ENDOTHELIAL GLYCOCALYX

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Consideration of the nature of endothelial glycocalyx has to be preceded by appreciation of the diversity of endothelial cells that boast a glycocalyx layer, and how endothelial cells bind to one another and the tissues they service. The capillaries that serve skin, connective tissues, muscles, lung parenchyma, nervous system *etc* are termed continuous non-fenestrated capillaries; they are connected to one another along their edges by near-continuous adherens junctions and tight junctions, and the luminal glycocalyx layer of adjacent endothelial cells is essentially continuous. Within the family of continuous capillaries there are of course some necessary specializations. For instance, central nervous system capillaries have very few interendothelial junction gaps and are almost impervious to solvent in health; the blood – brain barrier, while pulmonary capillaries are adapted to conducting high blood flow rates at low pressure. As we shall see, continuous capillaries are now known to filter solvent from the intravascular to extravascular space, creating an extracellular fluid circulation that includes the lymphatics. Contrary to the classic version of the Starling principle still presented in most physiology and medicine textbooks, reabsorption of filtered fluid does not occur in continuous capillaries; the steady state Starling principle. Another family of capillaries is the continuous fenestrated capillaries; they feature cartwheel-shape windows where the luminal endothelial cell membrane is fused to the abluminal. Fenestrations enable the bi-directional passage of fluid and are found in tissues where substantial absorption of fluid into a capillary is required, such as lymph node capillaries, capillaries supplying endocrine glands, the choroid plexus and gut mucosa. The glomerular capillary is so spectacularly fenestrated that it deserves to be in a family of its own. Creating 120 ml of filtrate per minute from around 1.2 litres of renal blood flow, it defines the glomerular fitration rate. Finally we meet the family of capillaries that supply the sinusoidal tissues such as liver, spleen and bone marrow. With many interendothelial gaps and discontinuous glycocalyx layer they do not filter soluble molecules, so their interstitial fluid has a similar protein concentration to plasma. The basic structure of the endothelial cells has long been appreciated by light microscopy techniques.

Electron microscopy was invented in 1931 by Knoll and Ruska. The first transmission electron microscope was available to researchers in 1939. The wavelength of an electron can be up to 100,000 times shorter than that of a photon, so electron microscopes can reveal the structure of objects invisible by light. It should not be surprising that the early electron microscopists were so captivated by cells and their intracellular organelles that relatively little attention was paid to the thin luminal lining of the endothelial cells until 1966, when J.H. Luft published his work on 'Fine structures of capillary and endocapillary layer as revealed by ruthenium red." Luft's endocapillary layer has achieved fame or notoriety as the endothelial glycocalyx (EG). In 1963, Bennett suggested 'glycocalyx' as the general term for an "extracellular sugary coating, wherever it may be found." The idea that the EG is a passive luminal gel that comes between circulating red cells and the endothelial cell membrane is giving way to the realization that it is a complex, multicomponent, biochemical structure that functions exquisitely as a molecular sieve, a lubrication layer for red blood cell motion, an inhibitor of inflammation and a sensor of plasma flow-induced fluid shear strain.

Older readers may recall the Fahreus effect; as they pass along in vivo capillaries, erythrocytes can be seen to concentrate along the axis of the vessel, leaving a gap between themselves and the capillary cell membrane. The faster the flow, the greater the gap. It became evident that the haematocrit of blood sampled from larger vessels could not be presumed to reflect the haematocrit of the blood within the capillaries. In 1990 Desjardins and Duling used heparanase to fragment the EG and showed that the capillary hematocrit was due to the presence of intraluminal EG. In 1997 Pries *et al* found that the resistance to blood flow in microvessels was much higher than in glass capillaries of the same diameter. The concept that emerges is that the EG is an intravascular gel phase that retards plasma flow near the vessel wall and excludes erythrocytes. The presence of the EG explains a lower central volume of distribution for erythrocytes than for plasma water. Fragmentation or thinning of the EG will lead to lower vascular resistance. Earlier electron micoscopic studies substantially underestimated the in vivo volume of the EG because fixation techniques dehydrated the layer. We now hold the EG of microvessels to be around 0.4-0.5 microns, or 15-20% 0f the radius of the smallest capillaries. The EG of larger vessels is often thicker. Measuring the volume of the intravascular gel phase with any precision is impossible because of frequent fluctuations and an ill-defined boundary with free-flowing plasma, but over one litre has been calculated for healthy adults, and less than a litre in adults with diabetes.

The backbone molecules of the EG are membrane-bound proteins (proteoglycans) and glycosylated proteins (glycoproteins). They are synthesized and assembled in a series of steps as they are shuttled in vesicles from the endoplasmic reticulum to the golgi apparatus and finally to the cell membrane. Glycoproteins are typically cell surface receptors, selectins, integrins, and other functionally dynamic proteins at the cell surface. Proteoglycans are more structural and are made of a core protein anchored to the cell membrane with long glycosaminoglycans (GAGs) attached to them. The fixed molecules are decorated by soluble molecules, some derived from the cell and some plasma constituents. Some GAGs are not assembled as part of protein synthesis but rather assembled extracellularly and later bound to surface proteins or receptors. One example is hyaluronic acid (HA), also called hyaluronan, which links to the endothelial surface receptor, CD44. This HA/CD44 interaction is now known to be a contributor responsible for what is termed the molecular sieve characteristic of the EG. This means long HA GAGs weave through the EG just above the cell surface and create a fence-like meshwork which contribute to size exclusion of plasma molecules. Heparan sulfate accounts for more than half of the EG GAGs, chondroitin sulphate being the next commonest. These soluble GAGs are responsible for the seamless meshwork that bridges the luminal surfaces of one endothelial cell to the next, thereby creating a semipermeable filter to large solutes. At the EG – plasma boundary there predominate plasma components, linked by soluble proteoglycans and GAGs. The most numerous plasma protein albumin is present within the EG, and the permeability of the capillary to albumin is found to be increased when albumin levels are very low. This would only be clinically relevant in the most extreme states of hypoalbuminaemia. The composition and thickness of the EG continuously fluctuate with changes in flow and with changes in circulating soluble molecules. The most recent electron microscopic studies show a quasi-periodic structure to the EG, like bushes planted by a fastidious gardener in a hexagonal array with 100nm spacing. This regularity appears to be due to anchoring of the EG structure to the intracellular actin cortical cytoskeleton (ACC).

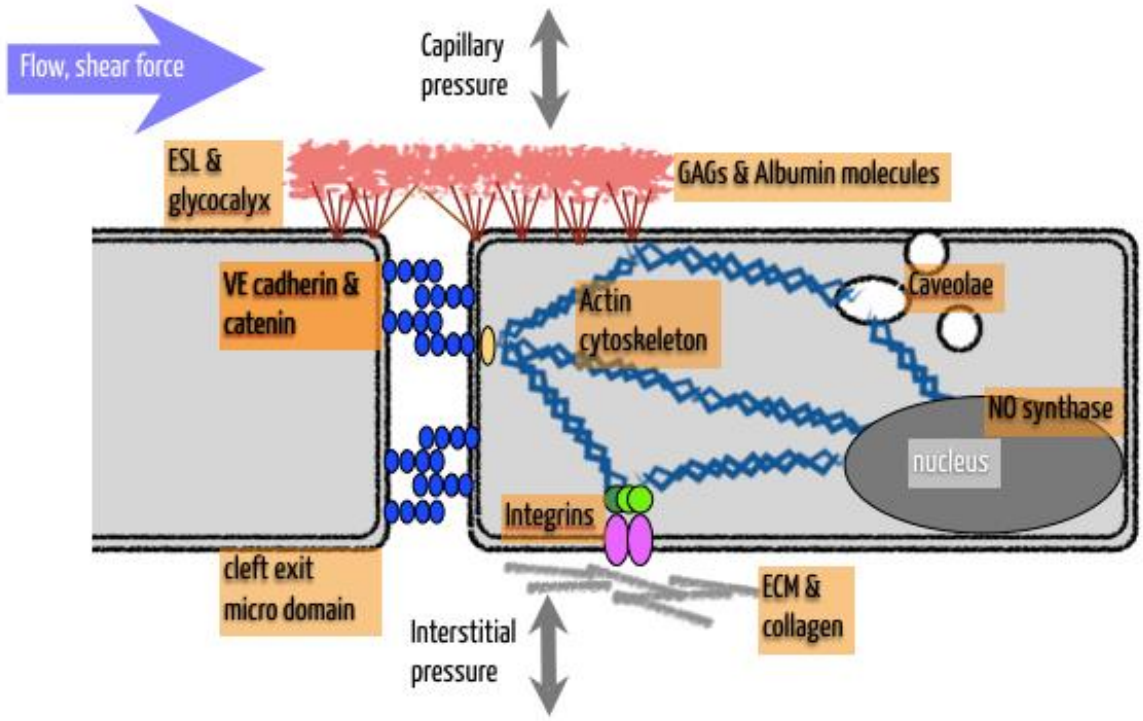

Figure 1: Endothelial glycocalyx and relation to other endothelial cell components. ESL = endothelial surface layer. GAG = glycosaminoglycan. ECM = extracellular matrix.

Fluid velocity within the intravascular gel phase (the EG) is greatly attenuated and the endothelial cell membrane experiences very little direct fluid shear strain. Intracellular responses to flow-induced fluid shear strain are largely due to a drag on the EG matrix fibres. When the EG is intact, fluid shear strain is transmitted to the cell through the core proteins, and the specific connections of these proteins to the ACC (syndecans) and the plasma membrane

(glypicans) mediate specific cell signaling such as NO production. However, this stress is also distributed to other regions of the cell, most notably the intercellular junctions and the basal adhesion plaques, where transduction to intracellular biochemical signals also occurs. When the EG is degraded fluid shear strain is transmitted closer to the plasma membrane and apical signaling can proceed by mechanisms that differ from those associated with the transmembrane core proteins of the EG. Remarkably this appears to ensure that the stresses delivered to the basal adhesion plaques will be pretty much the same with or without an intact EG. In other words, for a given fluid shear strain level, a basal adhesion plaque does not know if the cell has an intact EGL or not; it senses similar stress.

While we have noted that the EG does not buckle or crush in response to a moving erythrocyte, in circulatory arrest and erythrocyte stand-still the EG is crushed by the weight of the red cell. The EG is inflated while plasma is flowing and fluid escape from the gel phase is retarded. After plasma and erythrocyte flow is restarted, the crushed EG is restored to full thickness in about one second. The restorative mechanisms include oncotic forces of the adsorbed plasma proteins, flexural rigidity of the core proteins, and electrochemical repulsion amongst the fixed negative charges. A white cell is much larger, and does indent the EG as it passes, perhaps by as much as 80% in smaller capillaries.

Leucocytes deformed by emerging from a capillary whose diameter is smaller than their own, into a wider post-capillary venule are more likely to tether in that area because of activation of selectin molecules and their counterligands. The EG is therefore likely to play a critical role in the inflammatory response cascade, inhibiting primary leukocyte capture and systemic leukocyte extravasation and providing a physical barrier that can regulate and localize leukocyte recruitment to inflamed vascularized tissue.

EG can be fragmented by enzymes and by shear forces. Rehm at al. showed in 2007 evidence of EG shedding in human patients undergoing cardiac surgery. They suggested the cause was ischaemia/ reperfusion and examined plasma levels of syndecan-1 and heparan sulfate. Clinical experiments hint at glycocalyx protection for hydrocortisone therapy, isoflurane anaesthesia, and avoidance of excessively raised capillary pressure.

In 1980, Michel published “A Fiber Matrix Model of Capillary Permeability.” This rejected intercellular pore theory and suggested “the endocapillary layer is a three-dimensional network formed by the fibrous chains of the membrane glycoproteins of the endothelial cell coat reinforced by the absorption of plasma proteins.” Since the landmark study published by Adamson *et al* 2004 it is established that the EG is the primary molecular sieve for circulating soluble proteins. Indeed, it is the EG that retards the extracellular circulation of these solvents causing their higher up-stream concentration within plasma and lower downstream concentration within the interstitial gel phase. When the Starling principle is reconsidered, taking the EG as it covers interendothelial junction clefts to be the molecular sieve, we are led to some remarkable conclusions about fluid circulation and lymph flow that have been affirmed by experiments.

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