

THE GLYCOCALYX: THE GATEKEEPER OF THE ENDOTHELIUM

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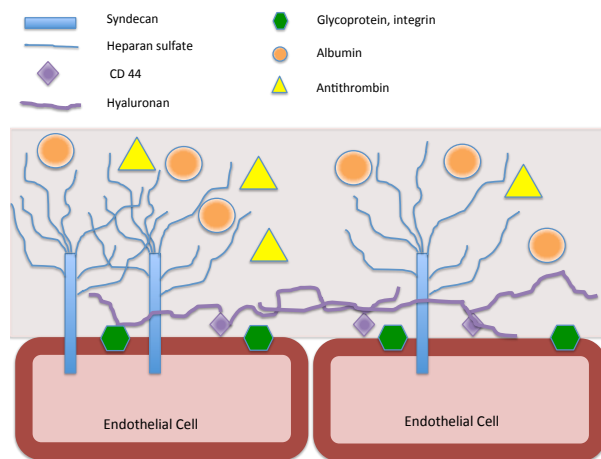
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Physiology of the glycocalyx

The endothelial glycocalyx (EG) is a carbohydrate-rich scaffold of proteoglycans and glycosaminoglycans on the luminal surface of endothelial cells.¹ The main proteoglycans are the syndecans (Figure 1). These structures are transmembrane and play a role in transmitting shear stress signals to the cytoskeleton of the endothelial cell and also changing their binding capacity according to intracellular signalling pathway activation. They also provide an anchor for glycosaminoglycans such as heparan sulfate. Another important glycosaminoglycan is hyaluronan, which weaves its way through the glycocalyx and is anchored by cell surface receptors such as CD44.

Figure 1 – The main components of the endothelial glycocalyx



The meshwork of carbohydrates and proteins creates an immobile plasma layer and barrier, preventing interaction between cells in circulation and endothelial cells. It does this by physically ‘hiding’ glycoproteins such as selectins, integrins and immunoglobulins.² This prevents adhesion of platelets and white blood cells. It also discourages thrombosis by housing anticoagulants such as antithrombin III and tissue factor pathway inhibitor.

The EG also plays a role in the endothelial response to shear stress. Increased flow leads to local nitric oxide production, with subsequent vasodilation, and increased vascular permeability, both mediated via the

EG.³ The EG can also change in thickness under certain flow rates. Evidence suggests that the EG becomes thicker under low flow rates, increasing vascular resistance, likely due to adherence of plasma components.⁴ Under high flow rates, the actual thickness of the EG appears unaffected if the blood is diluted with plasma.⁵ However, saline haemodilution decreases vascular resistance independent of a reduction in blood viscosity and shear-induced vasodilation. This effect is likely due to loss of the EG and an increase in intra-luminal diameter due to ‘washout’ of soluble glycocalyx constituents.

The EG plays an important role in fluid flux across the endothelium, which has led to a revision of the traditional Starling’s forces.⁶ Albumin molecules within the EG layer provides some oncotic pressure, and it is the oncotic balance between the intravascular (flowing) compartment and the EG compartment that affects fluid flux according to protein levels. This explains why raising oncotic pressure in the intravascular compartment does not actually draw fluid from the interstitial space, much to the disappointment of clinicians attempting to reduce peripheral oedema. However, delivering a fluid with iso-oncotic or hyper-oncotic properties serves to hold fluid in the intravascular compartment and diminish fluid flux through the EG layer. Therefore, this strategy of fluid therapy may still serve a purpose in critical care to maintain effective circulating blood volume while allowing restriction of crystalloid fluids in the oedematous patient. When choosing any combination of fluid types, it must always be tailored to the individual patient with recognition that no one strategy has been shown to be broadly effective over another.

Shedding of the glycocalyx due to injury

Pro-inflammatory mediators, such as tissue necrosis factor- α (TNF α), interleukin-1 β , lipopolysaccharide (LPS), toll-like receptor agonists and thrombin, have been shown to induce

shedding of the EG.⁷⁻¹⁸ Endocan has also been shown to be shed from endothelial cells by LPS.¹⁶ Syndecan-4 is also shed by TNF α from the glomerular endothelial cells.¹¹ Thrombin and plasmin have both been shown to cleave syndecan-1 and -4 in cell culture.¹²⁻¹⁴ It is likely that shedding of the EG is one of the first steps in inflammation and coagulation, which then allows interactions between endothelial cells and circulating leucocytes and platelets.^{4,19-22} The timing of this process, whether the EG sheds first or activated leucocytes promote EG shedding, has not been fully explored in a clinical model and may differ between circumstances. Loss of the glycocalyx will also have effects on mechanotransduction however the exact process is still being characterised.²³

Many *in vivo* experimental studies have demonstrated EG shedding in response to a variety of stimuli, including inflammatory mediators,^{15,17} endotoxin,¹⁸ and crystalloid fluid therapy.²⁴ In one study that mimicked a clinical scenario, a porcine trauma model, syndecan-1 increased within 15 minutes of injury.²⁵ Clinical research assessing glycocalyx biomarkers in critically ill people is limited to fairly small studies. Syndecan-1 has been shown to be higher in septic ICU populations, compared to healthy controls,^{26,27} post-abdominal surgery patients²⁸ and healthy men injected with LPS.²⁹ Syndecan-1 is also increased in trauma patients on admission to ED.³⁰⁻³² Endocan has been shown to be higher than healthy controls in patients with sepsis, according to severity,^{16,33} and in trauma patients.³⁴ Heparan sulfate has also been shown to be increased in sepsis³⁵⁻³⁷ with one study showing that levels were higher in non-survivors.³⁷ Interesting, in one study using healthy men, it took 24 hours for heparan sulfate levels to significantly increase after injection of TNF α .¹⁸ Hyaluronan has been shown to be increased in one small septic population³⁸ and in ICU patients receiving mechanical ventilation,³⁶ whereby higher levels were found in patients with a pulmonary source of sepsis compared to an extra-pulmonary source. People with out-of-hospital cardiac arrest had increased hyaluronan 48-72 hours after the event, but not in the first 6 hours, therefore, similar to heparan sulfate, this marker may also be slow to rise after the initial insult.³⁹

Common to the clinical studies previously mentioned is a limitation of cohorts to ICU patients; post-intervention and late in the disease process. Blood volume expansion is a key first-line intervention in many types of critical illnesses and fluid boluses have been shown to increase EG damage markers.^{24,40} Combined with the insult of inflammation, large-bolus fluid therapy may further enhance EG damage, and heavily influence the degree of marker increase in ICU populations.

Shedding of the glycocalyx due to intravenous fluid therapy

There is some evidence that rapid intravenous crystalloid fluid therapy, without coinciding injury or inflammation, can increase EG damage markers.^{24,40} In one study, healthy euvoalaemic men were bolused one of three fluid types; 7.1ml/kg of dextrans or albumin, or 21.4ml/kg of Ringer's acetate.²⁴ The Ringer's acetate group had an increase in an EG damage biomarkers, whereas the other two fluid types did not. Interestingly, the dextrans group showed the lowest levels 3 hours after the infusion. It is unknown if this response to blood volume expansion is related shear stress, release of atrial natriuretic peptide,^{41,42} dilution of EG constituents, or a combination of these factors. In studies using euvoalaemic models, it is difficult to separate the effects of hypervolaemia from the effects of the fluid infusion itself and the scenario is less applicable to emergency medicine. One controlled-haemorrhage shock model using mice compared fluid resuscitation with either Lactated Ringer's (three x shed volume) or fresh frozen plasma (FFP)(one x shed volume).⁴³ The group that received FFP had less lung hyperpermeability, less lung myeloperoxidase content, and less EG shedding. However, it is unknown if this effect was due to the provision of plasma constituents or the lower volume used for resuscitation.

Evidence of glycocalyx damage in the dog

Unfortunately, there is little information on the presence of glycocalyx damage in the dog. Research is limited by poor availability of quality assays to measure biomarkers of EG damage. One paper describing a sepsis model in dogs used assays that were not validated for the dog therefore results are questionable.⁴⁴ Our group has had success with measuring hyaluronan via ELISA in dogs in shock, but assays for syndecan-1 have been disappointing thus far. Sidestream darkfield microscopy shows some promise in estimating the thickness of the glycocalyx via imaging capillaries in the buccal mucosa. However, it is not currently validated for this use in the dog. Work is currently undergoing in this area.

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