being added. The membrane-bound mesh takes on a fluid, yet gel-like (slime?) quality that fills the spaces between the larger, membrane-bound proteins. This intricate, thin layer covers the endothelial cells of our blood vessels and is foundational to our vasculature’s barrier characteristics (keeping fluid in or out, based on physiologic need). In essence, the glycocalyx forms a kind of endothelial surface layer that performs vital chores for us, unless it gets perturbed, and like the fish noted earlier, vulnerability ensues.

Figure 1 is a microscopic image of the endothelial glycocalyx; the various constituents of the vascular lining are depicted as well. The wedding of glyco, in reference to the carbohydrate-dense molecules, and calyx, with its Greek roots for “cup” or “chalice,” suggests a kind of sweet/organic, protective envelope.

The negatively charged glycocalyx consists of a web of membrane-bound glycoproteins and proteoglycans that are associated with a variety of glycosaminoglycans. It is cooperatively connected to the vascular endothelium by a host of backbone molecules (primarily the aforementioned proteoglycans and glycoproteins) forming a network in which a variety of water-soluble molecules is incorporated. The glycosaminoglycan is able to bind water up to 10,000× its own weight, thus making a major contribution to the overall volume of the endothelial glycocalyx.

In a symphony of cooperativity, the glycocalyx performs its various functions in a dynamic manner. Figure 2 is a simplification of its presence in the vasculature as it creates an “exclusion zone,” facilitating erythrocyte navigation while preventing mechanical contact with the basement epithelial cell. Because we have so many blood vessels in the body (on average, 60,000 miles of vessels in an adult), in the aggregate, the endothelial glycocalyx constitutes a volume of 700 to 1,700 mL, ranging in thickness from 0.2 μm to 8.0 μm. Most of its volume is in the capillaries, because it is the capillaries that constitute the majority of the composite surface area of the human circulatory system. The variability in size and thickness depends on the individual, vessel location, and the “health” of the glycocalyx.

**Functional Importance of the Vascular Endothelial Glycocalyx**

The essential role of the glycocalyx in maintaining vascular homeostasis cannot be overemphasized. Its major functions are listed in Table 1 and will be briefly described here.

- **Regulation of Vascular Permeability.** This will be discussed in more detail later, but the intact vascular glycocalyx provides a double barrier beyond what the vascular endothelial cells provide. The glycocalyx is able to limit the movement of certain molecules to the endothelial cell membrane. Processes that impair or degrade the glycocalyx result in loss of barrier functionality and subsequent loss of fluid to the interstitium.

- **A Mechano-transducer Regulating Vascular Tone.** In
its movement through the body, blood produces physical forces against the vascular wall. These forces, known as shear stress, can induce the production and release of vasodilating molecules such as nitric oxide from the vascular endothelium. Recent work strongly implicates the glycocalyx as having an important role in mechano-transduction, finding that its core proteins are the key players in translating shear stress signals into target cells responsible for production of vasculomodulating chemicals.2

- **Moderating Leukocyte and Platelet Adhesion.** The glycocalyx contains core proteins such as heparin and chondroitin sulfates, and because of other chemical influences as well, adhesion of leukocytes and blood platelets to endothelial cells is prevented. The corollary to this is that damage to the glycocalyx alters this substantially, increasing the adhesiveness of both, promoting inflammation and clot formation.

- **Antithrombotic Effect in the Vasculature Due to “Enzyme Docking.”** The nature of the glycocalyx is such that it is ready and able to bind numerous plasma-derived molecules in a highly selective way. The “docking” (think of an agonist or antagonist molecule “docking” onto a receptor) adds a major vasculoprotective role to the glycocalyx. Examples of these are antithrombin III, heparin cofactor II, and tissue factor pathway inhibitor.

- **Repulses Red Blood Cells From the Vascular Endothelium.** When the glycocalyx is intact, it provides a smooth, gel-like (or slimy) surface that allows blood cells to flow smoothly without physically contacting the vascular endothelium. Think of it this way: frying an egg in a traditional iron skillet will invariably lead to the egg sticking; frying an egg in a pan with a nonstick coating results in the food not sticking. The glycocalyx is a nonstick lining.

- **Reduction in Oxidative Stress.** Endothelial glycocalyx is highly sensitive to oxidative stress, and when it occurs, it can induce substantial microvasculature dysfunction. The intact glycocalyx can bind quenchers of oxygen radicals (so-called free radicals) such as superoxide dismutase, reducing oxidative stress and maintaining nitric oxide bioavailability.

### Starling’s Description of Fluid Homeostasis—Revisited

Ernest Starling’s3 sentinel 1896 article, dryly titled “On the Absorption of Fluids From the Connective Tissue Spaces,” established that fluid movement due to filtration across the wall of a capillary is dependent on the balance between the hydrostatic pressure gradient and the oncotic pressure gradient across the capillary. His now classic formula established a relationship among 4 forces:

- Hydrostatic pressure in the capillary
- Hydrostatic pressure in the interstitium

### Table 1. Major Functions of the Vascular Endothelial Glycocalyx

<table>
<thead>
<tr>
<th>Function</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regulation of vascular permeability in peripheral vessels</td>
<td>Acts as a mechanotransducer detecting shear stress, moderating vascular tone</td>
</tr>
<tr>
<td>Moderating (obtunding) leukocyte and platelet adhesion</td>
<td>Antithrombotic effect in the vasculature due to “enzyme docking”</td>
</tr>
<tr>
<td>Antithrombotic effect in the vasculature due to “Enzyme Docking.”</td>
<td>Repulses red blood cells from the endothelium</td>
</tr>
<tr>
<td>Reduction in oxidative stress</td>
<td>Reduction in oxidative stress</td>
</tr>
</tbody>
</table>

**Figure 2. Red Blood Cell (RBC) in Vessel With the Glycocalyx Exclusion Zone**
The balance among these forces permitted the calculation of the end (net) driving pressure for transvascular fluid filtration. Figure 3 is a simplified rendering of the Starling principle (also called the Starling hypothesis) for fluid filtration.

Role of the Glycocalyx in Modifying the Starling Principle

An essential role of the glycocalyx, and one that has the most immediate importance to the anesthesia provider, is its active interface between the blood and capillary wall. A variety of recent and unique studies have markedly enhanced our understanding of the role or roles of the glycocalyx, and its dynamic function as a barrier is now well appreciated. The glycocalyx is semipermeable with respect to certain macromolecules such as the plasma proteins (eg, albumin), which are able to penetrate and incorporate into the glycocalyx, facilitating its nourishment and subsequent physiologic function. An intact glycocalyx cannot be penetrated by red blood cells or large molecules such as dextran 70.

The glycocalyx present on the vascular endothelial surface binds plasma proteins, forming an endothelial surface area with a resultant high intravascular oncotic pressure. The very low net fluid movement passing through the intact glycocalyx has extremely low protein content; therefore, the oncotic pressure underneath the glycocalyx is very low. An inward-directed oncotic pressure gradient results as the low concentrations of protein below the glycocalyx are cleared away into the interstitial space, eventually captured by the lymphatic system. An appreciation of the role of the glycocalyx advances our understanding of the physiology of vascular permeability, revising our thinking about the traditional Starling principle (Figure 4).

The bottom line is that the endothelial glycocalyx is a critical determinant of vascular permeability, serving as a gatekeeper to fluid and protein penetration. The glycocalyx is composed of numerous constituents, primarily the membrane-bound molecules noted earlier, resulting in a noncirculating, protein-rich, gel-like plasma. Given our understanding of the functionality of the glycocalyx, it is clear that damage to it will lead to protein extravasation, movement of fluid out of the intravascular compartment, and resultant interstitial edema. Revisions of traditional Starling principle applications to vascular permeability stress the important, modifying influences of the glycocalyx as a “double-barrier” based on a wealth of recent research. Readers interested in exploring this in greater detail should consult the reviews by Woodcock and Woodcock and also Rehm et al.

Phenomena that Threaten or Damage the Glycocalyx

Contrast healthy, functional vascular glycocalyx with that which has been damaged, as illustrated in Figure 5. The glycocalyx is highly sensitive to perturbations and is fragile in nature. There are numerous clinical scenarios that can set the stage for damaging the glycocalyx (Table 2).

Shedding is the term applied to loss of glycocalyx constituents. Like a person who gradually loses hair and becomes bald, or a rising river that erodes its bank, various processes can lead to loss (partial or complete) of the glycocalyx (Figure 6). These phenomena will be discussed individually here.

- Ischemia. Direct tissue damage secondary to ischemia is highly destructive to the glycocalyx. Microvascular dysfunction along with enhanced adhesion of leukocytes...
and platelets magnifies the injury, with endothelial cells eventually experiencing oxidative stress. Myocardial glycocalyx appears to be particularly sensitive, as does that of vessels nourishing central nervous system tissue. Near total loss of the glycocalyx in these tissues can occur very rapidly in the event of ischemia.

- **Inflammation and Trauma.** A host of insults that evokes an inflammatory response can lead to glycocalyx damage. Sepsis results in the release of a number of inflammatory mediators (eg, tumor necrosis factor-α, bacterial lipopolysaccharide) that can degrade the endothelial glycocalyx. Even in the absence of sepsis, surgical trauma itself can produce an impressive loss of glycocalyx. It is important for the anesthesia provider to appreciate that the inflammatory-induced loss of the glycocalyx may be followed by even more inflammatory processes as

---

**Figure 4. The Starling Principle Revisited: Influence of the Glycocalyx**

Abbreviations: EC, endothelial cell; HPI, hydrostatic P in interstitial space; HPvL, hydrostatic P in vascular lumen; OPesL, oncotic P in endothelial surface layer; OPI, oncotic P in interstitial space; OPvL, oncotic P in vascular lumen.

**Figure 5. Healthy, Functional Glycocalyx Versus Damaged Glycocalyx**

**Table 2. Factors That Threaten or Degrade the Glycocalyx**
the lost glycocalyx renders the now exposed endothelial cells targets for leukocyte attachment. This vicious cycle is the basis for systemic inflammatory response syndrome, which carries a mortality above 75%.8

• Atherosclerosis. High concentration of low-density lipoprotein (LDL) is a known risk factor for heart disease and atherosclerosis. Subendothelial deposits lead to inflammation and eventually to plaque formation. Studies have revealed that LDL degrades the glycocalyx and increases platelet stickiness.9 The sum of the research in the area strongly suggests that alteration of the glycocalyx plays an important and evolving role in the progression of atherosclerosis.

• Diabetes. We are all well aware that diabetes is frequently associated with profound vascular comorbidities. Attendant pathophysiology includes an increased vascular permeability, impairment of vasoresponsiveness, and a proatherogenic effect. Even acute hyperglycemia has been shown to reduce the volume of the glycocalyx with a resultant increased vascular permeability and albuminuria; the latter is a clear clinical sign of deterioration in vascular barrier function.10 A recent clinical study of male volunteers experiencing acute hyperglycemia, rapid glycocalyx perturbation mediating vascular dysfunction was observed.11 Here a 6-hour period of hyperglycemia in healthy subjects decreased the systemic glycocalyx volume by approximately 50% compared with control values.

• Intravenous Fluid Mismanagement. The type and volume of fluid we use in our patients can greatly affect the function of the glycocalyx and this understanding is transforming our thinking about perioperative fluid management. Overhydration resulting in hypervolemia is a pathogenic factor altering the glycocalyx. A large body of clinical research has emerged that clearly demonstrates that excessive fluid infusion provokes atrial natriuretic peptide (ANP) release, which in turn initiates shedding of the glycocalyx.12,13 Release of ANP into the circulation by the heart occurs in the face of both increased wall stress and hypervolemia. This detrimental effect of volume loading by ANP is well known to produce rapid shifts in intravascular fluid into the interstitial space and loss of glycocalyx.

Parallel to this is the substantial body of systematic work demonstrating the superiority of the natural colloid, albumin, for developing and maintaining the glycocalyx over other intravenous fluid choices. Crystalloids freely and rapidly distribute across the vascular endothelium with only about one-fifth of such an infusion remaining intravascular after about an hour. Recent work notes that for artificial colloid solutions, the negative charges on their surface molecules render them unavailable to the glycocalyx and thus unable to contribute to its integrity. The naturally occurring albumin has both positive and negative molecular surface charges and is able to maintain and actively contribute to the endothelial glycocalyx. This was recently discussed in the Anesthesia Patient Safety Foundation Newsletter, whose contributors suggested that the safety of hydroxyethyl starch solutions is now in doubt.14

On June 24, 2013, the US Food and Drug Administration (FDA) issued a boxed warning on the use of hydroxyethyl starch (HES) solutions in certain clinical settings.15 The FDA’s careful analysis of accumulated data and research revealed an increased risk of death and severe renal injury, as well as risk of bleeding, in critically ill, adult patients. Although the predominance was seen in those undergoing cardiac surgical procedures, there was sufficient concern to urge providers to “not use HES solutions in critically ill adult patients.”15 For related information, readers should consult the FDA website (http://

Figure 6. Shedding of the Glycocalyx
Protecting the Glycocalyx: Clinical Interventions to Optimize Its Function

First, there is a growing body of literature suggesting that there is a need to reconsider the widespread clinical use of nonphysiologic solutions as a substitute for natural plasma colloid. Not only is there solid literature noting unique contributions that albumin makes to the glycocalyx, but the literature also demonstrates an ability to at least partially restore degraded barrier properties. The simplest way to achieve protection may be to ensure sufficient concentration of plasma protein and make every effort to avoid overhydration and resultant hypervolemia.

Second, there is now good theoretical and basic science research suggesting that corticosteroids, in particular, hydrocortisone, may prevent shedding of the glycocalyx under surgical conditions, thus maintaining its barrier function and also preventing leukocyte and platelet adhesiveness. The composite result is a reduction in inflammation and tissue edema.

Third, clinical studies are under way that are looking at direct targets to inhibit the degradation of the glycocalyx. These involve specific drugs such as antioxidants, etanercept, ilomastat, antithrombin III, and other protease inhibitors. Antithrombin, as an inhibitor of the coagulation process and also possessing anti-inflammatory effects, has received particular scrutiny. It preserves glycocalyx volume and function, maintaining vascular barrier function and reducing interstitial edema.

The interaction of antithrombin with endothelial glycocalyx glycosaminoglycans is critical for its effects as a thrombin inhibitor. Related studies are in the early stages of clinical relevance and are beyond the scope of this review.

Fourth, there may also be anesthetic techniques that may better preserve the glycocalyx than others. Although research in this area is limited, it was recently found that sevoflurane-based general anesthesia was superior to propofol-based general anesthesia in protecting the endothelial glycocalyx during surgery.

The effects of general anesthesia vs regional anesthesia have yet to be systematically studied, but this research area is likely to be pursued in the near future.

Conclusion

The glycocalyx is a highly dynamic lining of the vascular endothelium. It plays a major role in regulating vascular permeability as well as maintaining vascular homeostasis throughout the circulatory system. It modulates nitric oxide synthesis and release via its mechanotransducer properties as well as inhibiting platelet adhesion. Damage to the glycocalyx results in not only vascular permeability but also a proatherogenic state. As our understanding grows, it is clear that our perioperative decision making and resultant interventions will, at least in part, deserve thoughtful consideration to the glycocalyx.

REFERENCES


AUTHOR
Chuck Biddle, CRNA, PhD, is a staff anesthetist and professor at Virginia Commonwealth University Medical Center in Richmond, Virginia. He is the editor-in-chief of the AANA Journal and had no oversight role in the peer review of this manuscript, which was handled by other members of the editorial board. Email: cbiddle@hsc.vcu.edu.