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Review

Glycocalyx and endothelial (dys) function: from mice to men

Bernard M. van den Berg[1], Max Nieuwdorp[2], Erik S.G. Stroes[2], Hans Vink[1,2]

[1]Department of Physiology, Cardiovascular Research Institute Maastricht (CARIM), Maastricht University, Maastricht, The Netherlands

[2]Department of Vascular Medicine, Academic Medical Center and University of Amsterdam, Amsterdam, The Netherlands

Correspondence: Hans Vink, e-mail: h.vink@fys.unimaas.nl

Abstract:
Located on the luminal surface of vascular endothelial cells, the glycocalyx is composed of a negatively charged mesh of proteoglycans, glycosaminoglycans, glycoproteins and glycolipids and harbors a wide array of enzymes that contribute in regulation of leukocyte-/thrombocyte adherence, with a principal role in plasma and vessel wall homeostasis. Glycocalyx disruption is accompanied by enhanced sensitivity of the vasculature towards atherogenic stimuli which emphasizes that not only the composition of the glycocalyx is important in facilitating these properties but that the contribution of its physical dimension and barrier properties should also be considered. In addition, similarities found between micro-versus macro vascular beds suggest common structural properties throughout the entire vascular bed that might be of importance in protective strategies against vascular perturbation. Collectively, these data lend support to a potential role of the glycocalyx as a first barrier in protection against atherogenic insults. Therefore, it will be a challenge to determine whether glycocalyx volume measurement, systemically or at the individual capillary level, is a feasible surrogate marker for cardiovascular disease, and whether it may prove to be of use to assess the impact of novel interventions aimed at glycocalyx restoration on atherosclerosis progression.

Key words:
glycocalyx, endothelium, composition, permeability, volume assessment

Abbreviations: ec-SOD – endothelial cell super oxide dismutase, LDL – low density lipoprotein, Lp – hydraulic conductivity, NO – nitric oxide, OPS imaging – orthogonal polarization spectral imaging

The endothelial surface glycocalyx

At the interface of flowing blood and the vascular endothelial lining, a glycocalyx shields the vascular wall from direct exposure to blood flow, contributes to the vascular permeability barrier and its anti-adhesive properties, and stimulates endothelial release of nitric oxide (NO) by mechanotransducing fluid shear stresses. Numerous studies have contributed in elucidating endothelial glycocalyx composition, as reviewed by Pries et al. [22], that resulted in the view of a negatively charged mesh of proteoglycans, glycosaminoglycans, glycoproteins and glycolipids on the luminal surface of vascular endothelial cells. Particularly, the endothelial glycocalyx harbors a wide array

of enzymes and proteins, e.g. endothelial nitric oxide synthase, extra cellular superoxide dismutase, angiotensin converting enzyme, anti-thrombin III, lipoprotein lipase, hepatic endothelial lipase, apolipoproteins, growth factors, and chemokines, that contribute in regulation of leukocyte-/thrombocyte adherence and with a principal role in plasma and vessel wall homeostasis.

The first electron micrographs revealed a small irregular shaped layer extending approximately 50- to 100 nm into the vessel lumen [15]. Subsequent approaches with varying perfusate contents or fixatives revealed stained structures on endothelial cell surfaces throughout diverse microvascular beds, arterial- and venular macrovessels with large variations in dimension and appearance [2, 3, 10, 25, 28, 34]. These studies, especially when specific approaches were applied that stabilize anionic carbohydrate structures to prevent loss- and or collapse of these structures, gave evidence for a thick endothelial surface layer (Fig. 1).

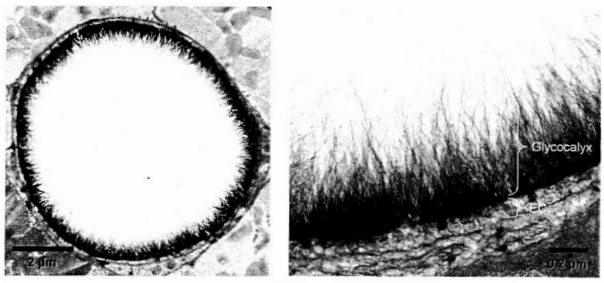

Fig. 1. Electron micrograph of a goat coronary capillary stained with Alcian blue

Intravital microscopy studies on cremaster muscle showed dramatic differences between microvascular- and systemic hematocrit [13], that could be abrogated upon enzymatic treatment of the microvascular network with heparinase or hyaluronidase [7, 11]. By comparing the width of the plasma column filled with fluorescein-labeled dextran to the luminal endothelial cell boundaries, evidence for a 0.4- to 0.5 µm thick continuous endothelial cell surface layer was provided [31]. Based on these observations, theoretical studies predicted a glycocalyx thickness of 0.5- to 1.0 µm accounting for the observed variations in red-cell motion through the micro vessels and the discrepancy between *in vivo* and *in vitro* estimates of resistance to blood flow [6, 8, 24]. Such an unexpectedly large dimension of the glycocalyx exceeds the dimensions of the endothelium and adhering leukocyte adhesion molecules several fold and argue for a protective role of the glycocalyx dimension under physiological conditions of the blood vessel. Indeed, various studies observed alterations in glycocalyx dimension upon ischemia/reperfusion [3], hypoxia [34], high-density- [21] and low-density lipoprotein [4, 30], and variations in wall shear stress [10, 27].

Endothelial glycocalyx and vascular permeability

Vascular barrier properties determine transport of fluid to and from the interstital space given a balanced equilibrium between opposing oncotic and hydrostatic pressures according to the Starling principle [26]. Consequently, there is a continuous turnover of fluid in the body, caused by the fact that fluid is filtered from blood to tissues at the arterial end of the circulation and reabsorbed at the venous end. Excess fluid not taken up at the venous end is removed from the tissue by the lymphatics.

One of the main permeability parameters that can be obtained is the hydraulic conductivity (Lp), i.e. the vessel wall permeability to water. In microvascular beds within the various organs, a very large variability in the Lp can be found as a result of the involvement of coordinated functions of a host of players to optimize blood flow and match exchange of solute flux with tissue demand. These variations are observed between the various vessel types involved (arterioles, capillaries and venules), up to the individual capillary level.

However, it was argued that when the molecular sieving properties of the capillary wall are determined by a fiber matrix, covering all endothelial channels and filling intracellular boundaries, molecular selectivity would remain constant. This was hypothesized as the fiber matrix concept developed by Curry and Michel [5]. In this concept it was suggested that the local protein concentration gradient which is necessary for the colloid osmotic pressure (or oncotic pressure), is localized across the glycocalyx and not between the plasma and tissue underlying the endothelium. Thus, the endothelial surface glycocalyx

maintains the fluid balance between blood and tissue that results in a balance between absorption and filtration of water. This hypothesis was confirmed by the remarkable similarity of protein permeability and reflection coefficient, i.e. rejection of molecular passage, in fenestrated and in continuous capillaries in spite of their differences in filtration coefficient and ultrastructure [23]. Recently, this was further illustrated by very similar estimates of the permeability parameter in relation to the presence of charge-selective properties between peripheral- and glomerular capillaries, although the renal glomeruli are the body's most active filtration units, producing about 180 liters of primary urine per day with a minimal loss of proteins [19].

Since glomerular capillaries consist of fenestrated endothelial cells, whereas peripheral endothelial cells are predominantly of the continues type, it appears that in most vascular beds the glycocalyx behaves as a molecular filter which allows the free exchange by convection and diffusion, of water, ions and small hydrophilic solutes between the plasma and tissue spaces, but limits the passage of especially charged macromolecules. These findings argue for equal selective permeability properties throughout the various vascular networks and organs, predominantly dependent on the high plasma concentration of negatively charged albumin the main contributor in oncotic pressure.

Endothelial glycocalyx in micro-versus macro vasculature

Studies in both micro- and macro vasculature demonstrated similarity in glycocalyx constituents such as hyaluronan [10, 11, 28], release of NO [9, 17, 35], and presence of endothelial cell super oxide dismutases (ec-SOD) [16], which are all involved in vascular homeostasis and protection against damage. Collectively, these observations are of particular interest since altered vascular permeability, attenuated NO-bioavailability and redox dysregulation are amongst the earliest characteristics of atherogenesis [14].

In spite of these observations, it has proven difficult to show direct relevance of the glycocalyx as a vasculoprotective paradigm for larger vessels. The latter is predominantly due to the fact that glycocalyx research has traditionally focused at the microvasculature, in which atherogenesis does not occur. However, several studies have emphasized that the relevance of the glycocalyx is not confined to smaller vessels [3, 27]. Thus, van Haaren et al recently visualized a thick endothelial glycocalyx in larger arteries in rats [29]. Interestingly, small glycocalyx dimensions that correlated significantly with local thickening of the intimal layer (Fig. 2) and is accompanied by significant swelling of the subendothelial matrix, lends direct support to a potential role of glycocalyx perturbation in making low-shear regions more susceptible

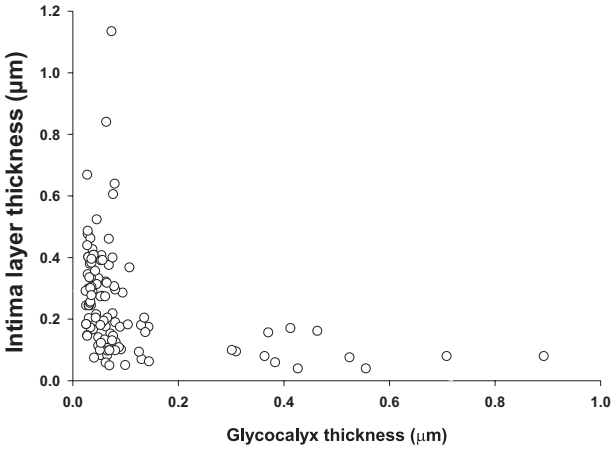

Fig. 2. Intimal layer thicknesses as a function of glycocalyx thickness in the murine carotid artery bifurcation segment. A significant correlation ($P < 0.001$; $\rho -0.523$), as assessed by means of two-tailed Spearman's non-parametric test, was observed

to atherogenesis [27, 33]. The glycocalyx in larger vessels has also been shown to decrease extravasation of low density lipoprotein (LDL) particles into the subendothelial space [1, 12]. Amongst others, these data imply that also in the macro vasculature the glycocalyx adds towards the vasculoprotective properties of the vessel wall.

Glycocalyx volume assessment in humans

To date, direct visualization of endothelial glycocalyx in humans has been unsuccessful, mainly due to the fact that the endothelial glycocalyx is a very delicate

structure depending critically on the presence of flowing plasma [22]. Since the endothelial glycocalyx provides limited access to plasma macromolecules and erythrocytes, the best way to measure the endothelial glycocalyx in humans is to compare intravascular volumes using a glycocalyx impermeable tracer, i.e. labeled autologous erythrocytes [20, 31] and a glycocalyx permeable tracer such as neutral Dextran 40 (MW 40 kDa), exemplified in Fig. 3. At present, such measurements in patients with type 1 diabetes revealed a profound reduced systemic glycocalyx volume compared with healthy age and sex-matched controls [18].

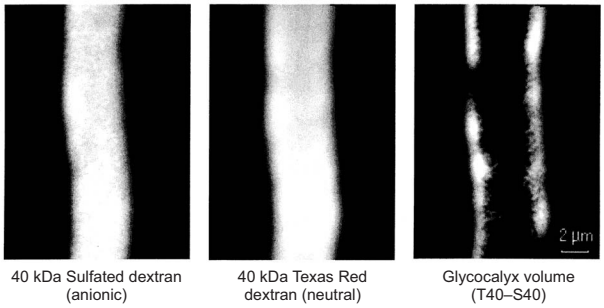

Fig. 3. 3D microscopic reconstruction of fluorescent tracer distributions in mouse cremaster tissue capillaries. Contributed by Vink H, Stace TM, and Damiano ER [32]

Visualization of the capillary endothelial glycocalyx in humans

The abovementioned reductions in systemic glycocalyx volume in diabetes patients were confirmed by reductions in glycocalyx dimension in individual capillary blood vessels. Such estimates of individual capillary glycocalyx dimensions were obtained using orthogonal polarization spectral (OPS) imaging of the sublingual microcirculation [18]. Images of capillary red cell columns were obtained in healthy control (Fig. 4, left panel) and type 1 diabetic subjects. The change in capillary red cell column width following capillary leukocyte passage (Fig. 4, center and right panels) can be used to provide an estimate of the capillary dimension by comparing the anatomic capillary diameter, i.e. the red cell width while glycocalyx is still compressed (Fig. 4, right panels), with the functional perfused capillary diameter, i.e. the red cell column width before leukocyte passage (Fig. 4, center panels). In line with the systemic glycocalyx volume measurements, capillary glycocalyx dimensions were reduced by 40% in diabetic patients.

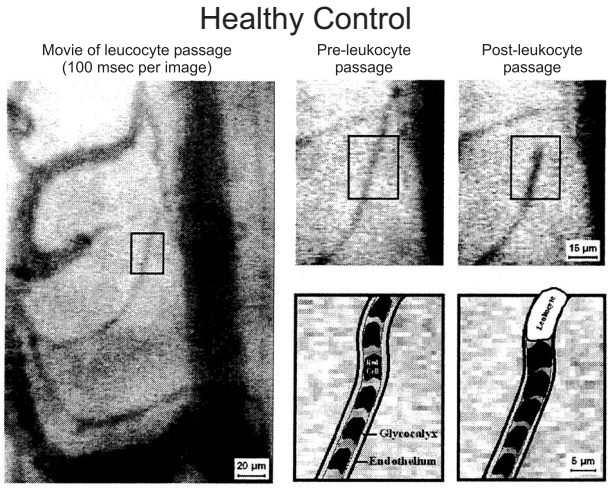

Fig. 4. Orthogonal polarization spectral (OPS) imaging of the sublingual microcirculation by Cytoscan (Cytometrics, Philadelphia, PA) in healthy control subject. Left, overal image of capillary red cell columns. Center, detailed image of capillary red cell column before leukocyte passage (upper panel), with illustration (lower panel). Right, detailed image of capillary red cell column following capillary leukocyte passage (upper panel), with illustration (lower panel)

Conclusions

Located at the interface of flowing blood and the vascular endothelial lining, currently available evidence shows that the endothelial glycocalyx exerts a wide array of functions to ensure maintenance of interstitial fluid balance and facilitating an anti-atherogenic vascular wall surface. Glycocalyx disruption is accompanied by enhanced sensitivity of the vasculature towards atherogenic stimuli which emphasizes that not only the composition of the glycocalyx is important in facilitating these properties but that the contribution of its physical dimension and barrier properties should also be considered. In addition, similarities found between micro-verses macro vascular beds suggest common structural properties throughout the entire vascular bed that might be of importance in protective strategies against vascular perturbation.

Collectively, these data lend support to a potential role of the glycocalyx as a first barrier in protection against atherogenic insults. Therefore, it will be a challenge to determine whether glycocalyx volume measurement, systemically or at the individual capillary level, is a feasible surrogate marker for cardiovascular disease, and whether it may prove to be of use to assess the impact of novel interventions aimed at glycocalyx restoration on atherosclerosis progression.

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