Glomerular endothelial cell fenestrations: an integral component of the glomerular filtration barrier

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Satchell SC, Braet F. Glomerular endothelial cell fenestrations: an integral component of the glomerular filtration barrier. Am J Physiol Renal Physiol 296: F947–F956, 2009. First published January 7, 2009; doi:10.1152/ajprenal.90601.2008.—Glomerular endothelial cell (GEnC) fenestrations are analogous to podocyte filtration slits, but their important contribution to the glomerular filtration barrier has not received corresponding attention. GEnC fenestrations are transcytoplasmic holes, specialized for their unique role as a prerequisite for filtration across the glomerular capillary wall. Glomerular filtration rate is dependent on the fractional area of the fenestrations and, through the glycocalyx they contain, GEnC fenestrations are important in restriction of protein passage. Hence, dysregulation of GEnC fenestrations may be associated with both renal failure and proteinuria, and the pathophysiological importance of GEnC fenestrations is well characterized in conditions such as preeclampsia. Recent evidence suggests a wider significance in repair of glomerular injury and in common, yet serious, conditions, including diabetic nephropathy. Study of endothelial cell fenestrations is challenging because of limited availability of suitable in vitro models and by the requirement for electron microscopy to image these sub-100-nm structures. However, extensive evidence, from glomerular development in rodents to in vitro studies in human GEnC, points to vascular endothelial growth factor (VEGF) as a key inducer of fenestrations. In systemic endothelial fenestrations, the intracellular pathways through which VEGF acts to induce fenestrations include a key role for the fenestral diaphragm protein plasmalemmal vesicle-associated protein-1 (PV-1). The role of PV-1 in GEnC is less clear, not least because of controversy over existence of GEnC fenestral diaphragms. In this article, the structure-function relationships of GEnC fenestrations will be evaluated in depth, their role in health and disease explored, and the outlook for future study and therapeutic implications of these peculiar structures will be approached.

fenestrations; fenestrae; glomerular endothelial cell; liver; hepatic; filtration; sieve; transendothelial permeability; microvascular; endothelial transport; sinusoidal; fenestral diaphragm; vascular endothelial growth factor

Fenestrations are the defining characteristic of the glomerular endothelial cell (GEnC) both morphologically and functionally. Although a proportion of other endothelial cells (EnC) possess these transcytoplasmic holes, those of the glomerular endothelium have a unique constellation of structural features (including absence of diaphragm but retention of a basal lamina). Furthermore, they perform a unique and vital physiological function in allowing filtration of the blood in the glomerulus. Without them, the kidney could not perform its primary function of clearing low-molecular-weight waste products from the circulation. Recently, podocytes, and in particular their foot processes, have received detailed scrutiny with regard to their importance as part of the permselectivity barrier (77). Despite evidence that the glomerular filtration barrier functions as a whole (52), the contribution of the glomerular endothelium and especially its fenestrations has been relatively neglected.

What are Fenestrations?

The systemic endothelium forms a lining of all blood vessels and represents the principal regulator of vascular permeability, in addition to its other important physiological functions. The following three main types of endothelium are recognized: continuous (lacking fenestrations), fenestrated, and “discontinuous” (82, 84). Fenestrations are round or ovoid transcellular holes through the most attenuated part of the EnC cytoplasm. They are found in the endothelium of organs where a higher rate of exchange between intra- and extravascular compartments is required. Generally, this is a higher rate of exchange of water and small solutes, as in the gastrointestinal or peritubular renal capillaries. These capillaries retain a low permeability to macromolecules (66). In discontinuous endothelia, larger fenestrations may allow passage of lipid particles and cellular debris.

Endothelial fenestrations can be grouped into three types according to type of endothelium and presence or absence of...
diaphragms (Table 1). The most common type is found in organs including endocrine tissue (e.g., pancreatic islets, adrenal cortex), gastrointestinal mucosa, and renal peritubular capillaries. These fenestrations are typically 60–70 nm in diameter and are traversed by a thin (3–5 nm) diaphragm. The protein “plasmalemmal vesicle-associated protein-1” (PV-1 or PLVAP), a type II transmembrane glycoprotein, is an integral part of this diaphragm (100, 101). PV-1 is the antigen recognized by the antibody “pathologische anatomie Leiden-endothelium,” long used as an endothelium-specific marker (74). At the ultrastructural level, it is believed that each individual fenestration is encircled by a cytoskeletal lattice (56). Furthermore, early studies demonstrated that cholesterol is enriched in close proximity to the fenestral opening, suggesting a role in stabilization of the plasma membrane in this area (96).

The second type of fenestration, occurring in endothelia that are often referred to as discontinuous (i.e., lacking a continuous basal lamina), is found in the spleen, bone marrow, and in liver sinusoidal endothelial cells (LSEC). However, fenestrations have been studied in detail only in the latter (16, 23, 115). These fenestrations do not possess diaphragms and the EnC do not express PV-1 in vivo. In perfusion-fixed rat livers, fenestrae have an average diameter of 100–175 nm (115), but in culture this figure is 200 nm (16). Fenestrae in human LSEC are of a similar diameter (103–113 nm) (116). LSEC fenestrations expand and contract under various stimuli, such as norepinephrine, vasoactive intestinal peptide, serotonin, nicotine, and alcohol (4, 23, 51). They have a well-defined fenestrae-associated cytoskeleton ring and actin filaments are found in close proximity (Fig. 1A) (14, 19, 51). However, only the smaller fenestrations have a cholesterol-rich ring (96). LSEC are also unusual in that they do not possess a basal lamina (23, 114).

LSEC fenestrations act as a sieving barrier to control the extensive exchange of material between the blood and the liver parenchyma (115). In contrast to fenestrae described in the kidney, pancreas, and brain, their biological relevance in various diseases such as fibrosis, cirrhosis, steatosis, hepatitis, inflammation, and metastasis has been described in detail (20, 23). Furthermore, it has been widely acknowledged that changes of the endothelial filter directly influence the transport of chylomicron remnants and other lipoproteins to the space of Disse, with implications for atherosclerosis (50). Recent LSEC studies showing a complex relationship between fenestrations, endothelial migration, tube formation and caveolin-1, orchestrated through Rho family GTPases in response to vascular endothelial growth factor (VEGF), further suggest that the importance of fenestrae in endothelial biology extends beyond modification of barrier properties (13, 120).

GEnC fenestrations constitute a third type. They are similar to the first type in size [diameter 60–80 nm (6, 60, 64, 88)] yet, like the second type, they generally do not express PV-1 (74, 101, 105, 119), and, it is generally asserted, do not possess diaphragms (12, 61, 80). We should note that a number of observations challenge this position (53, 88, 89). To some extent, appearance of this feature may be dependent on the fixation and labeling techniques used, since a diaphragm is seen in some preparations but not others. It could be either that some techniques destroy a very delicate diaphragm or that other techniques result in artefactual appearance of a diaphragm, perhaps through condensation/cross-linking and labeling of glycolcalyx, other plasma proteins, or the outer surface of the glomerular basement membrane. A recent, careful study demonstrated that 2% of glomerular capillary cross sections from mature rat glomeruli contain diaphragmed fenestrations (55). Along with the appreciation that the intraglomerular portion of efferent arterioles and direct tributaries may express fenestrated diaphragms (40), this goes a long way toward clarifying the position. An additional possible explanation is that it may be a reflection of dynamic changes in GEnC structure (see below) (55). GEnC fenestrations are concentrated in the peripheral cytoplasm, away from the cell body and arranged in clusters or sieve plates, separated by ridges of cytoplasm (Fig. 1B). GEnC fenestrations are thus in the part of the cell cytoplasm opposite podocyte foot processes and filtration slits across the glomerular basement membrane commensurate with their filtration function (Fig. 1C). At the ultrastructural level, each individual fenestration is surrounded by a network of actin microfilaments (110). GEnC fenestrations are the focus of the remainder of this review.

Are Glomerular Endothelial Fenestrations Empty Holes?

All endothelia possess a gelatinous surface coat, or glycolcalyx, composed principally of proteoglycans and sialoproteins (81, 113). Onto this glycolcalyx is absorbed a further layer of proteins, known as the endothelial surface layer, in equilibrium with circulating plasma. In combination, these layers have important roles in regulation of permeability of the endothelium as well as in modifying ligand-receptor and cellular interactions with the endothelium.

Preservation of glycolcalyx during fixation for electron microscopy (EM) requires specialized techniques. Such studies have shown that endothelial fenestrations in various organs contain glycolcalyx-like material often arranged as filamentous “sieve plugs” or “fasciae fenestrae” (53, 88, 89). In the glomerular endothelium, a 300-nm surface coat composed of very thin filaments was observed over both fenestral and interfenestral domains (Fig. 1D) (88). In a further development of the technique employing contrast enhancement, sieve plugs were also observed in GEnC fenestrations (89). In a murine study, infused Intralipid droplets were excluded from a layer immediately adjacent to the GEnC surface, and the mean

Table 1. Comparison of the three types of endothelial cell fenestrations

<table>
<thead>
<tr>
<th>Endothelium in Which Fenestrations are Expressed</th>
<th>Systemic capillaries, e.g., gastrointestinal and renal peritubular</th>
<th>“Discontinuous” endothelium, e.g., hepatic sinusoidal</th>
<th>Glomerular</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endothelial type</td>
<td>Fenestrated</td>
<td>Discontinuous</td>
<td>Fenestrated</td>
</tr>
<tr>
<td>Diaphragm</td>
<td>60–70</td>
<td>100–175</td>
<td>60–80</td>
</tr>
<tr>
<td>PV-1 expression</td>
<td>Yes</td>
<td>No (only in development)</td>
<td>No (only in development)</td>
</tr>
<tr>
<td>Cytoskeletal ring</td>
<td>?</td>
<td>Yes</td>
<td>?</td>
</tr>
<tr>
<td>Cholesterol ring</td>
<td>?</td>
<td>Yes</td>
<td>?</td>
</tr>
<tr>
<td>Basal lamina</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Glycolcalyx</td>
<td>Yes</td>
<td>?</td>
<td>Yes</td>
</tr>
</tbody>
</table>

PV-1, plasmalemmal vesicle-associated protein-1; ?, unknown.
distance between droplets and GEnC was reduced by glyocalyx-degrading enzymes (58). This also resulted in increased urinary albumin excretion, providing evidence that the glyocalyx forms a barrier to protein. Furthermore, the ability of GEnC glyocalyx to form a permeability barrier to macromolecules can be directly demonstrated in vitro (97). Analysis of the composition of the endothelial glyocalyx has revealed significant differences between vascular beds (9) and between fenestral and interfenestral regions of GEnC (7). The glyocalyx in the fenestrae has a higher ratio of heparan sulfates and hyaluronic acid to sialoproteins. The particular composition of glyocalyx in the fenestrae is likely to be crucial for its permeability properties.

These differences in fenestrations and glyocalyx are reminders that there are important differences between EnC of different organs and of different types of blood vessel (2, 32). Therefore, as we consider GEnC fenestrations, care is needed in assessing the relevance of observations from other vascular beds. In general, the microcirculation, consisting of capillaries and postcapillary venules, is concerned with exchange between the vascular and extravascular compartments, and it is in microvascular EnC that fenestrations are found in vivo. The macrocirculation’s role is as a conduit of blood, and macrovascular endothelium is much less involved in exchange processes and nowhere has fenestrations in vivo.

How are Endothelial Fenestrations Studied?

Because of the small size of fenestrations, the fragility of the attenuated cytoplasm in the sieve plates, and paucity of specific protein markers, study of EnC fenestrations is technically demanding and largely reliant on EM for both ex vivo and in vitro studies. (The limit of resolution of light microscopy is ~200 nm.) Because EM in general is performed on fixed tissues or cells, it produces static or “snapshot” images, making dynamic interpretations difficult.

In vitro studies, although vital for detailed analyses, are further complicated by relative paucity of fenestration expression in cultured EnC. Some EnC types, notably LSEC, retain numerous fenestrations in sieve plate structures when freshly isolated in primary culture (16, 17) but lose them over time and through subculturing (24, 41). Two reported LSEC lines continue to express fenestrations but at a lesser density than primary isolates and with an appearance dissimilar to cells in vivo (34, 90). Others, although expressing many fenestrations in vivo, have none in culture (45, 56). Fenestrations can be induced more easily in microvascular EnC, which express fenestrations in vivo, than in macrovascular cells (45, 102). However, even in these cells, the density of fenestrations induced may be very low compared with the in vivo situation (56). Primary cultures of human and rat GEnC under basal conditions express sparse pores, thought to be fenestrations (108, 111) as do conditionally immortalized human and murine GEnC lines (87, 93). Interestingly, fenestrations in these cells have a similar appearance to those expressed by the LSEC line referred to above (90).

How do Glomerular Endothelial Fenestrations Arise in Development?

Rodents are useful models in which to study aspects of glomerular development, since their glomeruli do not mature until 2 wk of age. During this period, all stages of glomerular development are apparent simultaneously. The earliest GEnC arise from mesenchyme (or angioblasts, endothelial precursors) migrating into the cleft of the developing glomerulus at
the S-shaped body stage. These earliest cells are cuboidal and lack fenestrations (55, 80). In the capillary loop stage, the endothelial cells proliferate, and the cytoplasm thins before formation of fenestrations. At this stage, podocytes are present on the opposite side of the developing glomerular basement membrane, but foot processes are poorly formed. The earliest fenestrations have diaphragms, but these disappear as the endothelium becomes progressively attenuated and as podocyte foot processes develop. Thus the formation of GEnC fenestrations parallels the maturation of podocyte foot processes (55, 78). Fenestrations in LSEC also have a diaphragm-like structure initially, at least in rodent development (10).

What Factors Stimulate the Development of Fenestrations?

The observation that high levels of VEGF expression are found in epithelial cells closely associated with fenestrated endothelia led to the hypothesis that VEGF induces endothelial fenestrations (25, 44). This hypothesis has been investigated and confirmed in a variety of in vivo and in vitro studies in both glomerular and other EnC. VEGFs (VEGF-A to -E) comprise a family of growth factors that act through VEGF receptors (VEGFR) 1–3. In EnC, they activate diverse signaling pathways effecting migration, proliferation, and permeability (75). VEGF-A, often referred to (as here) simply as VEGF, is the prototypic family member and is most widely expressed and studied. It arises in a variety of different isoforms denoted by the number of amino acids, with VEGF165 being the most common. The recognition of a family of inhibitory "b" splice variants has revealed a further layer of complexity in the VEGF family (11, 95).

Podocytes begin to express VEGF at the S-shaped stage, as GEnC are migrating into the cleft and before the development of fenestrations (78). GEnC express VEGFR2 from this stage and maintain expression through to maturity (83). VEGF expression is also maintained in the podocyte at high levels, suggesting an ongoing role for VEGF signaling in the mature glomerulus (26, 92). That VEGF is expressed before fenestrations appear is consistent with a role for VEGF in their induction.

The hypothesis is further supported by manipulation of VEGF levels in vivo and also by in vitro observations. Manipulation of the level of VEGF expression in podocytes using transgenic technology reveals that glomerular and GEnC phenotype is exquisitely sensitive to the dose of VEGF (42). In development, loss of one VEGF allele leads to GEnC thickening and loss of fenestrations, whereas overexpression causes collapse of capillary loops. Antibody blockade of VEGF in the adult animal also causes GEnC damage with thickening, detachment from the basement membrane, and regression of fenestrations (61, 106). VEGF also induces fenestrations in nonglomerular circulations in animal models (85, 86), and blockade of hepatic VEGF signaling in transgenic models reduces LSEC fenestrations (29). In vitro, fenestrations can be induced by VEGF in both glomerular (93) and nonglomerular EnC (45, 121) as well as by coculture of EnC with VEGF-producing cells (30, 44). In some assays, VEGF increases permeability of EnC monolayers consistent with fenestration formation (44, 91).

These observations beg the question of whether VEGF is the only important factor responsible for fenestration induction. Blockade of transforming growth factor-β (TGF-β) in developing rodent glomeruli prevents maturation of GEnC, including formation of fenestrations (67). Although this indicates the importance of TGF-β in glomerular development and suggests a permissive role in fenestration formation (47), TGF-β has not yet been shown to directly induce fenestrations per se. Similarly, blockade of TGF-β and VEGF caused regression of choroid plexus vasculature fenestrations, although neither intervention alone did so (71). In apparent contradiction, another report indicates that TGF-β inhibits development of fenestrations in macrovascular EnC in vitro (69). Glomerular basement membrane protein abnormalities can lead to failure of GEnC maturation (1), but again this does not necessarily imply that matrix proteins directly induce fenestrations. Fenestrations can be induced in some EnC by culturing them on certain extraacellular matrixes, including those produced by particular cell types (31, 72, 73). In a number of these cases, it appears that matrix-bound VEGF plays a significant role (31, 45, 109). The importance of the matrix my lie in amplifying the response to VEGF by providing additional signals, perhaps through integrin binding or through sequestration and presentation of VEGF.

Lack of the inhibitory VEGF165b isoform in glomeruli of patients with Denys-Drash syndrome is associated with glomerular swelling and loss of fenestrations despite expression of VEGF (95). It is not clear whether this indicates the importance of VEGF165b as such for fenestration formation or whether it simply reinforces the importance of fine control of VEGF dose in this process. VEGF, but not fibroblast growth factor 2, induces fenestrations in a murine corneal angiogenesis assay (28, 43). Because VEGF-C is expressed by podocytes in the glomerulus (48) and GEnC express the cognate receptors VEGFR2 and VEGFR3 (49, 61), these observations suggest that VEGF, VEGF165b, and VEGF-C may signal in a coordinated fashion to induce the unique fenestral phenotype seen in GEnC.

An isolated report describes the induction of fenestrations in GEnC by shear stress (76) and in nonglomerular EnC by leptin (27) and endocrine gland-derived VEGF (65). Fenestrations have also been induced pharmacologically in vitro in nonglomerular EnC using phorbol myristate acetate (PMA), an analog of the intracellular second messenger diacylglycerol (37, 68, 69, 70, 102) and various actin-depolymerizing agents (15, 19, 21, 22, 56, 103), and in rat GEnC with cytochalasin B (111).

How Does VEGF Induce Fenestration Formation?

Studies of fenestration formation have relied on overexpression of VEGF in in vivo models or on in vitro systems where VEGF or pharmacological agents are added exogenously. Such models provide at best an approximation of physiological conditions, including VEGF concentrations, and they do not fully recapitulate physiological fenestration development. Still less clear is their relevance to GEnC, which have yet to be examined in detail. In maturity, most of the important roles of VEGF are mediated through VEGFR2, although simultaneous VEGFR1 binding may be important in modulating VEGFR2 signaling to produce permeability-enhancing effects (123). Little work has been done to understand which of the signaling pathways activated through VEGFRs are required for fenestrae induction.
In the mouse corneal angiogenesis assay, inhibition of Rac, a member of the Rho family of small protein GTPases, prevented fenestration formation in new vessels induced by VEGF and resulted in vessels with a thicker endothelium than those induced by VEGF alone (43). Activation of Rac was subsequent to VEGFR2 phosphorylation, downstream of phosphatidylinositol-3-OH kinase (PI3-kinase) activation. Rac is well known to have important roles in regulation of the actin cytoskeleton and endothelial permeability (117). However, these reported effects of induction of fenestrations, along with increased trans-endothelial leakage of Evan’s blue dye, appear to contradict the established role of Rac in reducing endothelial permeability by stabilizing cell-cell junctions and reducing actinomyosin contractility (117). It is possible that the method of Rac inhibition used resulted in other confounding changes in Rho family, or other, intracellular messengers. Hence, the importance of Rac in fenestrae formation needs to be confirmed in other systems. Another group found that Rho regulates fenestrations through the actin cytoskeleton in rat LSECs (122).

The presence of a circumferential fenestral ring, and the arrangement of fenestrae into sieve plates, further suggests the importance of the cytoskeleton for fenestrae formation and maintenance of structural organization. Studies using the mouse brain EnC line bEND5 have confirmed the importance of actin rearrangement and in particular depolymerization of stress fibers for fenestration formation in response to latrunculin and VEGF (56). In ex vivo glomerular culture, cytochalasins prevent the loss of endothelial fenestrations that normally occurs after 48 h (3). Although this is again consistent with the importance of actin depolymerization in the maintenance of fenestrations, the effect could also be mediated indirectly through effects, for example, on podocyte to GEnC signaling through VEGF.

Strong evidence points to the importance of PV-1 in the formation of systemic endothelial fenestrations (in addition to its importance as a diaphragm component). PV-1 is highly expressed, along with VEGF, in tumor circulations, which have abundant fenestrations (86). PV-1 is upregulated in response to VEGF in a murine tumor angiogenesis model and in human umbilical vein EnC in culture (105). This upregulation is also dependent on VEGFR2 binding and PI3-kinase activation. Treatment of cultured EnC with PMA causes upregulation of PV-1 and fenestrations (102). Inhibition of PV-1 upregulation using small-interfering RNA prevented fenestration formation. In bEND5 cells, PV-1 seemed to be important for regulation of the size of fenestrations and their organization into sieve plates: knockdown of PV-1 did not reduce the density of fenestrations formed in response to latrunculin but resulted in disorganization of sieve plates with more variable distance between fenestrations and greater variation in fenestral size (20–400 nm) (56). Thus both PV-1 and actin rearrangement are important, but exactly how they interact in fenestration formation is yet to be established (Fig. 2). We should note that one group has reported negative regulation of PV-1 in response to VEGF signaling in murine alveolar EnC (54).

A further debate has concerned whether fenestrations arise from preexisting structures such as caveolae (plasmalemmal vesicles) or whether they arise de novo (Fig. 3). In the first scenario, it is suggested that they form by fusion of caveolae or vesiculo-vacuolar organelles (suggested to be clusters of interconnected uncoated vesicles and vacuoles) with both the apical and basal membranes of an EnC (39, 45). This is envisaged to occur as the endothelial cytoplasm thins such that the membranes come into contact (56, 61). This hypothesis is attractive given the association of PV-1 with caveolae in nonfenestrated EnC in culture (56) and the apparent reciprocity between the number of caveolae and of fenestrations (61). However, caveolin-1 is not found in fenestrations in GEnC in vivo (98) or adrenal cortical microvascular EnC in culture (45), and VEGF does not alter the distribution, amount, or phosphorylation of caveolin-1 (45). The fact that GEnC fenestrations appear to be normal in caveolin-1 knockout mice further argues against involvement of caveolae in fenestration formation, at least in GEnC (98). Similarly, although VEGF increases both fenestrations and caveolin-1 expression in LSEC, the caveolin-1 and fenestrations are not associated (121).

The alternative explanation is that fenestrations arise de novo from the fusion of the two cell membranes without

![Diagram showing proposed intracellular pathways of vascular endothelial growth factor (VEGF)-induced fenestration formation in glomerular endothelial cells (GEnC). VEGF-A binds to and activates VEGF receptor (VEGFR) 2, which leads, via small protein GTPases, to actin rearrangement required for formation of a fenestral cytoskeletal ring. VEGFR2 activation also leads to recruitment of plasmalemmal vesicle-associated protein-1 (PV-1) and PV-1 multimer assembly in the forming fenestration. Fenestral diaphragms and PV-1 disappear as the fenestration matures.](http://ajprenal.physiology.org/)
meet and fuse. The extent in particular regions where the opposing cell membranes eventually meet and fuse. C: as the cytoplasm thins, cell membranes fuse with membranes of preexisting intracellular organelles such as caveolae. These pathways are not necessarily mutually exclusive, and it may be that fenestrations form through a combination of pathways.

Fig. 3. Diagram showing three possible routes to fenestration formation. For a fenestration to form in the peripheral cytoplasm of GEnC, both attenuation of the cytoplasm and hole formation must occur. A: a pore forms first, and then the pore enlarges as the cytoplasm thins. B: the cytoplasm thins to a greater extent in particular regions where the opposing cell membranes eventually meet and fuse. C: as the cytoplasm thins, cell membranes fuse with membranes of preexisting intracellular organelles such as caveolae. These pathways are not necessarily mutually exclusive, and it may be that fenestrations form through a combination of pathways.

relation to preexisting caveolae. This appears to be the case at least in LSEC where fenestrations arise in fenestrae-forming centers (21). The exact mechanism of the required apical-basal membrane fusion in fenestration formation has not been investigated, but is likely to be dependent on fusion proteins and the initial formation of a fusion pore (57, 59). Although this has not been studied in GEnC, it indicates the possibility of an alternative generation process of fenestrations lacking diaphragms, PV-1.

How can this information be applied to GEnC? The role of VEGF in fenestration induction in GEnC is established as above; therefore, VEGFR2 and PI3-kinase, along with actin rearrangement, are likely to be important in other EnC, but what about PV-1? The presence of diaphragms and PV-1 initially during fenestration development suggests that PV-1 may be involved in the initial formation of GEnC fenestrations, but expression is lost as diaphragms are removed or remodeled (Fig. 3) (8, 55, 79). Evidence suggests that this process is reversed and then recapitulated during GEnC damage and repair when fenestrations become diaphragmed (or are lost completely), and GEnC reexpress PV-1 (55, 61, 119). The fact that blocking VEGF leads to loss of fenestrations confirms a role for VEGF in maintaining GEnC fenestrations in the absence of PV-1 expression or diaphragms (61).

How are Fenestrations Maintained and Regulated?

The above evidence points to a primary role for VEGF in maintenance of fenestrations as well as in their biogenesis. However, the question of whether GEnC fenestrations are dynamic structures is at present totally unexplored. The regression and reappearance of fenestrations with VEGF blockade and some disease states suggests that fenestrations may be regulated physiologically in a similar way (55, 61, 104). This might be important in regulating glomerular filtration rate (GFR) or “rotating” filtration over the glomerular filtration barrier surface. Control of fenestral size as in LSEC (51) could potentially modulate GFR even more precisely.

Although fenestrations in systemic capillaries are uniform in size (99), those of GEnC and LSEC are more variable (23, 61, 116). This size variability cosegregates with lack of PV-1, suggesting that PV-1, when present, dictates the size of fenestrations (56). Conversely, the absence of PV-1 in GEnC and LSEC may be necessary to allow dynamic regulation of the fenestral aperture. This concords with the observation that GEnC fenestral size is more uniform in that small proportion possessing diaphragms (55).

What is the Role of Glomerular Endothelial Fenestrations in Regulation of Glomerular Filtration Barrier Permeability?

A detailed discussion of the mechanisms of selective glomerular permeability is beyond the scope of this review and has been addressed in detail elsewhere (52). We will highlight the salient points in relation to GEnC fenestrations. Filtration of water and small solutes across the glomerular filtration barrier occurs via gaps through or between cells rather than through the cell cytoplasm. Therefore, the high hydraulic conductivity of the glomerular filtration barrier depends on the presence of endothelial fenestrae and filtration slits between adjacent podocyte foot processes. That is to say, fenestrations and filtration slits are essential for clearance of low-molecular-weight waste products from the circulation by filtration (52).

Resistance to filtration of water and small solutes at the level of the glomerular endothelium depends on the fractional area of the capillary surface occupied by fenestrations and on their contents. Changes in the fractional area of fenestrae are predicted to have profound effects on GFR (36). Biophysical models indicate that fenestral glycocalyx contributes 50% to the overall hydraulic resistance of the glomerular filtration barrier (38). Therefore, changes in the amount or composition of glycocalyx within the fenestrae potentially also has significant effects on GFR.

Macromolecules, and in particular albumin, are largely excluded by the glomerular filtration barrier. There has long been speculation about which layer of the glomerular filtration barrier is most restrictive to the passage of albumin. However, there is now growing appreciation that the filtration barrier functions as a whole in this respect and that each layer makes a significant contribution. In this context, it is recognized that the fenestral glycocalyx is an important part of the barrier to albumin permeability (52, 97) and that damage to it is a potential mechanism of the microalbuminuria characteristic of conditions including diabetes (94).

What is the Clinical Significance of GEnC Fenestration Dysfunction?

The reduction in GFR associated with loss of fenestral area predicted by biophysical models does indeed occur in pre-eclampsia where there is good evidence that this is the mechanism of acute renal failure (63). In this condition, increased circulating levels of soluble VEGFR1 bind to podocyte-produced VEGF and reduce availability for endothelial signaling (104). This results in endothelial thickening and a reduction in...
both size and density of fenestrations, “endotheliosis.” GFR recovers as the condition resolves and fenestrations reappear.

In a number of other instances, the glomerular endothelium is similarly damaged. These include both animal models [experimental diabetes (35, 46), uranyl nitrate-induced acute renal failure (6), cyclosporine nephropathy (62), serum sickness nephritis (33), and Thy-1 nephritis (55)] and human disease [diabetic nephropathy (107) and transplant glomerulopathy (112, 119)]. Although the impact of fenestral changes in these conditions has not been analyzed as carefully as in preeclampsia, it is inevitable that they contribute to the observed reduction in GFR in similar way. Neither the endothelium nor its fenestrations have been studied in detail in the majority of human glomerular diseases.

Interestingly, all of the above conditions are associated with proteinuria despite the reduction in GFR, whereas, in many cases, podocyte foot processes appear relatively normal. Taken together, these observations suggest that dysfunction or loss of the endothelial glycocalyx, as well as fenestrations, may be contributing to the increased passage of albumin across the glomerular filtration barrier. In human diabetic nephropathy, fenestration loss correlates with albuminuria (107), and, in transplant glomerulopathy, reexpression of PV-1 in GEnC is associated with loss of fenestrations and also correlates with proteinuria (119). Deterioration in GFR in diabetic nephropathy correlates with endothelial function (5), and there is strong evidence for glomerular as well as generalized endothelial dysfunction in this condition (94). Another possible explanation for the link between reduced fenestrations and proteinuria is that this GEnC dysfunction results in disturbed signaling to, and dysfunction of, other glomerular cells.

Conclusions and Outlook

Our review highlights the physiological and pathological importance of GEnC fenestrations as a key component of the glomerular filtration barrier. However, the study of GEnC fenestrations is at a relatively early stage, and a number of questions remain to be resolved. Refinements of cell culture models will allow more precise dissection of the pathways to fenestration formation, more detailed study of the relationship between fenestrations and permeability properties, and application in pharmacokinetic analyses. Necessary improvements will include adjusting experimental conditions to allow induction of fenestrations with a greater density and with a closer resemblance to those seen in GEnC in tissue sections. Although technically demanding, this may be achievable through culture of very freshly isolated GEnC, as used successfully with LSEC, or through an appropriate cocktail of mediators and physicochemical factors.

Novel imaging techniques, or new applications of existing ones, will help, particularly for dynamic imaging. For example, atomic force microscopy has the required resolution to study fenestrations, can be used in live cells, and may enable imaging of fenestration formation. Similarly, the latest iteration of low-vacuum environmental scanning electron microscopy will allow live, unfixed cells to be studied at varying stages of fenestration development. Although PV-1 is a well-characterized component of diaphragmed fenestrae, so far no proteins specifically associated with GEnC fenestrations have been identified, and their structural components remain obscure. Correlative microscopy techniques will help to address this.

The evidence that VEGF is important in GEnC fenestration formation is strong, but it is unlikely to be the sole factor. The possibility that other aspects of the physicochemical environment within the glomerulus are important has been raised, but the exact role of shear stress and the specialized glomerular basement membrane have yet to elucidated.

A greater understanding of the relevance of GEnC fenestrations in glomerular physiology and pathology naturally raises the question of whether it will be possible to manipulate them therapeutically. For example, promotion of GEnC fenestration formation would be desirable in severe preeclampsia to avoid renal failure and may increase GFR in a number of other conditions, including diabetic nephropathy. VEGF has been used to restore fenestrations in LSEC and reduce portal hypertension in an animal model of cirrhosis, supporting the concept of therapeutic fenestration manipulation (118). The restoration of endothelial health in diabetic glomerular disease holds promise for increasing GFR through increased fenestral density and decreasing proteinuria through restored glycocalyx.

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Review


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