The Endothelial Glycocalyx

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Overview

The glycocalyx is a complex gel layer that coats all healthy vascular endothelium. This layer exists in dynamic interaction between flowing blood and the endothelial cell wall. It plays a pivotal role in vascular protection, modulation and haemostasis.

Components

The endothelial glycocalyx is essentially made up of glycoproteins or proteoglycans but there is great diversity in structure and function within these two groups.

Proteoglycans have a protein core to which are attached negatively charged glycosaminoglycan (GAG) side chains. There are different types of proteoglycans and they vary according to: the core protein size, the number of GAG side chains and whether they are bound or not bound to the cell membrane. Syndecans and glypicans are first bound to the cell membrane and the GAG side chains are attached later. Other proteoglycans (perlecans, versicans, decorins, biglycans, mimecans) are first bound to GAGs and then secreted by the endothelial cells.

There are five types of GAG side chains: heparan sulphate makes up 50–90%, with the remainder composed of hyaluronic acid and chondroitin, dermatan and keratin sulphates. Hyaluronic acid is the only GAG not usually bound to a core protein and forms viscous solutions with water.

Glycoproteins act as adhesion molecules and contribute to the coagulation, fibrinolytic and haemostatic systems. These include E and P selectin which are expressed after stimulation by histamine, thrombin, interleukin-1 and tumour necrosis factor (TNF-α). Ligands for leucocyte and platelet adhesion ICAM-1, ICAM-2, VCAM-1, PECAM-1 are also expressed within the glycocalyx.

Structure

The glycocalyx provides a luminal mesh for the binding of proteins and other active molecules. The composition and dimensions of the glycocalyx fluctuate as it continuously replaces material sheared off by flowing plasma. The thickness of the glycocalyx thickness varies throughout the vasculature, between 0.1 and 1 µm.

The glycocalyx has a net negative charge that is dependent on GAG side chain sulphation which is modified by physiological and pathophysiological stimuli. This affects protein binding and vascular permeability. The charged mesh of the glycocalyx acts as a macromolecular sieve, repelling negatively charged molecules, as well as white and red blood cells and platelets. Macromolecules larger than 70 kDa are excluded from the glycocalyx. Albumin is 67 kDa and thus leaks through the glycocalyx despite its mostly negative charge. However it does also carry positive charge, and this amphoteric nature helps it to also bind to the glycocalyx. This binding reduces the hydraulic conductivity across the vascular barrier.

The binding of plasma constituents to the inert framework of glycocalyx produces the physiologically active endothelial surface layer (ESL). The ESL regulates vascular permeability, influences blood cell-vessel wall interactions, affects rheology and controls the microenvironment.

Physiology

Models of fluid movement across the vascular barrier, traditionally based on the principles derived by Starling in 1896, have been modified by our understanding of the endothelial surface layer.

Starling, who was not aware of the existence of the glycocalyx, described four forces that regulate fluid homoeostasis. The sum of tissue oncotic and luminal hydrostatic pressures forces fluid out of the vessel into the tissue. The sum of tissue hydrostatic and luminal oncotic pressures forces fluid out of the tissue into the vessel. According to this model there is a great movement of fluid both out of and into vessels. However, experiments over the past 25 years do not show this great fluid movement.

To resolve this problem, three key components of Starling’s equation were revised.

1) Sustained venous reabsorption does not occur in most tissues, only in specialized tissues like the kidney and intestines. This was shown by Levick in 1996. In contrast the net force between venular hydrostatic pressure and tissue hydrostatic and oncotic pressures favours venous filtration. The alternate route for fluid to return to the circulation is the lymphatic system. However, measurements of lymphatic flow showed it was too slow to clear fluid produced by the capillary filtration rate according to Starling’s model.

\[ F/A = C_H \left( (P_c - P_{is}) - \sigma (\pi_c - \pi_{is}) \right) \]

*Figure 1*: The Starling principle (*Jv/A* = volume filtered per unit area, *Lp* = hydraulic conductance, *Pc* = capillary hydrostatic pressure, *Pis* = interstitial hydrostatic pressure, *σ* = osmotic reflection coefficient, *πc* = capillary oncotic pressure, *πis* = interstitial oncotic pressure)
2) Capillary filtration rate is much less than Starling predicted. This is a logical conclusion since a scenario of no venous reabsorption, low lymphatic flow and high capillary filtration should lead to tissue oedema, but it doesn’t.

Why is the capillary filtration rate it less than Starling predicted? And how is this possible?

3) The endothelial surface layer reduces hydraulic conductivity. Adamson et al. showed that oncotic forces are only set up across the endothelial surface layer on the luminal aspect of the endothelial cell, not across the whole capillary wall. Capillary filtration rate is tightly regulated. The oncotic pressure differences across the endothelial surface layer reach equilibrium very quickly, thereby leading to less fluid filtration.

The glycocalyx model presents an efficient system that resolves all three of these problems. Fluid filtration is regulated at the point at which it begins – within the capillary lumen by the endothelial surface layer.

**Revised Starling Equation**

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F/A = C_H \left[ (P_c - P_{is}) - \sigma (\pi_p - \pi_{sg}) \right]
\]

*Figure 2*: Revised Starling principle *Jv/A* = volume filtered per unit area, *Lp* = hydraulic conductance, *Pc* = capillary hydrostatic pressure, *Pis* = interstitial hydrostatic pressure, \(\sigma\) = osmotic reflection coefficient, \(\pi_p\) = oncotic pressure on plasma-side of ESL, \(\pi_{sg}\) = oncotic pressure in subglycocalyx space

**Pathology**

There are certain pathological processes that affect glycocalyx integrity and leads to shedding and disruption of constituents.

This list broadly includes:

1) Ischaemia/reperfusion
2) Sepsis
3) Volume loading
4) Hyperglycaemia
5) Atherosclerosis
6) Trauma/cardiac and aortic bypass surgery

Effects of shedding:

- capillary leak
- oedema
- accelerated inflammation
- platelet aggregation
- hypercoagulability
- loss of vascular responsiveness
Assessment of the glycocalyx

The glycocalyx is a difficult structure to visualize because it is destroyed by conventional dyeing and fixation techniques. Dynamic assessment of the glycocalyx is also difficult and current technologies are not readily available and can only assess the glycocalyx in easily assessible parts of the circulation, eg. sublingual microcirculation. These novel technologies are: Orthogonal polarization spectral imaging (OPS) and side-stream dark field imaging (SDF).

Therapies

These therapies may provide benefit due to their effects on the glycocalyx:

- Avoiding hyperglycaemia
- Albumin
- Steroids in septic shock
- Sevoflurane
- Avoiding hypervolaemia
- Statins

Therapies such as TNFα inhibition, antithrombin III and infusions of glycocalyx constituents are still experimental.

References

1. Available on request: email: csalphonsus@gmail.com