Journal of Pathology
J Pathol 2012; **226:** 562–574
Published online in Wiley Online Library
(wileyonlinelibrary.com) DOI: 10.1002/path.3964

INVITED REVIEW

Endothelial glycocalyx dysfunction in disease: albuminuria and increased microvascular permeability

Andrew HJ Salmon[1,2] and Simon C Satchell[1]*

[1] *Academic Renal Unit, University of Bristol, Southmead Hospital, Bristol, UK*
[2] *Microvascular Research Laboratories, Bristol Heart Institute, School of Physiology and Pharmacology, Southwell Street, University of Bristol, UK*

**Correspondence to: Simon C Satchell, Academic Renal Unit, University of Bristol, Southmead Hospital, Bristol BS10 5NB, UK.*
e-mail: s.c.satchell@bristol.ac.uk

Abstract

Appreciation of the glomerular microcirculation as a specialized microcirculatory bed, rather than as an entirely separate entity, affords important insights into both glomerular and systemic microvascular pathophysiology. In this review we compare regulation of permeability in systemic and glomerular microcirculations, focusing particularly on the role of the endothelial glycocalyx, and consider the implications for disease processes. The luminal surface of vascular endothelium throughout the body is covered with endothelial glycocalyx, comprising surface-anchored proteoglycans, supplemented with adsorbed soluble proteoglycans, glycosaminoglycans and plasma constituents. In both continuous and fenestrated microvessels, this endothelial glycocalyx provides resistance to the transcapillary escape of water and macromolecules, acting as an integral component of the multilayered barrier provided by the walls of these microvessels (ie acting in concert with clefts or fenestrae across endothelial cell layers, basement membranes and pericytes). Dysfunction of any of these capillary wall components, including the endothelial glycocalyx, can disrupt normal microvascular permeability. Because of its ubiquitous nature, damage to the endothelial glycocalyx alters the permeability of multiple capillary beds: in the glomerulus this is clinically apparent as albuminuria. Generalized damage to the endothelial glycocalyx can therefore manifest as both albuminuria and increased systemic microvascular permeability. This triad of altered endothelial glycocalyx, albuminuria and increased systemic microvascular permeability occurs in a number of important diseases, such as diabetes, with accumulating evidence for a similar phenomenon in ischaemia–reperfusion injury and infectious disease. The detection of albuminuria therefore has implications for the function of the microcirculation as a whole. The importance of the endothelial glycocalyx for other aspects of vascular function/dysfunction, such as mechanotransduction, leukocyte–endothelial interactions and the development of atherosclerosis, indicate that alterations in the endothelial glycocalyx may also be playing a role in the dysfunction of other organs observed in these disease states.

Keywords: glycocalyx; endothelium; albuminuria; proteinuria; permeability; diabetic nephropathy; diabetes; glomerulus; ischaemia–reperfusion injury; dengue

Received 14 September 2011; Revised 4 November 2011; Accepted 5 November 2011

No conflicts of interest were declared.

Introduction

Exchange of water and solutes across the vessel wall is the primary function of the microcirculation. Physiological regulation of this exchange is a fundamental homeostatic process achieved by modulation of aspects of haemodynamics, including pressure, flow rate and the surface area available for exchange, as well as the permeability of the microvascular walls themselves. Microvascular exchange, which can be measured as flux or clearance, is therefore distinct from permeability, which describes the intrinsic property of the capillary wall to impede the movement of fluid or solutes.

Capillaries throughout the body have common features but often possess unique adaptations for their particular role in microvascular exchange. All capillaries are lined by endothelial cells supported by a basement membrane, but differ in the details of this basic structure, as well as in the type of supporting cell they possess. The consequent multilayer arrangement of capillary walls ensures that they function as multicomponent, composite exchange barriers. We will outline the components of those barriers in continuous and fenestrated capillaries, and focus in particular on the glomerular capillary wall as a uniquely adapted composite barrier, in both anatomical and functional terms: in the glomerulus, the selective permeability properties of capillaries in general are harnessed to allow filtration of plasma and hence excretion of waste solutes. One consequence of this composite arrangement is that defects in any one of the components may result in disordered permeability.

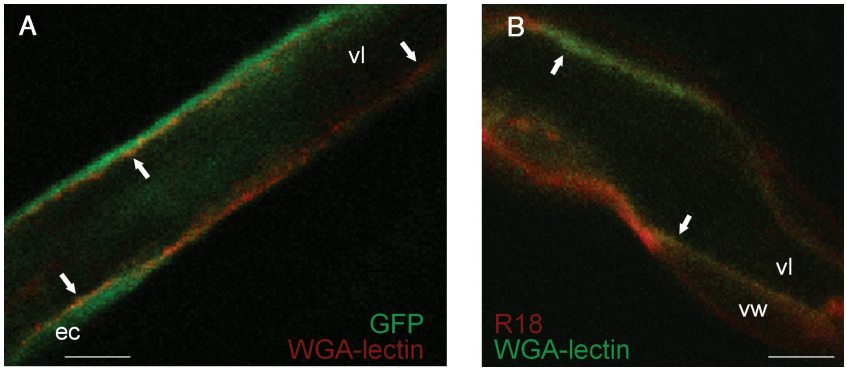

Figure 1. Endothelial glycocalyx labelling *in vivo* and *ex vivo* with fluorescently-tagged lectin. (A) Systemic application of Alexa-594-tagged wheat germ agglutinin (WGA) lectin (red) *in vivo* labels the endothelial glycocalyx (arrows) lining the luminal aspect of endothelial cells (ec) in a mesenteric microvessel of a mouse that overexpresses green fluorescent protein (GFP) in endothelial cells, under control of the Tie2 promoter. (B) Efferent arteriole of a manually dissected wild-type mouse glomerulus, cannulated and perfused with the plasma membrane label R18 (red) and FITC-tagged WGA–lectin (green). WGA–lectin again labels the endothelial glycocalyx covering the luminal aspect of the arteriolar wall (arrows). Confocal microscopy images of the endothelial glycocalyx appear similar in these *in vivo* and *ex vivo* preparations; vl, vessel lumen; vw, vessel wall; scale bars = 5 μm.

The luminal surface of vascular endothelium throughout the body is covered with a layer of glycocalyx (Figure 1): a hydrated mesh rich in carbohydrates and in dynamic equilibrium with plasma constituents [1,2]. The endothelial glycocalyx has important roles in transduction of shear stress, regulation of leukocyte–endothelial cell interactions, regulation of clotting and complement cascades, growth factor binding and, of particular interest here, it contributes to the permeability of the capillary wall [3,4]. We review the evidence that altered endothelial glycocalyx results in disordered microvascular permeability, which in the kidney manifests as albuminuria. We discuss the hypothesis that generalized damage to the endothelial glycocalyx occurs in a number of diseases characterized by both albuminuria and altered systemic permeability, and address the contribution that damage to the endothelial glycocalyx in those conditions may make to other facets of those diseases.

The structure of the capillary wall and transmural pathways for fluid and solute exchange

The capillaries and postcapillary venules of the microcirculation are the site of solute exchange between the intra- and extravascular compartments. The endothelium of these vessels may be described as continuous, fenestrated or discontinuous [5,6]. In continuous endothelia, one cell directly contacts the next at intercellular junctions. Continuous endothelia may possess fenestrations, transcellular cytoplasmic holes, which may or may not contain diaphragms [7]. The mature glomerular endothelium is characterized by fenestrations without diaphragms [8]. In discontinuous endothelial there are significant gaps between adjacent cells where basement membrane may also be absent.

Continuous capillaries

Endothelial glycocalyx

Multiple constituents of the endothelial glycocalyx are recognized [1,3,4], including cell surface-anchored components such as proteoglycans (PGs) and sialoproteins, and adsorbed components such as albumin, orosomucoid and lumican [9]. PGs consist of a core protein, eg syndecan or glypican, and glycosaminoglycan (GAG) side-chains. Heparan sulphate and chondroitin sulphate GAGs are prominent in the endothelial glycocalyx and are largely responsible for its anionic charge. Hyaluronan is a non-sulphated GAG which may be anchored to the cell surface by various receptors and interacting proteins or simply adsorbed onto the cell surface-anchored components [4]. Sialoproteins, possessing a number of neuraminic acid residues, are also prominent. Adsorbed components of the endothelial glycocalyx are essential for normal function of the layer [9,10]. Reports on the depth of the endothelial glycocalyx vary greatly, but the layer extends up to 1 μm from the endothelial cell membrane and varies in composition and appearance between different blood vessels and vascular beds. Some of these differences are at least partly due to variation introduced by preparation and imaging methods [3,11,12]. Little is known regarding the regulation of glycocalyx composition but this is likely to be determined by a the balance between stimuli that increase glycocalyx biosynthesis (eg shear stress [13]) and those that lead to its degradation (eg inflammation [14])

Cell–cell junctions

The principle pathway of fluid and solute movement across continuous endothelia is paracellular, ie through interendothelial clefts, which occupy approximately 0.2% of the endothelial cell surface [15] (Figure 2A). Junctional adhesion molecules form adherens and tight junctions which are interspersed within the clefts,

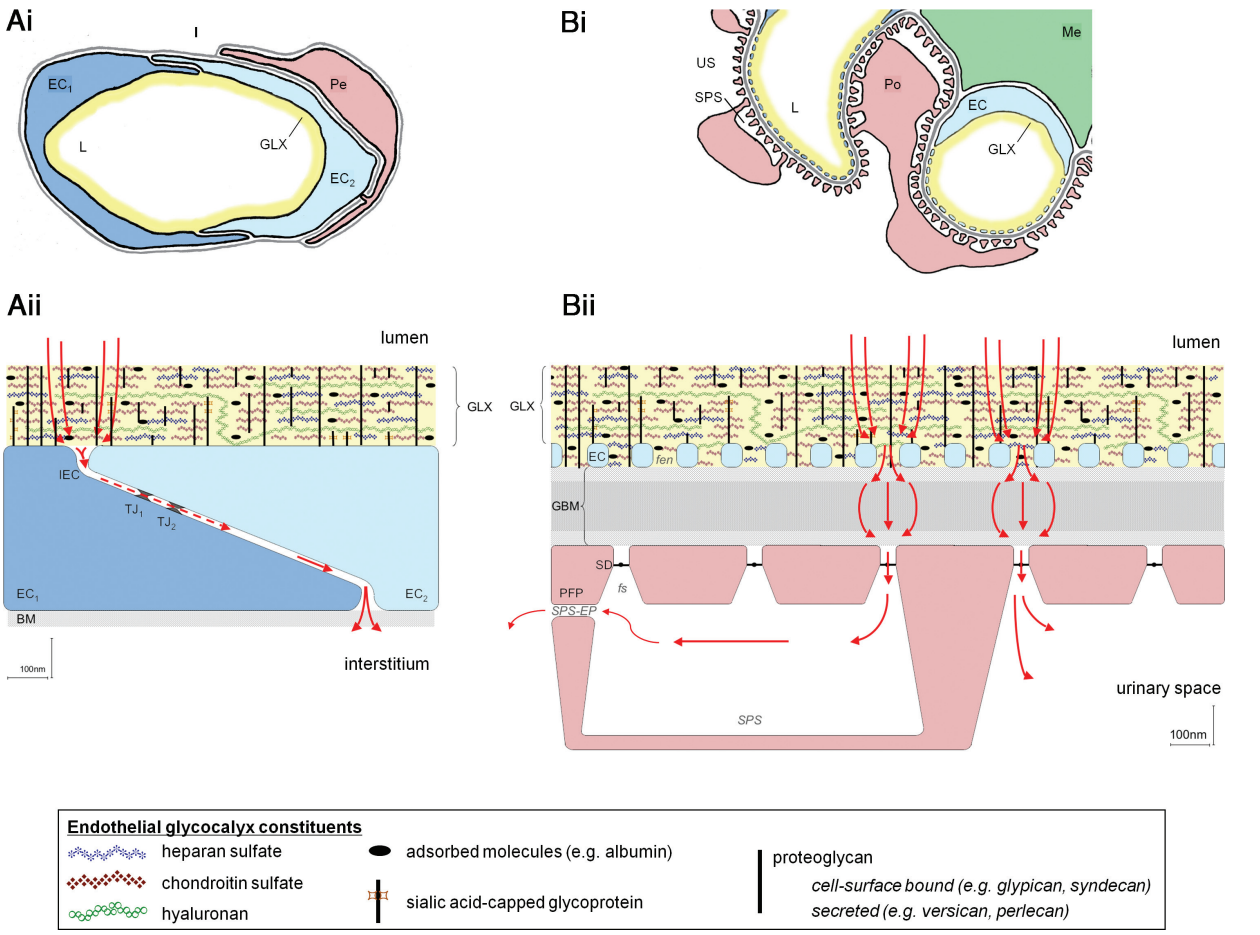

Figure 2. Pathways across capillary walls. (A) Continuous microvessel; (Ai) representation of the wall of a continuous microvessel, formed by two endothelial cells (EC_1, EC_2) and an associated pericyte (Pe). A continuous endothelial glycocalyx layer (GLX: yellow) forms the interface between the endothelial cells and the vessel lumen (L). Endothelial cells and the pericyte are invested by basement membrane (grey) and embedded within interstitium (I). (Aii) 2D scaled representation of a cross-section across a continuous capillary wall. Endothelial glycocalyx (GLX) overlies two adjacent endothelial cells (EC), including the entrance to an interendothelial cleft (IEC) containing two tight junction strands (TJ). The major route of fluid and solute flux (red arrows) is between the fibres of the GLX, through the IEC and between gaps in the TJ strands, and finally across the basement membrane (BM) to reach the interstitium; scale bar = 100 nm. (B) Fenestrated glomerular capillary: (Bi) representation of the walls of two adjacent glomerular capillaries. Endothelial glycocalyx (GLX; yellow) forms the interface between fenestrated endothelial cells (EC) and the vessel lumen (L). Glomerular basement membrane (grey) is interposed between endothelial cells and the foot processes of podocytes (Po), which are either covered by podocyte cell bodies (subpodocyte space: SPS) or revealed directly to the urinary space (US). Central-facing portions of these glomerular capillaries abut mesangial areas (Me). (Bii) 2D scaled representation of a cross-section across a glomerular capillary wall. GLX is present above and within endothelial cell (EC) fenestrae (fen). The glomerular basement membrane (GBM) is interposed between endothelial cells and podocyte foot processes (PFP). Slit diaphragms (SD) bridge the filtration slit (fs) between adjacent PFPs; 60% of filtration slits are covered by restrictive spaces beneath podocyte cell bodies [sub-podocyte space (SPS)], from which filtered fluid must exit via SPS exit pores (SPS-EP). The major route of fluid and solute flux (red arrows) is between the fibres of the GLX, through fenestrae, and in divergent streams across the GBM before being channelled through filtration slits into either SPS and thence via exit pores into urinary spaces, or directly into urinary spaces. Scale bar = 100 nm.

restricting the flux of water and solutes through them [16]. The organization and abundance of these cell–cell junctions varies throughout the vascular tree [16] and confers specialized permeability properties, eg tight junctions predominate in specialized circulations with particularly low permeability (eg retina and brain) [17–19].

Basement membrane

All endothelial cells are in direct association with a basement membrane—a specialized 50–100 nm layer of extracellular matrix [20,21]. Basement membranes are composed of a meshwork of type IV collagen and laminin with entactin (nidogen) acting as a bridging protein between them. Proteoglycans, in particular perlecan, are another important component. The exact composition of basement membranes varies, with particular isoforms of collagen type IV and laminin chains characterizing individual locations [22].

Supporting cells

Pericytes are distributed at intervals along capillaries and postcapillary venules within the basement membrane. The pericyte coverage ranges from 22% in

cerebral cortex capillaries [23] to as much as 95% in skeletal muscle capillaries [24]. Pericytes have long cytoplasmic processes, which directly contact endothelial cells forming communicating gap junctions [25]. Complex cell–cell communication between these cells and endothelial cells via soluble mediators, as well as direct contact via gap junctions, is vital for the functional stability of the microvessel [26].

Fenestrated capillaries

Fenestrations Fenestrated capillaries share all of the above features with continuous capillaries (endothelial glycocalyx, intercellular junctions, basement membrane and pericytes) but also have round or ovoid transcellular holes (60–70 nm diameter) through the most attenuated part of the endothelial cell cytoplasm [7]. They are found in the endothelium of organs, where a higher rate of exchange between intra- and extravascular compartments is required including endocrine tissue (eg pancreatic islets, adrenal cortex), gastrointestinal mucosa, joint synovium and renal peritubular capillaries. Fenestrae are typically both filled and covered with endothelial glycocalyx [27] and are traversed by a thin (3–5 nm) diaphragm [28].

Glomerular capillaries Glomerular capillaries also exhibit the above structural features but with particular adaptations (Figure 2B). Enzyme digestion and histochemical studies confirm that rat glomerular endothelial glycocalyx is anionic and made up principally from sialoproteins in the non-fenestrated regions, and from heparan sulphate, hyaluronic acid and sialoproteins in the fenestrae [29].

Glomerular endothelial fenestrations cover up to 20–50% of the capillary surface area [30,31] and do not contain diaphragms in the mature glomerulus [8,32]. The mature glomerular basement membrane is significantly thicker than others at 240–370 nm [33] and is characterized by α3, α4 and α5 chains of type IV collagen, α5, β2 and γ1 chains of laminin [34] and the proteoglycans perlecan and agrin [1,35].

The glomerulus is supported by podocytes and mesangial cells, both of which have some pericyte-like properties. The interdigitating foot processes of podocytes, spanned by a specialized intercellular junction termed the slit diaphragm [36–38], are covered by the restrictive subpodocyte space in 60% of the glomerulus [39–41]. Podocytes therefore form the outer surface of the glomerular capillary wall. Mesangial cells regulate glomerular structure and distensibility [1] and contribute to the essential cell–cell communication within the glomerulus [42].

Contribution of the endothelial glycocalyx to fluid and solute movement across the microvascular wall

In both systemic continuous and glomerular capillary beds, the structures that comprise the vascular wall act in concert to determine the permeability of the vessel wall to fluid and solute movement. Detailed reviews of microvascular permeability are available [1,31,43–45]. Here we summarize evidence relevant to understanding the contribution of the endothelial glycocalyx.

Continuous capillaries

Tight junction bands within the interendothelial cleft of continuous capillaries are incomplete: breaks in these bands occur over 3–10% of their length [46,47]. By measuring the frequency of these breaks in electron micrographs of individually perfused microvessels, modelling water flow through these breaks and measuring actual rates of water flow across the walls of the same vessels, Adamson and Michel [46] demonstrated that these breaks would allow more fluid to cross the vessel wall than is actually observed. This indicates that overall resistance to water flow is provided by tight junction strands within the cleft in combination with other components of the vessel wall, such as the endothelial glycocalyx [48]. In addition, the endothelial glycocalyx overlying the cleft entrance, together with breaks in the tight junction strand within the cleft, act together to form a 'fibre matrix–junction break' pathway that accounts for the degree of macromolecular sieving exerted by continuous capillary walls [49,50]. Hu *et al* [51] and Adamson *et al* [52] have demonstrated in frog and rat mesenteric (continuous) microvessels, respectively, that changing the oncotic pressure gradient in the interstitium immediately adjacent to the vessel wall has a minimal effect on steady-state transvascular fluid flux, in contrast to the symmetrical predictions of the traditional Starling equation. This supports a model in which the endothelial glycocalyx reflects a considerable proportion of albumin molecules back into the vessel lumen (ie high reflection coefficient), such that the concentration of albumin molecules immediately beneath the endothelial glycocalyx is considerably lower than the concentration of albumin in plasma. In addition, fluid crossing the endothelial glycocalyx enters the interendothelial cleft and streams towards breaks in tight junction strands, thereby washing out albumin beneath the endothelial glycocalyx and preventing albumin from diffusing back up the cleft from the interstitium. The oncotic pressure gradient is therefore set up across the endothelial glycocalyx, rather than across the entire capillary wall; hence, changing the oncotic pressure in the interstitium has little effect on transvascular fluid flux [44,53]. This combination of reflection of albumin by the endothelial glycocalyx and continuous wash-out of the interendothelial cleft provides an explanation for the observation that microcirculatory networks perfused in the steady state do not reabsorb fluid [54], even at the venous end of the network [44].

Fenestrated capillaries

The surface area available for fluid exchange through fenestrae significantly exceeds that available via intercellular clefts, although there are large differences between capillary beds (~0.65% fenestral area in synovium, 15.7% in renal peritubular capillaries) [15]. There is a strong linear relation between hydraulic conductance and fenestral density (correlation coefficient 0.99) [15]. However, the geometry of the cellular and diaphragmatic portions of the fenestral pathway predicts hydraulic conductance across fenestrated capillary walls that greatly exceeds experimental measurements. The same discrepancy is also apparent for small solute permeability across fenestrated capillaries. Additional resistance across the fenestral pathway, attributable to overlying endothelial glycocalyx and/or underlying basement membrane, is required to reconcile physiological measurements with biophysical predictions [15].

Glomerular capillaries

Fenestral density is very high in the glomerulus (~ 22.5% [15]) and, accordingly, glomerular hydraulic conductivity is also very high [55]. Mathematical models indicate that glycocalyx-filled fenestrae contribute about one-quarter of the overall resistance of the glomerular capillary wall [31]. As with other types of capillaries, the layers of the glomerular capillary wall act in concert to hinder fluid movement: the hydraulic resistance of individual layers summate, and the structure and composition of each layer modifies the resistance of (or the pathways available for movement across) other layers [31].

The resistance of the glomerular capillary wall to the passage of macromolecules is fundamentally different—the contributions of each layer are multiplied together [31] in contrast to the addition of hydraulic resistances of individual layers. Thus, an order of magnitude change in the macromolecular permeability of an individual layer results in the same order of magnitude change in the macromolecular permeability of the entire barrier. Whilst tubular function modifies the precise amount of albumin in final urine, the majority of current evidence indicates that changes in glomerular permeability are responsible for significant albuminuria [1]. Glomerular sieving coefficients (the ratio of macromolecule concentrations in urine and plasma, relative to that of a freely-filtered molecule) decreases as glomerular filtration rate (GFR) increases [56–58]. If a downstream structure (such as the podocyte slit diaphragm) was the main site of macromolecular resistance, then elevated flow rates would result in accumulation of macromolecules on the upstream side of this resistance, shortening the effective distance for diffusion of these molecules into the urinary space and hence elevating the sieving coefficient. That the converse situation is observed (fall in sieving coefficient with increased GFR) indicates that molecules are predominantly excluded by upstream layers of the barrier (eg endothelial glycocalyx) and the concentrated layer of macromolecules does not form, partly because of continuous stirring by flowing blood [59]. Sieving by upstream layers of the glomerular capillary wall is therefore analogous to the situation in continuous capillaries, where the majority of albumin is reflected by the innermost layer (endothelial glycocalyx). An inverse relation between sieving coefficient and GFR has been demonstrated for dextran [56], Ficoll [58] and albumin [57]. It is noteworthy, however, that this inverse relation between sieving coefficient and GFR is no longer apparent when the charges on albumin are neutralized [57], supporting evidence from enzyme degradation studies that upstream layers of the barrier (eg endothelial glycocalyx) make an important contribution to charge selectivity.

Similarity of endothelial glycocalyx in different capillary beds

Whilst hydraulic conductivity varies with the proportion of the endothelial surface available for fluid exchange, the reflection coefficient varies little between fenestrated and continuous capillary beds [60], which may imply that a structure common to continuous and fenestrated capillary beds, such as the endothelial glycocalyx (Figure 3), provides a common degree of macromolecular sieving in these diverse microcirculations [45]. Whilst some endothelial glycocalyx labelling techniques (eg cationic ferritin) have revealed differences in the gross appearance of the endothelial glycocalyx in different capillary beds [61], selective filtration by the endothelial glycocalyx occurs at the level of spaces between the individual nanofibres that comprise the matrix. The endothelial glycocalyx is predicted to act as an efficient barrier to macromolecules if the fibres of the matrix are regularly arranged and if the spacings between the matrix fibres are sufficient to exclude macromolecules (and particularly albumin) [45]. Squire *et al* [62] used autocorrelation analysis of electron micrographs of the endothelial glycocalyx of continuous capillaries to study the arrangement of these individual fibres of the endothelial glycocalyx, and identified both a regular arrangement of matrix fibres and inter-fibre spacing sufficient to exclude albumin molecules. Recently, this regularity within the endothelial glycocalyx was shown to be strikingly similar across fenestrated and continuous capillaries, including above the fenestrae of glomerular capillaries [63], supporting the notion that the endothelial glycocalyx provides a relatively constant degree of macromolecular exclusion throughout the microcirculation. The final degree of macromolecular exclusion exhibited by individual capillaries, particularly in specialized microcirculations such as the glomerulus, is likely to be achieved by specializations of the structure and composition of the endothelial glycocalyx within these microcirculations, coupled with specialized anatomy (eg additional resistance to macromolecular movement

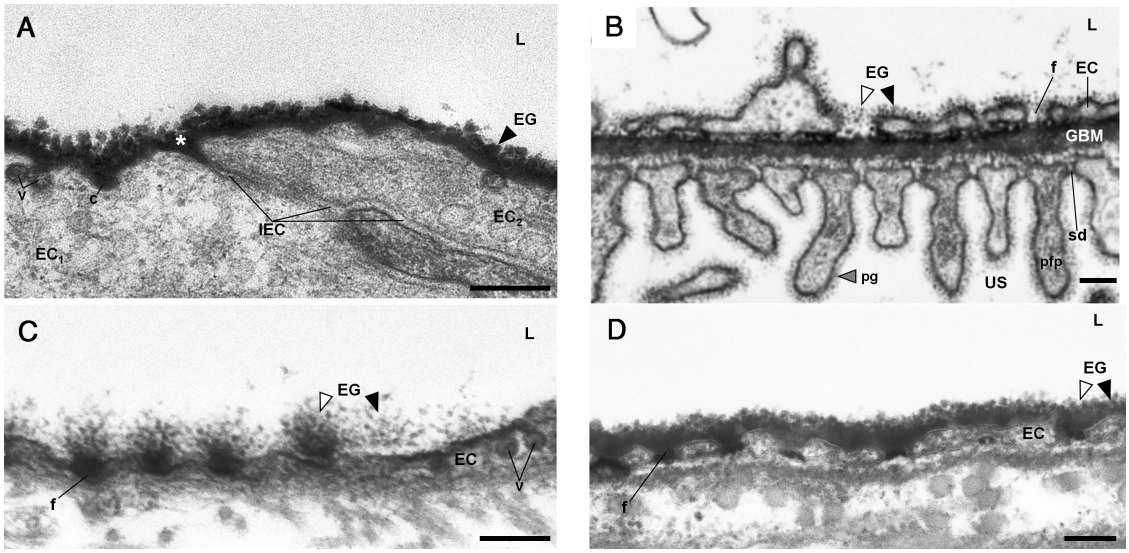

Figure 3. Endothelial glycocalyx lines the luminal surface of continuous and fenestrated microvessels. Electron micrographs of the walls of microvessels from four different organs, using oxygenated fluorocarbon perfusion to preserve the endothelial glycocalyx. (A) Continuous microvessel from psoas muscle. Endothelial glycocalyx (EG) forms an uninterrupted layer over the surface of two adjacent endothelial cells (EC_1, EC_2), including across the luminal aperture ($*$) of the interendothelial cleft (IEC), and across the luminal surface of caveolae (c); v, vesicles; L, lumen of vessel; scale bar = 200 nm. (B) Glomerular capillary wall. Endothelial glycocalyx (EG) again forms an uninterrupted layer with uniform appearance, covering endothelial cell (EC) cytoplasmic surfaces (black arrowhead) and filling fenestrae (f, white arrowhead). Note that the technique also reveals a continuous layer of podocyte glycocalyx (pg, grey arrowhead) covering podocyte foot processes (pfp); L, lumen of vessel; GBM, glomerular basement membrane; sd, slit diaphragm; US, urinary space; scale bar = 200nm. (C) Fenestrated microvessel from fundus of stomach. A plaque of four fenestrae (f) and an adjacent, non-fenestrated segment of endothelial cell (EC) surface both exhibit endothelial glycocalyx (EG) coverage. Whilst EG covering fenestrae (white arrowhead) is arranged into 'sieve plugs', whereas that overlying cell cytoplasm (black arrowhead) is more diffuse, autocorrelation analysis reveals a common arrangement of fibres in both fenestrated and non-fenestrated regions [63]; L, lumen of vessel; v, vesicles; scale bar = 200 nm. (D) Fenestrated capillary from retinal choroidal microvessel. Endothelial glycocalyx (EG) appears uniform, both within fenestrae (f, white arrowhead) and overlying endothelial cell (EC) cytoplasmic surfaces (black arrowhead); L, lumen of vessel; scale bar = 200 nm. Images courtesy of Arkill, Rostgaard, Qvortrup and colleagues (analysed in [63]). (A) has previously appeared in [141] and is used here with permission.

imposed by relatively thick basement membrane combined with podocyte slit diaphragms in the glomerulus) and/or by specialized haemodynamics (eg high filtration rate across the glomerular capillary wall, reducing the magnitude of sieving towards a minimum value approximating the reflection coefficient of albumin) [64].

Altered microvascular permeability in disease states

In disease states, defects in any of the multiple components of composite microvascular wall barriers may result in changes in permeability: this is true for all of the aforementioned types of capillary beds. In both animal models and patients with diabetes, for example, a reduction in tight junctions within the interendothelial cleft of retinal microvessels has been described [65,66], and this corresponds to the diabetes-induced increase in retinal microvessel permeability [66]. Inflammatory mediators also increase endothelial cell–cell junction width, and in some cases induce para-junctional transcellular holes, associated with increased permeability [67]. A number of mediators combine to effect endothelial cell junctions in conditions such as adult respiratory distress syndrome [68].

In the glomerulus the importance of the podocyte for the integrity of the barrier to macromolecules is clearly demonstrated by the profound proteinuria which results from genetic abnormalities of key proteins associated with the slit diaphragm, eg nephrin and podocin [69,70]. Although the GBM is thought to make a relatively small direct contribution to the barrier to macromolecules [71], GBM abnormalities do lead to proteinuria through disrupting the cellular contribution to the barrier [34]. The reduction in GFR associated with loss of endothelial fenestral area predicted by biophysical models does indeed occur in pre-eclampsia, and there is good evidence that this is the mechanism of acute renal failure in this condition [72]. The glomerular endothelium is similarly damaged in a number of other conditions, including diabetic nephropathy [73] and transplant glomerulopathy [74,75].

It should be noted that multiple layers of the microvascular wall may be affected simultaneously and that dysfunction of one layer may compromise that of another, including by altered signalling via soluble mediators. For example, in the glomerulus, damage to the podocytes may result in reduced vascular endothelial growth factor production, which leads to glomerular endothelial cell dysfunction and potentially also to glycocalyx disruption [32,76].

Experimental manipulation of the endothelial glycocalyx results in altered permeability

Damage to the endothelial glycocalyx also results in abnormal permeability, both in continuous and fenestrated capillary beds. Adamson [48] found that partial removal of the endothelial glycocalyx in frog mesenteric capillaries caused a greater than two-fold increase in L_P. A similar approach was used to demonstrate that the endothelial glycocalyx contributes to the barrier to solute permeability in swine coronary arterioles [77]. Conversely, the endothelial growth factor angiopoetin-1 increased the endothelial glycocalyx thickness, caused a corresponding decrease in hydraulic conductivity and increased retention of albumin in mesenteric microvessels [78].

In glomeruli, enzymatic degradation of GAG reduces glomerular filtration barrier anionic sites and increases the clearance of albumin in anaesthetized mice and in cooled isolated perfused kidneys [79,80]. Long-term administration of hyaluronidase also caused proteinuria [81]. Notably, chondroitinase increased the clearance of charged macromolecules, but not of neutral molecules of an equivalent size [80]. Removal of sialic acid residues in the glomerulus also results in loss of anionic sites and albuminuria [82,83]. Elution of non-covalently bound components of the endothelial glycocalyx caused a 12-fold increase in the fractional clearance of albumin [9], supporting evidence from continuous capillaries [10,48] that both the soluble and bound components of the endothelial glycocalyx determine permeability. Similar effects of disruption of heparan sulphate and sialic acid components of the endothelial glycocalyx have been observed in monolayers of human glomerular endothelial cells, in which potential confounding effects of enzymatic degradation on the glomerular basement membrane are eliminated [84,85]. Overall, these studies provide additional support for the concept that the endothelial glycocalyx is a functionally important layer in the glomerular filtration barrier.

Disruption of glomerular endothelial glycocalyx coincides with albuminuria in glomerular disease

The observation that experimental damage to the glomerular endothelial glycocalyx results in increased clearance of unmodified, negatively-charged albumin is recapitulated in albuminuric disease, and particularly in the early albuminuric phase of diabetic nephropathy. Jeansson *et al* [86] reported a change in a number of molecular components of the endothelial glycocalyx in diabetic animals, coupled with an increase in the fractional clearance of albumin. Loss of glomerular labelling of glycosaminoglycans (specifically hyaluronan and heparan sulphate) within the glomerular capillary wall has been demonstrated in streptozotocin-induced diabetic rats with albuminuria [87], and similar observations in Zucker fatty rats [88] included demonstrable changes in the endothelial glycocalyx. Heparan sulphate biosynthesis and endothelial glycocalyx structure of human glomerular endothelial cell monolayers were both severely disrupted by exposure to high glucose concentrations, associated with increased passage of albumin across the monolayer [89]. These changes in glomerular endothelial glycocalyx in early diabetic nephropathy (and high glucose exposure) are consistent with the early loss of charge (but not size) selectivity that occurs in animal models of diabetic nephropathy, and in individuals with type 1 and type 2 diabetes and microalbuminuria [86,90,91].

Recently, changes in glomerular endothelial glycocalyx have also been demonstrated in non-diabetic nephropathy. Administration of adriamycin to mice caused a reduction in endothelial glycocalyx depth and change in a number of endothelial glycocalyx components, and these changes preceded demonstrable damage to other layers of the glomerular capillary wall and coincided with increased glomerular clearance of albumin [92].

Triad of damage to the endothelial glycocalyx, albuminuria and widespread increase in microvascular permeability in disease states

The evidence outlined above indicates that localized damage to the glomerular endothelial glycocalyx may result in albuminuria, and that localized damage to the endothelial glycocalyx of continuous capillaries may result in increased systemic microvessel permeability. However, the diabetic milieu is imposed on the endothelial glycocalyx of all blood vessels: if damage to the endothelial glycocalyx is generalized, then changes in glomerular permeability (ie albuminuria) and systemic permeability may coincide. This is consistent with the Steno hypothesis, which held that albuminuria in diabetes is indicative of widespread vascular damage [93]. However, the evidence reviewed points to endothelial glycocalyx damage as that aspect of endothelial dysfunction that is responsible for albuminuria, rather than (or possibly in addition to) other extracellular matrix defects, as the Steno hypothesis originally proposed. What evidence is there that this triad of damage to the endothelial glycocalyx, albuminuria and increased systemic permeability occurs in diabetes, and is there evidence indicating that the same triad occurs in other diseases?

Diabetes

Albuminuria is the hallmark of diabetic nephropathy, but the presence of albuminuria is associated with vascular dysfunction and disease outside the kidney. Albuminuria-associated vascular diseases in diabetes include microvascular complications, such as retinopathy and neuropathy, as well as macrovascular disease, such as adverse cardiovascular events [32,94,95]. Permeability coefficients, ie the amount of substance exchanged across a microvessel wall per unit pressure per unit area, have only been assessed in animal

models of diabetes, since these studies permit control of confounding factors that cannot be achieved in humans [96]. In these models, the apparent solute permeability coefficient of albumin is elevated in coronary microvessels of diabetic pigs [97], and hydraulic conductivity is elevated in frog mesenteric microvessels exposed to hyperglycaemia [98]. The closest approximation to these studies in humans was achieved by Jaap *et al* [99,100], who measured capillary filtration coefficient (CFC: the product of L_P and available exchange area) using a sensitive plethysmography technique. Increased CFC was noted in individuals with type 1 diabetes, particularly in those with microalbuminuria.

This demonstration of increased permeability in diabetes, with a greater increase in diabetic subjects with albuminuria, closely resembles the demonstration by Nieuwdorp and colleagues [101] that there is widespread loss of endothelial glycocalyx in patients with type 1 diabetes, and that the endothelial glycocalyx is more disrupted in diabetic subjects with albuminuria. Subsequent studies in patients with type 2 diabetes have demonstrated that these patients also have a reduction in endothelial glycocalyx [102]. Whilst many methods of endothelial glycocalyx detection are limited to some extent (eg [103]), these studies have used a range of detection methods to assess endothelial glycocalyx volume, all of which have indicated a reduction in endothelial glycocalyx depth in diabetic subjects. Thus, the triad of damage to the endothelial glycocalyx, albuminuria and increased systemic permeability occurs in type 1 [99–101] and possibly type 2 [102] diabetes.

The importance of the endothelial glycocalyx to widespread changes in microvascular permeability (including albuminuria) in diabetes does not exclude the possibility that functionally important changes in other capillary wall components are also relevant. In the glomerulus, thickening of the glomerular basement membrane and broadening of podocyte foot processes have been reported in early diabetic nephropathy [32], both of which may contribute to increased glomerular clearance of albumin because of the multiplicative nature of sieving by individual layers of the glomerular capillary wall [31]. In non-renal capillaries, changes in tight and adherens junction composition and structure occur [104]. Disruption of endothelial cell molecular cascades, including those resulting from increased oxidative stress [32,104], affect the integrity of interendothelial junctions [105] as well as the endothelial glycocalyx [88,106].

The diabetes-induced changes in endothelial glycocalyx may, in part, reflect direct suppression of the turnover of endothelial glycocalyx components by hyperglycaemia [107]. However, there is accumulating evidence that the same triad of damage to the endothelial glycocalyx, albuminuria and widespread changes in permeability also occurs in other diseases, independent of hyperglycaemia.

Ischaemia–reperfusion

Ischaemia–reperfusion, in response to aortic cross-clamp during abdominal aortic aneurysm repair, results in significant, transient albuminuria in humans [108,109]. Similarly, aortic clamping increased the renal clearance of albumin 16-fold in rats. Notably, no changes in the structure of podocytes, glomerular basement membrane or endothelial cell bodies was noted in these rats, implicating a change in the endothelial glycocalyx [110]. Ischaemia–reperfusion also results in an increase in the plasma levels of syndecan-1 and heparan sulphate, both of which are important components of the endothelial glycocalyx [111,112]. Damage to the endothelial glycocalyx following ischaemia–reperfusion has been directly demonstrated with electron microscopy in isolated guinea-pig hearts [111] and with a brightfield microscopy-exclusion technique in human kidneys donated for transplantation after cardiac death [112]. Ischaemia-reperfusion induced by systemic hypotension in rats causes near-complete loss of microvascular endothelial glycocalyx in the small intestine (the only organ bed studied) [113]. These findings demonstrate endothelial glycocalyx damage in various organs in response to either organ-specific ischaemia–reperfusion or systemic hypotension. As with the development of albuminuria in kidneys exposed to ischaemia–reperfusion [110], increased coronary fluid flux is observed in isolated perfused heart preparations subjected to ischaemia–reperfusion [114]. Moreover, agents that restore the endothelial glycocalyx reduce the degree of coronary fluid flux [114,115]. No physiological assessments of microvascular permeability were made in the systemic hypotension model [113], but significant thickening of the wall of lung alveoli was noted, which is compatible with increased transcapillary fluid flux. Notably, plasma (but not crystalloid) resuscitation resulted in both partial restoration of the endothelial glycocalyx and a significant reduction in alveolar thickness following hypotension, which is consistent with the notion that plasma components (including albumin and orosomucoid) are required to maintain the structure and function of the endothelial glycocalyx and thereby regulate microvascular permeability [9,10].

Infectious disease

The same triad may also occur in a number of infectious diseases. Wills and colleagues propose that damage to the microvascular endothelial glycocalyx occurs in dengue infection, and that this is the pathophysiological mechanism that explains the profound plasma leakage and consequent hypovolaemia that occurs in the shock phase of dengue fever [116]. Whilst clinically apparent sequestration of fluid in the abdomen and thorax occurs during this shock phase, subclinical extravascular fluid accumulation has been noted early in the febrile phase of the disease [117]. Patients with dengue infection also display a 50% increase in CFC,

suggesting that plasma leak may be a consequence of increased microvascular permeability (or surface area available for exchange), rather than as a primary result of changes in haemodynamics [118].

This apparent increase in systemic permeability in dengue infection is accompanied by an increase in urinary clearance of albumin and other macromolecules. Increased fractional clearances of four macromolecules (antithrombin, albumin, transferrin and IgG) of varying size (59–150 kDa) and charge (neutral to anionic) was observed in children with active dengue infection, relative to the clearance of these molecules after 1 month of convalesence in the same individuals [119]. An increase in the urinary clearance of heparan sulphate, a constituent of the endothelial glycocalyx, was also noted during dengue infection [119]. Whilst this evidence for dengue-induced changes in the endothelial glycocalyx is currently circumstantial, it is certainly compatible with widespread changes in microvascular permeability resulting from damage to the endothelial glycocalyx.

A similar phenomenon has also been proposed to occur in other infectious diseases, such as meningococcal septicaemia [120], in which systemic vascular leak, increased urinary protein leak, and increased plasma and urine concentrations of GAGs have been noted. A correlation between urinary GAG and urinary protein leak was also observed, leading the authors to suggest that infection-induced loss of GAG from the glomerular endothelial glycocalyx contributes to albuminuria. Again, direct examination of the endothelial glycocalyx in patients with and without meningococcal septicaemia remains outstanding. Shedding of the endothelial glycocalyx has been demonstrated more widely in experimental and clinical studies of sepsis in general [121–124]. The concomitant occurrence of microalbuminuria in these settings [125] suggests that altered microvascular permeability as a consequence of glycocalyx disruption plays a role in many pathophysiological states.

Implications of the relationship between damage to the endothelial glycocalyx and widespread permeability dysfunction

Because the endothelial glycocalyx is an important component of the permeability barrier throughout the body, widespread damage to the endothelial glycocalyx would be predicted to result in albuminuria and systemic changes in permeability. However, the endothelial glycocalyx also mediates a host of other important vascular functions, such as mechanotransduction and leukocyte adhesion [4]. If damage to the endothelial glycocalyx is an important pathophysiological phenomenon in disease states, then the third component of the damage to the endothelial glycocalyx/albuminuria/widespread change in permeability triad could be substituted for disruption of any other endothelial glycocalyx-dependent function, such as mechanotransduction and flow-mediated vasodilatation. Whilst direct experimental support is currently lacking, damage to the endothelial glycocalyx provides a theoretical explanation for the association between albuminuria and disruption of flow-mediated vasodilatation [126]. In addition, leukocyte adhesion to microvascular endothelium is regulated by the endothelial glycocalyx, with endothelial glycocalyx removal enhancing adhesion [127,128]. Endothelial glycocalyx damage in diabetes may therefore also contribute to leukocyte adhesion in the retina [129] and kidney [130]. Certainly factors that restore endothelial glycocalyx (such as angiopoietin-1) [78] decrease leukocyte adhesion in the retina [129], although again the role of the endothelial glycocalyx needs to be considered alongside other factors that regulate leukocyte adhesion in diabetes [129,131].

Cardiovascular disease and microalbuminuria are both closely associated with endothelial dysfunction, and so it has been proposed that endothelial glycocalyx dysfunction explains the link between microalbuminuria and cardiovascular disease [32]. This is supported by the observation that modification of the endothelial glycocalyx accelerates atherosclerosis in apolipoprotein-E-deficient mice [132]. However, a significant component of the cardiovascular disease associated with albuminuria, particularly in diabetes, is a consequence of microvascular dysfunction leading to cardiomyopathy, diastolic dysfunction and cardiac failure [133–135]. Indeed cardiomyopathy may develop in diabetic patients in the absence of significant coronary artery disease [136]. This appreciation suggests an alternative (and possibly complementary) hypothesis of endothelial glycocalyx dysfunction in the coronary microcirculation contributing to diabetic cardiomyopathy. As in other vascular beds, control of coronary microvascular permeability is a fundamental homeostatic process in which the endothelial glycocalyx plays an important role [137,138]. Arguably this control is particularly significant in the heart, which is exquisitely sensitive to increases in microvascular permeability and consequent myocardial interstitial oedema: only a few percent increase in interstitial fluid volume compromises cardiac function [139]. Diabetes-induced damage to the endothelial glycocalyx resulting in increased coronary microvascular permeability would be predicted to result not only in accelerated atherosclerosis [140] but also myocardial oedema, and therefore may directly contribute to the impaired cardiac function frequently observed in individuals with diabetes.

Conclusion

Endothelial glycocalyx makes a significant contribution to the permeability barrier in both systemic and glomerular capillaries. Understanding the glomerulus as a specialized microcirculation, including by

applying physiological insights from the systemic microcirculation to the glomerulus, affords greater understanding of glomerular function and pathophysiology. Damage to any layer of capillary walls, including the endothelial glycocalyx, frequently results in altered permeability: in the glomerulus, this is clinically apparent as albuminuria. Widespread damage to the endothelial glycocalyx would be predicted to result in albuminuria and systemic changes in permeability, and this triad of damage to the endothelial glycocalyx, albuminuria and widespread change in permeability occurs in diabetes, with accumulating evidence for a similar phenomenon in a number of other diseases. Widespread damage to the endothelial glycocalyx may also contribute to the associations between albuminuria and other facets of vascular dysfunction and disease, such as impaired flow-dependent vasodilatation, increased leukocyte adhesion and predisposition to atherosclerosis. Hence, whilst the microcirculation as a whole provides a basis to understand glomerular filtration, the glomerular circulation provides a unique 'window' on the systemic circulation: in certain circumstances, microalbuminuria, resulting from disturbed glomerular filtration barrier function, potentially provides an index of glycocalyx-dependent vascular functions as a whole.

Acknowledgment

AHJS was funded by a Medical Research Council clinician scientist award (No. G0802829).

Author contributions

Both authors contributed substantially to the design and conception of the article, to drafting the article and to revising it critically for important intellectual content. Both authors approved the final version.

Abbreviations

CFC, capillary filtration coefficient; GAG, glycosaminoglycans; GBM, glomerular basement membrane; GFR, glomerular filtration rate; L_P, hydraulic conductivity; PG, proteoglycans.

References

1. Haraldsson B, Nystrom J, Deen WM. Properties of the glomerular barrier and mechanisms of proteinuria. *Physiol Rev* 2008; **88**: 451–487.
2. Pries AR, Secomb TW, Gaehtgens P. The endothelial surface layer. *Pflugers Arch* 2000; **440**: 653–666.
3. Reitsma S, Slaaf DW, Vink H, *et al*. The endothelial glycocalyx: composition, functions, and visualization. *Pflugers Arch* 2007; **454**: 345–359.
4. Weinbaum S, Tarbell JM, Damiano ER. The structure and function of the endothelial glycocalyx layer. *Annu Rev Biomed Eng* 2007; **9**: 121–167.
5. Risau W. Development and differentiation of endothelium. *Kidney Int Suppl* 1998; **67**: S3–6.
6. Roberts WG, Palade GE. Endothelial fenestrae and fenestral diaphragms. In *Morphogenesis of Endothelium*, Risau W, Rubanyi GM (eds). Harwood Academic: Amsterdam, 2000; 23–41.
7. Satchell SC, Braet F. Glomerular endothelial cell fenestrations: an integral component of the glomerular filtration barrier. *Am J Physiol Renal Physiol* 2009; **296**: F947–956.
8. Ichimura K, Stan RV, Kurihara H, *et al*. Glomerular endothelial cells form diaphragms during development and pathologic conditions. *J Am Soc Nephrol* 2008; **19**: 1463–1471.
9. Friden V, Oveland E, Tenstad O, *et al*. The glomerular endothelial cell coat is essential for glomerular filtration. *Kidney Int* 2011; **79**: 1322–1330.
10. Adamson RH, Clough G. Plasma proteins modify the endothelial cell glycocalyx of frog mesenteric microvessels. *J Physiol* 1992; **445**: 473–486.
11. Ebong EE, Macaluso FP, Spray DC, *et al*. Imaging the endothelial glycocalyx *in vitro* by rapid freezing/freeze substitution transmission electron microscopy. *Arterioscler Thromb Vasc Biol* 2011; **31**: 1908–1915.
12. Luft JH. Fine structures of capillary and endocapillary layer as revealed by ruthenium red. *Fed Proc* 1966; **25**: 1773–1783.
13. Arisaka T, Mitsumata M, Kawasumi M, *et al*. Effects of shear stress on glycosaminoglycan synthesis in vascular endothelial cells. *Ann NY Acad Sci* 1995; **748**: 543–554.
14. Mulivor AW, Lipowsky HH. Inflammation- and ischemia-induced shedding of venular glycocalyx. *Am J Physiol Heart Circ Physiol* 2004; **286**: H1672–1680.
15. Levick JR, Smaje LH. An analysis of the permeability of a fenestra. *Microvasc Res* 1987; **33**: 233–256.
16. Dejana E, Tournier-Lasserve E, Weinstein BM. The control of vascular integrity by endothelial cell junctions: molecular basis and pathological implications. *Dev Cell* 2009; **16**: 209–221.
17. Abbott NJ, Patabendige AA, Dolman DE, *et al*. Structure and function of the blood–brain barrier. *Neurobiol Dis* 2010; **37**: 13–25.
18. Abbott NJ, Ronnback L, Hansson E. Astrocyte–endothelial interactions at the blood–brain barrier. *Nat Rev Neurosci* 2006; **7**: 41–53.
19. Erickson KK, Sundstrom JM, Antonetti DA. Vascular permeability in ocular disease and the role of tight junctions. *Angiogenesis* 2007; **10**: 103–117.
20. Simionescu M, Simionescu N. Ultrastructure of the microvascular wall: functional correlations. In *Handbook of Physiology*, Renkin EM, Michel CC (eds). American Physiological Society: Bethesda, MD, 1984; 41–101.
21. LeBleu VS, Macdonald B, Kalluri R. Structure and function of basement membranes. *Exp Biol Med (Maywood)* 2007; **232**: 1121–1129.
22. Kruegel J, Miosge N. Basement membrane components are key players in specialized extracellular matrices. *Cell Mol Life Sci* 2010; **67**: 2879–2895.
23. Frank RN, Dutta S, Mancini MA. Pericyte coverage is greater in the retinal than in the cerebral capillaries of the rat. *Invest Ophthalmol Vis Sci* 1987; **28**: 1086–1091.
24. Tilton RG, Kilo C, Williamson JR. Pericyte–endothelial relationships in cardiac and skeletal muscle capillaries. *Microvasc Res* 1979; **18**: 325–335.
25. Allt G, Lawrenson JG. Pericytes: cell biology and pathology. *Cells Tissues Organs* 2001; **169**: 1–11.
26. Kutcher ME, Herman IM. The pericyte: cellular regulator of microvascular blood flow. *Microvasc Res* 2009; **77**: 235–246.
27. Rostgaard J, Qvortrup K. Electron microscopic demonstrations of filamentous molecular sieve plugs in capillary fenestrae. *Microvasc Res* 1997; **53**: 1–13.

28. Stan RV, Tkachenko E, Niesman IR. PV1 is a key structural component for the formation of the stomatal and fenestral diaphragms. *Mol Biol Cell* 2004; **15**: 3615–3630.
29. Avasthi PS, Koshy V. The anionic matrix at the rat glomerular endothelial surface. *Anat Rec* 1988; **220**: 258–266.
30. Bulger RE, Eknoyan G, Purcell DJd, *et al*. Endothelial characteristics of glomerular capillaries in normal, mercuric chloride-induced, and gentamicin-induced acute renal failure in the rat. *J Clin Invest* 1983; **72**: 128–141.
31. Deen WM, Lazzara MJ, Myers BD. Structural determinants of glomerular permeability. *Am J Physiol Renal Physiol* 2001; **281**: F579–596.
32. Satchell SC, Tooke JE. What is the mechanism of microalbuminuria in diabetes: a role for the glomerular endothelium? *Diabetologia* 2008; **51**: 714–725.
33. Kriz W, Kaissling B. Structural organization of the mammalian kidney. In *The Kidney: Physiology and Pathophysiology*, Seldin D, Giebisch G (eds). Raven: New York, 1992; 707–777.
34. Miner JH. Glomerular basement membrane composition and the filtration barrier. *Pediatr Nephrol* 2011; **26**: 1413–1417.
35. Miner JH. Renal basement membrane components. *Kidney Int* 1999; **56**: 2016–2024.
36. Pavenstadt H, Kriz W, Kretzler M. Cell biology of the glomerular podocyte. *Physiol Rev* 2003; **83**: 253–307.
37. Gagliardini E, Conti S, Benigni A, *et al*. Imaging of the porous ultrastructure of the glomerular epithelial filtration slit. *J Am Soc Nephrol* 2010; **21**: 2081–2089.
38. Rodewald R, Karnovsky MJ. Porous substructure of the glomerular slit diaphragm in the rat and mouse. *J Cell Biol* 1974; **60**: 423–433.
39. Neal CR, Crook H, Bell E, *et al*. Three-dimensional reconstruction of glomeruli by electron microscopy reveals a distinct restrictive urinary subpodocyte space. *J Am Soc Nephrol* 2005; **16**: 1223–1235.
40. Neal CR, Muston PR, Njegovan D, *et al*. Glomerular filtration into the subpodocyte space is highly restricted under physiological perfusion conditions. *Am J Physiol Renal Physiol* 2007; **293**: F1787–1798.
41. Salmon AH, Toma I, Sipos A, *et al*. Evidence for restriction of fluid and solute movement across the glomerular capillary wall by the subpodocyte space. *Am J Physiol Renal Physiol* 2007; **293**: F1777–1786.
42. Eremina V, Baelde HJ, Quaggin SE. Role of the VEGF—a signaling pathway in the glomerulus: evidence for crosstalk between components of the glomerular filtration barrier. *Nephron Physiol* 2007; **106**: 32–37.
43. Curry FR, Adamson RH. Vascular permeability modulation at the cell, microvessel, or whole organ level: towards closing gaps in our knowledge. *Cardiovasc Res* 2010; **87**: 218–229.
44. Levick JR, Michel CC. Microvascular fluid exchange and the revised Starling principle. *Cardiovasc Res* 2010; **87**: 198–210.
45. Michel CC, Curry FE. Microvascular permeability. *Physiol Rev* 1999; **79**: 703–761.
46. Adamson RH, Michel CC. Pathways through the intercellular clefts of frog mesenteric capillaries. *J Physiol* 1993; **466**: 303–327.
47. Bundgaard M. The three-dimensional organization of tight junctions in a capillary endothelium revealed by serial-section electron microscopy. *J Ultrastruct Res* 1984; **88**: 1–17.
48. Adamson RH. Permeability of frog mesenteric capillaries after partial pronase digestion of the endothelial glycocalyx. *J Physiol* 1990; **428**: 1–13.
49. Curry FE, Michel CC. A fiber matrix model of capillary permeability. *Microvasc Res* 1980; **20**: 96–99.
50. Weinbaum S. 1997 Whitaker Distinguished Lecture: models to solve mysteries in biomechanics at the cellular level; a new view of fiber matrix layers. *Ann Biomed Eng* 1998; **26**: 627–643.
51. Hu X, Adamson RH, Liu B, *et al*. Starling forces that oppose filtration after tissue oncotic pressure is increased. *Am J Physiol Heart Circ Physiol* 2000; **279**: H1724–1736.
52. Adamson RH, Lenz JF, Zhang X, *et al*. Oncotic pressures opposing filtration across non-fenestrated rat microvessels. *J Physiol* 2004; **557**: 889–907.
53. Curry FR. Microvascular solute and water transport. *Microcirculation* 2005; **12**: 17–31.
54. Michel CC, Phillips ME. Steady-state fluid filtration at different capillary pressures in perfused frog mesenteric capillaries. *J Physiol* 1987; **388**: 421–435.
55. Deen WM, Troy JL, Robertson CR, *et al*. Dynamics of glomerular ultrafiltration in the rat. IV. Determination of the ultrafiltration coefficient. *J Clin Invest* 1973; **52**: 1500–1508.
56. Chang RL, Ueki IF, Troy JL, *et al*. Permselectivity of the glomerular capillary wall to macromolecules. II. Experimental studies in rats using neutral dextran. *Biophys J* 1975; **15**: 887–906.
57. Lund U, Rippe A, Venturoli D, *et al*. Glomerular filtration rate dependence of sieving of albumin and some neutral proteins in rat kidneys. *Am J Physiol Renal Physiol* 2003; **284**: F1226–1234.
58. Rippe C, Asgeirsson D, Venturoli D, *et al*. Effects of glomerular filtration rate on Ficoll sieving coefficients (θ) in rats. *Kidney Int* 2006; **69**: 1326–1332.
59. Deen WM, Robertson CR, Brenner BM. Concentration polarization in an ultrafiltering capillary. *Biophys J* 1974; **14**: 412–431.
60. Michel CC. Capillary permeability and how it may change. *J Physiol* 1988; **404**: 1–29.
61. Bankston PW, Milici AJ. A survey of the binding of polycationic ferritin in several fenestrated capillary beds: indication of heterogeneity in the luminal glycocalyx of fenestral diaphragms. *Microvasc Res* 1983; **26**: 36–48.
62. Squire JM, Chew M, Nneji G, *et al*. Quasi-periodic substructure in the microvessel endothelial glycocalyx: a possible explanation for molecular filtering? *J Struct Biol* 2001; **136**: 239–255.
63. Arkill KP, Knupp C, Michel CC, *et al*. Similar endothelial glycocalyx structures in microvessels from a range of mammalian tissues: evidence for a common filtering mechanism? *Biophys J* 2011; **101**: 1046–1056.
64. Michel CC. Capillary exchange. In *The Kidney: Physiology and Pathophysiology*, Seldin DW, Giebsich G (eds). Raven: New York, 1992; 61–91.
65. Ishibashi T, Inomata H. Ultrastructure of retinal vessels in diabetic patients. *Br J Ophthalmol* 1993; **77**: 574–578.
66. Wallow IH, Engerman RL. Permeability and patency of retinal blood vessels in experimental diabetes. *Invest Ophthalmol Vis Sci* 1977; **16**: 447–461.
67. Adamson RH, Zeng M, Adamson GN, *et al*. PAF- and bradykinin-induced hyperpermeability of rat venules is independent of actin–myosin contraction. *Am J Physiol Heart Circ Physiol* 2003; **285**: H406–417.
68. Kumar P, Shen Q, Pivetti CD, *et al*. Molecular mechanisms of endothelial hyperpermeability: implications in inflammation. *Expert Rev Mol Med* 2009; **11**: e19.
69. Boute N, Gribouval O, Roselli S, *et al*. *NPHS2*, encoding the glomerular protein podocin, is mutated in autosomal recessive steroid-resistant nephrotic syndrome. *Nat Genet* 2000; **24**: 349–354.
70. Kestila M, Lenkkeri U, Mannikko M, *et al*. Positionally cloned gene for a novel glomerular protein—nephrin—is mutated in congenital nephrotic syndrome. *Mol Cell* 1998; **1**: 575–582.
71. Jarad G, Cunningham J, Shaw AS, *et al*. Proteinuria precedes podocyte abnormalities inLamb2$^{-/-}$ mice, implicating the

glomerular basement membrane as an albumin barrier. *J Clin Invest* 2006; **116**: 2272–2279.
72. Lafayette RA, Druzin M, Sibley R, *et al*. Nature of glomerular dysfunction in pre-eclampsia. *Kidney Int* 1998; **54**: 1240–1249.
73. Toyoda M, Najafian B, Kim Y, *et al*. Podocyte detachment and reduced glomerular capillary endothelial fenestration in human type 1 diabetic nephropathy. *Diabetes* 2007; **56**: 2155–2160.
74. Yamamoto I, Horita S, Takahashi T, *et al*. Glomerular expression of plasmalemmal vesicle-associated protein-1 in patients with transplant glomerulopathy. *Am J Transpl* 2007; **7**: 1954–1960.
75. Wavamunno MD, O'Connell PJ, Vitalone M, *et al*. Transplant glomerulopathy: ultrastructural abnormalities occur early in longitudinal analysis of protocol biopsies. *Am J Transpl* 2007; **7**: 2757–2768.
76. Eremina V, Jefferson JA, Kowalewska J, *et al*. VEGF inhibition and renal thrombotic microangiopathy. *N Engl J Med* 2008; **358**: 1129–1136.
77. Huxley VH, Williams DA. Role of a glycocalyx on coronary arteriole permeability to proteins: evidence from enzyme treatments. *Am J Physiol Heart Circ Physiol* 2000; **278**: H1177–1185.
78. Salmon AH, Neal CR, Sage LM, *et al*. Angiopoietin-1 alters microvascular permeability coefficients *in vivo* via modification of endothelial glycocalyx. *Cardiovasc Res* 2009; **83**: 24–33.
79. Jeansson M, Haraldsson B. Glomerular size and charge selectivity in the mouse after exposure to glucosaminoglycan-degrading enzymes. *J Am Soc Nephrol* 2003; **14**: 1756–1765.
80. Jeansson M, Haraldsson B. Morphological and functional evidence for an important role of the endothelial cell glycocalyx in the glomerular barrier. *Am J Physiol Renal Physiol* 2006; **290**: F111–116.
81. Meuwese MC, Broekhuizen LN, Kuikhoven M, *et al*. Endothelial surface layer degradation by chronic hyaluronidase infusion induces proteinuria in apolipoprotein E-deficient mice. *PLoS One* 2010; **5**: e14262.
82. Gelberg H, Healy L, Whiteley H, *et al*. *In vivo* enzymatic removal of α2–6-linked sialic acid from the glomerular filtration barrier results in podocyte charge alteration and glomerular injury. *Lab Invest* 1996; **74**: 907–920.
83. Bakker WW, Borghuis T, Harmsen MC, *et al*. Protease activity of plasma hemopexin. *Kidney Int* 2005; **68**: 603–610.
84. Singh A, Satchell SC, Neal CR, *et al*. Glomerular endothelial glycocalyx constitutes a barrier to protein permeability. *J Am Soc Nephrol* 2007; **18**: 2885–2893.
85. Lennon R, Singh A, Welsh GI, *et al*. Hemopexin induces nephrin-dependent reorganization of the actin cytoskeleton in podocytes. *J Am Soc Nephrol* 2008; **19**: 2140–2149.
86. Jeansson M, Granqvist AB, Nystrom JS, *et al*. Functional and molecular alterations of the glomerular barrier in long-term diabetes in mice. *Diabetologia* 2006; **49**: 2200–2209.
87. Satoh M, Kobayashi S, Kuwabara A, *et al*. *In vivo* visualization of glomerular microcirculation and hyperfiltration in streptozotocin-induced diabetic rats. *Microcirculation* 2010; **17**: 103–112.
88. Kuwabara A, Satoh M, Tomita N, *et al*. Deterioration of glomerular endothelial surface layer induced by oxidative stress is implicated in altered permeability of macromolecules in Zucker fatty rats. *Diabetologia* 2010; **53**: 2056–2065.
89. Singh A, Friden V, Dasgupta I, *et al*. High glucose causes dysfunction of the human glomerular endothelial glycocalyx. *Am J Physiol Renal Physiol* 2011; **300**: F40–48.
90. Deckert T, Kofoed-Enevoldsen A, Vidal P, *et al*. Size- and charge selectivity of glomerular filtration in type 1 (insulin-dependent) diabetic patients with and without albuminuria. *Diabetologia* 1993; **36**: 244–251.
91. Lemley KV, Blouch K, Abdullah I, *et al*. Glomerular permselectivity at the onset of nephropathy in type 2 diabetes mellitus. *J Am Soc Nephrol* 2000; **11**: 2095–2105.
92. Jeansson M, Bjorck K, Tenstad O, *et al*. Adriamycin alters glomerular endothelium to induce proteinuria. *J Am Soc Nephrol* 2009; **20**: 114–122.
93. Deckert T, Feldt-Rasmussen B, Borch-Johnsen K, *et al*. Albuminuria reflects widespread vascular damage. The Steno hypothesis. *Diabetologia* 1989; **32**: 219–226.
94. Daneman D. Type 1 diabetes. *Lancet* 2006; **367**: 847–858.
95. Stehouwer CD. Endothelial dysfunction in diabetic nephropathy: state of the art and potential significance for non-diabetic renal disease. *Nephrol Dialysis Transpl* 2004; **19**: 778–781.
96. Tooke JE. Microvascular function in human diabetes: a physiological perspective. *Diabetes* 1995; **44**: 721–726.
97. Yuan SY, Ustinova EE, Wu MH, *et al*. Protein kinase C activation contributes to microvascular barrier dysfunction in the heart at early stages of diabetes. *Circ Res* 2000; **87**: 412–417.
98. Perrin RM, Harper SJ, Corrall R, *et al*. Hyperglycemia stimulates a sustained increase in hydraulic conductivity *in vivo* without any change in reflection coefficient. *Microcirculation* 2007; **14**: 683–696.
99. Jaap AJ, Shore AC, Gartside IB, *et al*. Increased microvascular fluid permeability in young type 1 (insulin-dependent) diabetic patients. *Diabetologia* 1993; **36**: 648–652.
100. Jaap AJ, Shore AC, Tooke JE. Differences in microvascular fluid permeability between long-duration type I (insulin-dependent) diabetic patients with and without significant microangiopathy. *Clin Sci (Lond)* 1996; **90**: 113–117.
101. Nieuwdorp M, Mooij HL, Kroon J, *et al*. Endothelial glycocalyx damage coincides with microalbuminuria in type 1 diabetes. *Diabetes* 2006; **55**: 1127–1132.
102. Broekhuizen LN, Lemkes BA, Mooij HL, *et al*. Effect of sulodexide on endothelial glycocalyx and vascular permeability in patients with type 2 diabetes mellitus. *Diabetologia* 2010; **53**: 2646–2655.
103. Michel CC, Curry FR. Glycocalyx volume: a critical review of tracer dilution methods for its measurement. *Microcirculation* 2009; **16**: 213–219.
104. Yuan SY, Breslin JW, Perrin R, *et al*. Microvascular permeability in diabetes and insulin resistance. *Microcirculation* 2007; **14**: 363–373.
105. Schreibelt G, Kooij G, Reijerkerk A, *et al*. Reactive oxygen species alter brain endothelial tight junction dynamics via RhoA, PI3 kinase, and PKB signaling. *FASEB J* 2007; **21**: 3666–3676.
106. Rubio-Gayosso I, Platts SH, Duling BR. Reactive oxygen species mediate modification of glycocalyx during ischemia–reperfusion injury. *Am J Physiol Heart Circ Physiol* 2006; **290**: H2247–2256.
107. Singh A, Friden V, Dasgupta I, *et al*. High glucose causes dysfunction of the human glomerular endothelial glycocalyx. *Am J Physiol Renal Physiol* 2011; **300**: F40–48.
108. Norwood MG, Sayers RD. Urinary albumin : creatinine ratio (ACR) and the prediction of postoperative complications after abdominal aortic aneurysm repair. *Eur J Vasc Endovasc Surg* 2005; **30**: 353–358.
109. Smith FC, Gosling P, Sanghera K, *et al*. Microproteinuria predicts the severity of systemic effects of reperfusion injury following infrarenal aortic aneurysm surgery. *Ann Vasc Surg* 1994; **8**: 1–5.
110. Andersson M, Nilsson U, Hjalmarsson C, *et al*. Mild renal ischemia–reperfusion reduces charge and size selectivity of the glomerular barrier. *Am J Physiol Renal Physiol* 2007; **292**: F1802–1809.
111. Rehm M, Bruegger D, Christ F, *et al*. Shedding of the endothelial glycocalyx in patients undergoing major vascular surgery with global and regional ischemia. *Circulation* 2007; **116**: 1896–1906.
112. Snoeijs MG, Vink H, Voesten N, *et al*. Acute ischemic injury to the renal microvasculature in human kidney transplantation. *Am J Physiol Renal Physiol* 2010; **299**: F1134–1140.

113. Kozar RA, Peng Z, Zhang R, *et al*. Plasma restoration of endothelial glycocalyx in a rodent model of hemorrhagic shock. *Anesth Analg* 2011; **112**: 1289–1295.
114. Chappell D, Jacob M, Hofmann-Kiefer K, *et al*. Hydrocortisone preserves the vascular barrier by protecting the endothelial glycocalyx. *Anesthesiology* 2007; **107**: 776–784.
115. Chappell D, Hofmann-Kiefer K, Jacob M, *et al*. TNFα-induced shedding of the endothelial glycocalyx is prevented by hydrocortisone and antithrombin. *Basic Res Cardiol* 2009; **104**: 78–89.
116. Trung DT, Wills B. Systemic vascular leakage associated with dengue infections—the clinical perspective. *Curr Top Microbiol Immunol* 2010; **338**: 57–66.
117. Statler J, Mammen M, Lyons A, *et al*. Sonographic findings of healthy volunteers infected with dengue virus. *J Clin Ultrasound* 2008; **36**: 413–417.
118. Bethell DB, Gamble J, Pham PL, *et al*. Noninvasive measurement of microvascular leakage in patients with dengue hemorrhagic fever. *Clin Infect Dis* 2001; **32**: 243–253.
119. Wills BA, Oragui EE, Dung NM, *et al*. Size and charge characteristics of the protein leak in dengue shock syndrome. *J Infect Dis* 2004; **190**: 810–818.
120. Oragui EE, Nadel S, Kyd P, *et al*. Increased excretion of urinary glycosaminoglycans in meningococcal septicemia and their relationship to proteinuria. *Crit Care Med* 2000; **28**: 3002–3008.
121. Marechal X, Favory R, Joulin O, *et al*. Endothelial glycocalyx damage during endotoxemia coincides with microcirculatory dysfunction and vascular oxidative stress. *Shock* 2008; **29**: 572–576.
122. Nelson A, Berkestedt I, Schmidtchen A, *et al*. Increased levels of glycosaminoglycans during septic shock: relation to mortality and the antibacterial actions of plasma. *Shock* 2008; **30**: 623–627.
123. Steppan J, Hofer S, Funke B, *et al*. Sepsis and major abdominal surgery lead to flaking of the endothelial glycocalix. *J Surg Res* 2011; **165**: 136–141.
124. Chappell D, Westphal M, Jacob M. The impact of the glycocalyx on microcirculatory oxygen distribution in critical illness. *Curr Opin Anaesthesiol* 2009; **22**: 155–162.
125. De Gaudio AR, Adembri C, Grechi S, *et al*. Microalbuminuria as an early index of impairment of glomerular permeability in postoperative septic patients. *Intens Care Med* 2000; **26**: 1364–1368.
126. Stehouwer CD, Smulders YM. Microalbuminuria and risk for cardiovascular disease: analysis of potential mechanisms. *J Am Soc Nephrol* 2006; **17**: 2106–2111.
127. Constantinescu AA, Vink H, Spaan JA. Endothelial cell glycocalyx modulates immobilization of leukocytes at the endothelial surface. *Arterioscler Thromb Vasc Biol* 2003; **23**: 1541–1547.
128. Mulivor AW, Lipowsky HH. Role of glycocalyx in leukocyte–endothelial cell adhesion. *Am J Physiol Heart Circ Physiol* 2002; **283**: H1282–1291.
129. Joussen A, Poulaki V, Tsujikawa A, *et al*. Supression of diabetic retinopathy with angiopoietin-1. *Am J Pathol* 2002; **160**: 1683–1693.
130. Galkina E, Ley K. Leukocyte recruitment and vascular injury in diabetic nephropathy. *J Am Soc Nephrol* 2006; **17**: 368–377.
131. Gamble JR, Drew J, Trezise L, *et al*. Angiopoietin-1 is an antipermeability and anti-inflammatory agent *in vitro* and targets cell junctions. *Circ Res* 2000; **87**: 603–607.
132. Nagy N, Freudenberger T, Melchior-Becker A, *et al*. Inhibition of hyaluronan synthesis accelerates murine atherosclerosis: novel insights into the role of hyaluronan synthesis. *Circulation* 2010; **122**: 2313–2322.
133. Liu JE, Robbins DC, Palmieri V, *et al*. Association of albuminuria with systolic and diastolic left ventricular dysfunction in type 2 diabetes: the Strong Heart Study. *J Am Coll Cardiol* 2003; **41**: 2022–2028.
134. Arnold JM, Yusuf S, Young J, *et al*. Prevention of heart failure in patients in the Heart Outcomes Prevention Evaluation (HOPE) Study. *Circulation* 2003; **107**: 1284–1290.
135. Katare RG, Caporali A, Oikawa A, *et al*. Vitamin B1 analog benfotiamine prevents diabetes-induced diastolic dysfunction and heart failure through Akt/Pim-1-mediated survival pathway. *Circ Heart Fail* 2010; **3**: 294–305.
136. Fang ZY, Prins JB, Marwick TH. Diabetic cardiomyopathy: evidence, mechanisms, and therapeutic implications. *Endocr Rev* 2004; **25**: 543–567.
137. Becker BF, Chappell D, Bruegger D, *et al*. Therapeutic strategies targeting the endothelial glycocalyx: acute deficits, but great potential. *Cardiovasc Res* 2010; **87**: 300–310.
138. Jacob M, Bruegger D, Rehm M, *et al*. The endothelial glycocalyx affords compatibility of Starling's principle and high cardiac interstitial albumin levels. *Cardiovasc Res* 2007; **73**: 575–586.
139. Dongaonkar RM, Stewart RH, Geissler HJ, *et al*. Myocardial microvascular permeability, interstitial oedema, and compromised cardiac function. *Cardiovasc Res* 2010; **87**: 331–339.
140. Noble MI, Drake-Holland AJ, Vink H. Hypothesis: arterial glycocalyx dysfunction is the first step in the atherothrombotic process. *QJM* 2008; **101**: 513–518.
141. Rostgaard J, Qvortrup K, Poulsen SS. Improvements in the technique of vascular perfusion–fixation employing a fluorocarbon-containing perfusate and a peristaltic pump controlled by pressure feedback. *J Microsc* 1993; **172**: 137–151.