Glomerular filtration into the subpodocyte space is highly restricted under physiological perfusion conditions

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The rate of formation and composition of primary filtrate is determined by the transport of fluid and solutes across the glomerular filtration barrier (GBF), consisting of the endothelial cells, the glomerular basement membrane, and the podocyte foot processes (9). Until recently, it had been considered that there was no significant resistance to fluid and solute flux downstream of the podocyte foot processes (6). However, we recently showed that cellular processes anchor the podocyte directly to the glomerular basement membrane (GBM) (13). These “anchoring processes” were sufficiently common that some of them formed boundaries of a space underneath the podocyte, the sub-podocyte space (SPS), which appeared to be relatively restrictive to flow of fluid. Furthermore, this space covered ~60% of the filtering portion of the glomerular filtration surface and described a tortuous pathway before it reached a further constriction between the SPS and a fluid space of greater dimensions between the podocytes, the interpodocyte space (IPS) (see Fig. 1). This constriction we have termed the SPS exit pore (or SEP). The wider channels of the IPS drain both the regions covered by SPS and the remainder of the glomerular center, and efflux is into the space between the parietal cells (lining Bowman’s capsule) and the peripheral podocytes, termed the peripheral urinary space (PUS). For peripheral podocytes (those situated around the edge of the glomerulus), flow from the SPS and uncovered regions of the filtration barrier would be directly into the PUS (see Fig. 1). These findings suggested that up to two-thirds of the GBF might be covered by a higher resistance pathway, at least under conditions of no filtration (immersion fixed) and hyperfiltration (perfusion fixed with no colloid). Provisional estimates, based on a deliberately simplified assumption, were that the increases in resistance were up to 900- to 14,500-fold compared with uncovered areas, assuming that the basement membrane resistance was similar to the resistance of the exit pore of the SPS. While it was clear that this was an upper estimate of the relative resistance, it did suggest that fluid flow through the SPS was low, which provided the basis for a resistance to fluid flow, resulting in an increased pressure in the SPS relative to other urinary spaces. This pressure would be expected to be between the capillary pressure and the proximal tubular pressure. The physiological significance of the SPS was not determined, but it was suggested that it may enable the podocyte to act as a sensor of glomerular filtration rate or enable backwashing of the membrane, preventing clogging with plasma constituents, or that it was an inevitable consequence of such a cellular structure covering the GBF.

The SPS may enable the podocyte to regulate the overall GBF resistance and so to control fluid or solute flux into the proximal tubule and subsequently regulate urine formation and production. In particular, the dimensions of the SEP, the constriction between the SPS and the IPS or PUS, provided a significant proportion of the calculated resistance when the glomerulus was fixed under conditions of high or no filtration. In our previous study of the SPS, kidneys were either immersed (perfusion fixed with no colloid). Provisional estimates, based on a deliberately simplified assumption, were that the increases in resistance were up to 900- to 14,500-fold compared with uncovered areas, assuming that the basement membrane resistance was similar to the resistance of the exit pore of the SPS. While it was clear that this was an upper estimate of the relative resistance, it did suggest that fluid flow through the SPS was low, which provided the basis for a resistance to fluid flow, resulting in an increased pressure in the SPS relative to other urinary spaces. This pressure would be expected to be between the capillary pressure and the proximal tubular pressure. The physiological significance of the SPS was not determined, but it was suggested that it may enable the podocyte to act as a sensor of glomerular filtration rate or enable backwashing of the membrane, preventing clogging with plasma constituents, or that it was an inevitable consequence of such a cellular structure covering the GBF.

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Fig. 1. A: transglomerular montage from 8 micrographs from 1 section (immersion fixed kidney). The subpodocyte spaces (SPS; yellow) are shown underneath the podocyte cell bodies and processes, they cover a large proportion of the glomerular filtration barrier (GFB). The exit from these SPS is through the exit pores (SEP; red) into the interpodocyte spaces (IPS; orange), forming an anastomosing network of urinary spaces that drain filtrate away from the GFB. The peripheral urinary space (PUS; blue) between Bowman’s capsule (BC) and the edge of the glomerulus should further channel filtrate toward the urinary pole and the renal tubular system. B: just the urinary spaces and SEP from A are shown and clearly display the dendritic, tortuous, narrow nature of the urinary spaces, with the SPS being the narrowest. In B, SEP shown in red are enlarged for clarity. Scale bars = 50 μm.

METHODS

All experiments were carried out in accordance with the Animals (Scientific Procedures) Act 1986 of the United Kingdom. Wistar-Kyoto rats (n = 3; 300-g males) were terminally anesthetized intraperitoneally with pentobarbital sodium (G. M. Loveridge, Southampton, UK). Immediately after death, the abdominal aorta upstream of the renal arteries was perfused at 100 mmHg with mammalian Ringer solution. The plastic aortic cannula was shaped with a bulbous end wider than the aorta, allowing rapid cannulation and perfusion without the cannula slipping out. The vena cava was cut to allow egress of perfusate and blood during fixation. Kidneys were first perfused with 6.5% Ficoll 400 in mammalian HEPES-Ringer solution at room temperature (pH 7.35) containing 10 IU/ml heparin (Monoparin, CP Pharmaceuticals). This solution had a colloid osmotic pressure of 25 mmHg (measured on a modified Hansen oncometer with a 10-kDa Amicon membrane). Once the kidneys changed color with the clear perfusate, then the initial flush solution was changed to 6.5% Ficoll 400 in mammalian HEPES-Ringer with 2.5% glutaraldehyde (pH 7.35) at room temperature for ~5 min, followed by excision and further fixation for 2–3 h on ice and then stored at 4°C before processing for electron microscopy. Small pieces (0.5- to 1-mm-diameter cubes) of kidney cortex were excised and washed in 0.1 M cacodylate buffer washes (4, 15). Dehydration was with ethanol, and tissues were infiltrated and embedded in Araldite resin (Agar Scientific).

Sectioning and reconstruction. Survey sections (500-nm thick) were cut from each kidney and stained with toluidine blue [1% in 1% (aqueous) borax] for light microscopy. Glomeruli were identified before the cut surface was trimmed to include one to three glomeruli (clustered) in a smaller block face suitable for ultrathin serial sectioning. Serial section runs of 20–250 sections long and 100-nm thickness were cut and laid on consecutive Formvar grids and stained with 3% (aqueous) uranyl acetate and Reynolds’ lead citrate solution (4, 15). Digital micrographs were taken on a Philips 100CS microscope at ×2,600 and ×3,400 to show the disposition of a few podocytes in each field of view from section to section. The sectioned shapes of capillaries, mesangial matrix, and red blood cells were used as positional cues to line up podocyte profiles from section to section.

Three-dimensional visualization of the SPS. Micrographs of the same region on consecutive sections were used to reconstruct areas underneath the podocyte as previously described (13). In brief, Adobe Photoshop software (Adobe Systems, San Jose, CA) was used to highlight and reconstruct the SPS from these micrographs.

In addition, serial section micrographs of the same area were collated in sequence and imported into the Reconstruct program (John C. Fiala). Micrographs were adjusted by shearing, stretching, and altering the magnification to a very minor degree so that they aligned with “key” micrographs. Subpodocyte space, podocytes, and under-
lying capillaries were outlined and color coded. Reconstruct was then used to display these features to emphasize the position of the SPS between the podocyte cell body and the GBM. These models were used to develop still-frame views, freely rotatable VRML files and AVI video files (see online supplement; all supplemental material can be found in the online version of this article on the journal web site).

**Analysis of SPS characteristics.** Measurements were made on the SPS Adobe Photoshop reconstructions and micrographs to determine the restriction to fluid flow out of the SPS. The width of SEPs was measured on each micrograph where the podocyte profiles were followed from the IPS or PUS into the SPS, and the narrowest separation was measured directly on the micrograph. For all sections (micrographs) encompassing a pore, the width of the pore was measured on a micrograph from random points within the pore. Additionally, the pore length (6), if in the sectioning direction, was estimated by counting the number of sections encompassing the whole pore and multiplying by the section thickness (0.1 μm) to give the sectioning diameter (in μm). The area of the GBM underlying an SPS where the exit pores were situated (A	extsubscript{GBM}) was estimated by summing all the measured lengths of GBM underlying SPS from each section and multiplying by section thickness (0.1 μm). Thus the term “width” refers to the axial direction across the face of the pore in either the vertical or horizontal direction, depending on the lesser. In our previous publication, this was described as both the width or the length of the pore (13). The length referred to here is the longitudinal axis of the pore, which was not previously measured. The height of the SPS through which fluid must pass to get to the SPS exit pores at the periphery of the podocyte was estimated at two points for each profile, each at approximately one third the way from one SPS exit pore to another. The separation between the podocyte cell body or process and the GBM or foot process lying over the GBM was measured. These SPS height measurements also included zero height when anchoring processes coincided. The pathlength for fluid from the filtration membrane through the SPS to PUS/IPS was estimated by randomly selecting 20 points on the GBM of an SPS and tracing the least distance for each point to an SPS exit pore from the three dimensional reconstruction.

**Mathematical modeling and statistics.** Mathematical models detailed in Appendix A were used to calculate actual and relative resistances. Measurements are presented as means ± SE unless otherwise stated. Significant differences were detected by parametric statistics (paired or unpaired t-test as required). To determine the power of the statistics, G-Power was used to determine effect size (d) and power (1-β) based on a probability cut-off of 5% (P < 0.05) being significant. This analysis showed that for all statistics power was >90%.
GLomerular Filtration is Restricted by the Subpodocyte Space

RESULTS

SPS dimensions. The SPS after immersion and perfusion fixation with or without colloid is extensive, covers the majority of the GBM and is convoluted and tortuous. Representative electron micrographs of serial sections from which reconstructions were made of the SPS and SEP from kidneys are shown in Fig. 2. It can be seen that the procedure mimicking the in vivo pressures (perfusion with colloid-containing solutions) shows the narrowest set of urinary spaces (Fig. 2, E and F) with all urinary space dimensions reduced. Figure 3, A–C, shows three different views of an SPS and an accompanying podocyte fully reconstructed from serial sections using Reconstruct software. The reconstruction has been pared down so that just the podocyte cell body, the GFB, and SPS can be seen. Figure 4 shows the same podocyte and SPS shown in Fig. 3, but all the neighboring podocytes and capillaries have been included. All the podocytes in this view cover an SPS (full rotating model of these structures can be seen in the online supplement). Assessment of four glomeruli from two rats (Table 1) showed that under colloid perfusion conditions the mean SPS height was 0.34 ± 0.1 μm (n = 73) from reconstructed SPS, significantly lower than during immersion fixation (mean 0.53 ± 0.03 μm, P < 0.001, n = 102 from 5 glomeruli of 3 rats, Fig. 5A). However, the distribution of SPS heights was monomodal, compared with multimodal in the absence of colloid (Fig. 5A). The SPS height showed colloid perfusion-fixed podocytes (Colloid) more closely opposed to the GFB than immersion-fixed podocytes (Immersion), i.e., Colloid, mode 0.3 μm; Immersion, modes 0.5 and 0.9 μm P < 0.001, Fisher’s exact test. Reconstructed SEPs were the same length in colloid perfusion-fixed SPS (0.25 ± 0.07 μm, n = 5 glomeruli) compared with colloid-free fixed SPS (0.21 ± 0.03 μm, n = 3 glomeruli). However, random measurements of SEP diameters showed them to be 0.15 ± 0.05 μm (n = 4 glomeruli), indicating that the SEP width was reduced by colloid perfusion compared with immersion fixation (0.33 ± 0.043, n = 6, P < 0.05). The mean pathlength through the SPS was narrowest (Colloid, 0.59 ± 0.06 μm, n = 29, P < 0.05) and PUS was narrowest (Perfused, 8.5 ± 3.4 μm; Colloid, 2.1 ± 0.35 μm, P < 0.01) when kidneys were fixed under colloid perfusion. Mathematical modeling of SPS resistances. The pressure inside the SPS will depend on the relative resistances of the

Table 1. SPS parameters under different perfusion conditions

<table>
<thead>
<tr>
<th></th>
<th>Colloid</th>
<th>Colloid-Free</th>
<th>Immersion</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>SPS height, μm</strong></td>
<td>0.34±0.1</td>
<td>0.73±0.03</td>
<td>0.53±0.03</td>
</tr>
<tr>
<td><strong>SEP length, μm</strong></td>
<td>0.25±0.07 (n = 5)</td>
<td>0.21±0.03 (n = 3)</td>
<td>ND</td>
</tr>
<tr>
<td><strong>SEP width, μm</strong></td>
<td>0.15±0.05 (n = 4)</td>
<td>0.21±0.03 (n = 5)</td>
<td>0.33±0.043 (n = 6)</td>
</tr>
<tr>
<td><strong>SPS pathlength, μm</strong></td>
<td>6.7±2.0 (n = 6)</td>
<td>ND</td>
<td>4.7±1.3 (n = 6)</td>
</tr>
</tbody>
</table>

Values are means ± SE. SPS, subpodocyte space; SEP, SPS exit pore; ND, not determined.
SPS and the GBM (13). To estimate these pressures based on a circular flow model, we calculated the relative resistances of the SPS, SEP, and GBF, as shown in Fig. 6.

**Contribution of SPS and SEP parameters to total SPS resistance.** The total resistance of the SPS was calculated for differing SPS heights and different SEP parameters. Figure 7 shows the relationship between SPS resistance and increasing SPS height and includes the effect of various lengths of exit pores with differing SEP dimensions. The SPS parameters used to generate the relationships shown in Fig. 7 are as follows. Pathlength (a; for fluid movement from the center of the SPS) was calculated as 20.1 μm (20.1 × 10^{-6} m), and the GBM thickness as 0.2 μm (2 × 10^{-7} m). The mean hydraulic conductivity of the glomerular capillary wall (k) was assumed to be 30 × 10^{-2} μm·s^{-1}·cmH2O^{-1} (3 × 10^{-4} m·s^{-1}·Pa^{-1}) (9). The number of SEP per SPS (n) was assumed to be 22 as found before (13). The reference value of $R_{GBM}$ was calculated from the specific hydraulic conductance or Darcy permeability (κ) of the GBM, 1.2 nm² (1.2 × 10^{-18} m²) (2, 3).

Figure 7A shows an electron micrograph with the relevant dimensions shown and the variables also used in Eqs. 6, 9, and 11 in Appendix A to derive Fig. 7, B and C. It can be seen in Fig. 7B that the resistance of the SPS with no exit pore restriction (i.e., SEP length δ = 0) is only sensitive to the height of the SPS <0.25 μm, i.e., below measured SPS heights (shaded region). However, the contribution of the SEP to this resistance is highly significant. Figure 7B also shows the effect of incorporating the SEP lengths (δ) on the SPS height calculations, and it can be seen that the exit pore adds significant resistance to the SPS. The total resistance of the combined SPS and SEP is $6.5 \times 10^{17}$ Pa·s·m^{-3}, when mean values for SPS height, SEP length, and SEP width are used ($h = 0.34$ μm, $δ = 0.25$ μm, $b = 0.15$ μm, Fig. 7B, arrow). This is sevenfold greater than that of the GBM alone ($0.92 \times 10^{17}$ Pa·s·m^{-3}). Figure 7B also shows that within a range of SPS heights from 0.4 to 0.15 μm the resistance of the SPS (including the SEP) is relatively unaffected by the SPS height, but a shortening of the exit pore to 0.1 μm (1 SE from the mean) results in a significant reduction in resistance of the SPS (including the SEP).

Thus inclusion of the SEP, which is a significant narrowing of the SPS as it approaches the interface between it and other urinary spaces (IPS and PUS), appeared to have a highly significant effect. Moreover, the SEP became narrower with colloid perfusion, suggesting it may regulate total SPS resistance. To determine the effects of this changing exit pore width, we have calculated the effect of the SEP length and diameter on the SPS resistance from Eq. 9b.

The effect of altering the SEP width (b) and length (δ) on the resistance of the entire SPS (including the SEP) is shown in Fig. 7C and D, for different lengths of the SEP (δ varies from 0.10 (C) to 0.25 μm (D)). It can be seen that the resistance of the SPS is highly sensitive to the width and length of the SEP. Figure 7C shows that the range of measured SEP widths encompasses the most sensitive part of the SEP resistance.
Figure 7D shows that the curves are shifted to the right by extending the length of the SEP to 0.25 μm, that measured in colloid-perfused and immersion-fixed glomeruli. Thus decreasing the SEP width from 0.18 μm (Fig. 7C, arrow) by just 30 nm to 0.15 μm (mean measured SEP width) or increasing the length by just 150 nm to 0.25 μm (Fig. 7D) results in a highly significant elevation of the resistance of the SPS, so that it far exceeds that of the GBM and is the major site of fluid resistance even in the presence of a relatively high SPS (0.4 μm).

Increased resistance of the SPS and SEP over the GFB. To determine the relative contribution of the whole of the SPS to resistance to glomerular filtration, the $R_{\text{SPS}}$ and $R_{\text{SEP}}$ combined can be expressed as a proportion of the $R_{\text{GFB}}$ (see Fig. 6). Figure 8A shows the relationship between SPS height and the relative resistance of the SPS, so that it far exceeds that of the GBM and is the major site of fluid resistance even in the presence of a relatively high SPS (>0.4 μm).

Effect of SEP on pressure and flow in the subpodocyte space. Figure 8C shows the effect of this change in relative resistance due to SEP diameter and length on the predicted SPS pressure calculated from Eq. 18A (APPENDIX A), assuming a pressure in the vasculature of 60 mmHg and the peripheral urinary space/interpodocyte space having a pressure of 20 mmHg (12). Again, and as a consequence, it can be seen that the pressure in the SPS is exquisitely sensitive to the size of the SEP. Thus at the mean height of the SPS (0.34 μm), mean length (0.25 μm), and width (0.15 μm) of the SEP, the pressure in the SPS is calculated to be 48 mmHg, or almost 30 mmHg greater than Bowman’s space. Moreover, the most sensitive part of the relationship between the SEP and the

![Fig. 5. Dimensions of the SPS after fixation under normal-, negligible-, and high-filtration conditions (colloid-perfused, immersion, and colloid-free perfused, respectively). A: SPS height was measured at random locations under the podocyte in the SPS. Data are displayed as a frequency distribution emphasizing that the colloid-perfused fixed SPS (●) has a uniform height with a near normal distribution compared with the other 2 fixation protocols [immersion (□) and colloid-free perfusion (▲)]. B: width of the exit pores measured from micrographs of SPS from colloid-perfused (CP) and immersion (I)-fixed kidneys [colloid-free perfused (P) similar to I]. *P < 0.05. C: pathlength was measured from random locations on the GFB to the nearest SPS exit pore. Colloid-free perfused (P) and immersion (I) are shown together (no difference in pathlength).
through the SPS becomes less, and the most sensitive part of the curve is again in the middle of the SEP range. At higher SEP dimensions, there is still less flow through the SPS than naked GFB ($Q_{SPS}/Q_{GFB} < 100\%$) as the resistance of the SPS contributes.

DISCUSSION

Glomerular filtration has generally been considered to be regulated by control of blood flow through the glomerulus, and the composition of the filtrate regulated by a combination of the podocyte slit diaphragms between the foot processes, the endothelial fenestrations, the GBM, and the glycoproteins on the podocytes and the endothelial cells (3). The identification in 2005 of the extent and possible significance of the subpodocyte space (13) indicated that regulation of this space by the podocyte might alter the driving pressures regulating the rate of glomerular filtration across at least a part of the GFB. That previous study, however, along with the majority of studies of glomerular ultrastructure, were carried out under conditions of abnormal glomerular perfusion. The SPS component was shown to be altered when two abnormal conditions were compared, high filtration and no filtration pressures. This indicated that the podocyte could respond to changes in filtration, either by detecting pressure differences or by flow rates. The mechanisms by which podocytes can respond to changes in pressure or flow are not yet clearly determined, but there is increasing evidence that podocytes do respond to altered pressure or flow (11, 16). The results described here, however, show that under normal perfusion conditions, the SPS assumes...
characteristics that place the podocyte in an ideal position to exquisitely regulate the resistance. Here we show that the resistance of the SPS relative to the GFB can be fine tuned by slight alterations in the SPS width or length, which would move the pressure in the SPS (and therefore that opposing filtration) from almost the same as that in the peripheral urinary (Bowman’s) space (proximal convoluted tubule pressure; when SPS $h$ is $>0.4 \mu m$ and SPS $b$ is $>0.2 \mu m$) to a pressure that is closer to capillary pressure (SEP $b = 0.10 \mu m$). Furthermore, under conditions of physiological perfusion pressures, the width of the SEP sits between these two boundary points.

When we first described the SPS, we made estimates of the increase in resistance due to the SPS compared with uncovered areas based on deliberately simplified assumptions and calculated what we indicated to be a gross overestimate of the relative resistance of the SPS. The current modeling allows us to make more detailed estimates of the increase in resistance for fluid traveling through the GFB and the SPS relative to that traveling through the GFB alone. The relative values shown in Fig. 8 indicate that the parallel resistance through the SPS, i.e. $(R_{SPS} + R_{SEP} + R_{GFB})/R_{GFB}$, varies from 1.34 when the SPS is large ($0.4 \mu m$) and the SEP loose ($0.1 \mu m$ long $\times 0.2 \mu m$ wide) to 26 when the SPS is small ($0.15 \mu m$) and the SEP is tight ($0.5 \mu m$ long $\times 0.1 \mu m$ wide). Although this is obviously much lower than the 900- to 14,000-fold based on the over-simplified assumptions stated in our previous paper (13), it does indicate that glomerular filtration through the SPS is between 75 (loose SEP) and 4% (tight SEP) of that through uncovered regions, with a mean of 29%.

**Problems with the model.** The model we have assumed makes assumptions that need to be addressed. This model assumes that the height of the SPS is uniform along the SPS. The new modeling described here allows the calculation of fluid flows into the SPS incorporating the increase in flow rate along the SPS from a “center of gravity” at which point flow is 0 (the position at which the resistances of that point in the SPS to its nearest point are identical). However, it is clear from the figures discussed above that the SPS height is not uniform along the vessel wall. Current sampling estimates described here have not identified where the narrowest parts of the SPS are but have estimated the height from random positions in the SPS. It is clear that under conditions of high filtration for instance, there is a significant reduction in height of the SPS at areas closer to the SEP and ballooning of the SPS in the center, where resistance is less affected by SPS height. Thus the SPS geometry is ideally suited to detecting changes in flow, in that
the areas of highest shear stress (close to the SEP) are the exact areas where control of SPS height is most sensitive for flow regulation. There is a significant nonnormal skew of the SPS size distribution [mean range of relative areas to GFB (0.2–8%)] under immersion conditions, whereas the SPS characteristics all appear more uniform under colloid-perfused conditions. One result of this is that the mean calculated area of SPS relative to the GFB in the model (0.16%) is at the lowest part of the range seen in immersion-fixed glomeruli.

Second, it was modeled that fluid approaching the exit pore could exit anywhere along the outer boundary of the SPS (at $r = a$). Since the exit pores occur discretely as a result of the arrangement of the anchoring processes along the edge of the SPS, there is a further restriction to flow. In other words, channeling caused by the discrete nature of the exit pores was not incorporated. This further reinforces the argument that the resistances calculated here are on the conservative side.

Finally, we have assumed that flow through the SEP is laminar and follows Poiseuille’s principles of resistance and flow, and that the SEP is cylindrical. Previous studies have shown that there is a substantial glycocalyx on the urinary side of the GBM, overlying the podocytes (7, 8, 14). In an examination of published information on cryofoxed glomeruli showing preservation of a podocyte glycocalyx, the glycocalyx was ~70 nm high, extending away from the podocyte plasma membrane (5, 10, 17, 18) and coats both the lower and upper surfaces of what appears to be the subpodocyte space, cell body, and foot processes. This means that the true SEP width in terms of laminar flow properties could be up to 140 nm (0.14 $\mu m$) less than that calculated, and hence in many cases resulting in Darcy flow rather than laminar Poiseuille flow, as the SEP may be almost, or completely, filled with such a glycocalyx, which has a gel-like structure. The conductance of the podocyte glycocalyx is not known, but the conductivity of the endothelial glycocalyx is 6 nm$^2$ (1), or about five times that of the GBM. There are no conductance studies on the glycocalyx of the podocyte. In addition, the SEPs are not cylindrical but have an elliptical or even c-shaped entrance and may vary their diameter, narrowing in the middle, and opening out again at the nanometer scale, within the pore described here. The geometry of such a pore is particularly difficult to model without further reconstruction or random sampling of such pores and awaits further investigation.

**Effect of SPS resistance on measured glomerular permeability.** The impact of SPS resistance on our understanding of glomerular physiology can be illustrated by calculating the effect of the SPS on the glomerular permeability measurements previously carried out, notwithstanding the above caveats. It has been assumed during all measurements of the permeability of the GFB that the GFB had homogenous permeability properties throughout the glomerulus. Previous estimates have shown that the SPS covers ~60% of the total glomerular area. The results described here indicate that previous estimates of glomerular permeability have significantly underestimated the true permeability of the GFB. The extent of this underestimation can be estimated using Eq. 15 in Appendix A. Figure 9 shows the relationship between SPS area coverage and glomerular permeability with the SPS relative to that with no SPS included. As the SPS area coverage increases, the underestimate of true glomerular permeability in previous models becomes more significant, such that for typical measured values, and a surface area coverage of 60%, the permeability is underestimated by 57%; i.e., the true permeability is almost double that previously calculated.

In summary, we show here that the SPS, which covers >60% of the glomerular filtration surface, has a resistance that can be similar to, or greater than, the GFB that lies underneath it. The resistance through the SPS can be altered by subtle changes in the width and length of the SEP, regulating pressure downstream of the GFB.

**GLOSSARY**

**List of Symbols with SI Units**

- $a$: Radius of subpodocyte drainage area (m)
- $b$: Diameter of exit pore (m)
- $B = (R_{SPS} + R_{SEP})/R_{GFB}$
- $G$: Dimensionless parameter defined in Eq. 7
- $h$: Height (thickness) of SPS (m)
- $I_{nl}(x)$: Modified Bessel function of first kind of order $m$
- $k$: Hydraulic permeability of capillary wall (endothelium plus GBM; m$^3$·s$^{-1}$·Pa$^{-1}$)
- $l$: Thickness of GBM (m)
- $n$: Number of exit pores per SPS
- $P(r)$: Local radial pressure in SPS (Pa)
- $P_{BS}$: Pressure in remainder of Bowman’s space, i.e., IPS and PUS spaces (Pa)
- $P_{CAP}$: Net intracapillary pressure, hydraulic minus oncotic (Pa)
- $P_{SEP}$: Pressure at upstream end of exit pore (Pa)
- $P_{SPS}$: Mean pressure in SPS (Pa)
- $Q$: Flow rate through one SPS (m$^3$·s$^{-1}$)
- $r$: Radial position in drainage area of SPS (m)
- $R_{GFB}$: Flow resistance of capillary wall (endothelium plus GBM plus filtration slits; Pa·s·m$^{-3}$)
- $R_{GBM}$: Flow resistance of bare GBM (Pa·s·m$^{-3}$)
- $R_{SEP}$: Flow resistance of n exit pores (Pa·s·m$^{-3}$)
- $R_{SPS}$: Flow resistance of SPS (Pa·s·m$^{-3}$)
- $U(r)$: Mean radial velocity in SPS, averaged over channel height (m·s$^{-1}$)

Fig. 9. SPS resistance results in a significant underestimation of the true permeability of the GFB. If there was no SPS, the measurements of GFB permeability are correct ($K_{tot}/K_1 = 1$). However, due to the relative flow through the SPS being subjected to an increased resistance, a mean fractional area coverage of 60% would indicate that the permeability of the GFB is only half that without an SPS; thus it would be underestimated by 2-fold ($K_{tot}/K_1$ of 0.54, vertical arrow). If the entire glomerulus was covered by SPS at the typical dimensions, the permeability would be underestimated by 76% ($K_{tot}/K_1$ of 0.24, horizontal arrow).
**APPENDIX A: RESISTANCE CALCULATIONS**

**Pressure-Resistance Relationships**

Any filtrate that traverses the subpodocyte space (SPS) must cross the glomerular filtration barrier (GFB), flow through the SPS, and pass through one or more exit pores (SEP) into Bowman’s space (BS; in this study equivalent to the remainder of the urinary space, i.e., the IPS and PUS). Here, GFB means the endothelium, the glomerular basement membrane (GBM), and the podocyte slit diaphragms. The overall driving pressure for filtration is \( P_{\text{CAP}} - P_{\text{BS}} \), where \( P_{\text{CAP}} \) is the mean intracapillary pressure (hydrostatic minus oncotic), and \( P_{\text{BS}} \) is the pressure in Bowman’s space. Intermediary between \( P_{\text{CAP}} \) and \( P_{\text{BS}} \) are the average pressure in the SPS and the pressure at the upstream end of the SEP (or outlet of the SPS), denoted as \( P_{\text{SPS}} \) and \( P_{\text{SEP}} \), respectively. In summary, under conditions of flow from the capillary to the SPS, \( P_{\text{CAP}} > P_{\text{SPS}} > P_{\text{SEP}} > P_{\text{BS}} \). The intermediate pressures are determined by the relative flow resistances upstream and downstream from the given location. Let \( R_{\text{GFB}} \) be the resistance of the GFB (including endothelium, basement membrane, and podocyte slit diaphragm), \( R_{\text{SPS}} \) that of the SPS, and \( R_{\text{SEP}} \) that of the exit pores from the SPS. If the flow rate through a representative SPS is denoted as \( Q \), the resistances are defined such that

\[
Q = \frac{P_{\text{CAP}} - P_{\text{SPS}}}{R_{\text{GFB}}} = \frac{P_{\text{SPS}} - P_{\text{SEP}}}{R_{\text{SPS}}} = \frac{P_{\text{SEP}} - P_{\text{BS}}}{R_{\text{SEP}}}
\]  

(1)

It follows that the pressures and series resistances are related as

\[
\frac{P_{\text{CAP}} - P_{\text{SPS}}}{P_{\text{CAP}} - P_{\text{BS}}} = \frac{R_{\text{GFB}}}{R_{\text{GFB}} + R_{\text{SPS}} + R_{\text{SEP}}} \tag{2a}
\]

\[
\frac{P_{\text{SPS}} - P_{\text{SEP}}}{P_{\text{SPS}} - P_{\text{BS}}} = \frac{R_{\text{GFB}}}{R_{\text{GFB}} + R_{\text{SPS}} + R_{\text{SEP}}} \tag{2b}
\]

\[
\frac{P_{\text{SEP}} - P_{\text{BS}}}{P_{\text{SEP}} - P_{\text{CAP}}} = \frac{R_{\text{SPS}}}{R_{\text{GFB}} + R_{\text{SPS}} + R_{\text{SEP}}} \tag{2c}
\]

These expressions give the fraction of the overall pressure drop that occurs in each segment of the flow path. If the external pressures (\( P_{\text{CAP}} \) and \( P_{\text{BS}} \)) and the three resistances are specified, then \( R_{\text{SPS}} \) and \( R_{\text{SEP}} \) can be found from Eq. 2. The evaluation of the resistances is discussed next.

**\( R_{\text{GFB}} \)**

In estimating the resistances of the GFB and SPS, we modeled the latter as a circular region of radius \( a \) and height (or thickness) \( h \), as shown in Fig. 6. Cylindrical coordinates \((r, z)\) were used, with \( z = 0 \) corresponding to the lower boundary of the SPS (downstream edge of GFB) and \( z = h \) the podocyte surface that is the upper boundary. The outer edge of the idealized SPS was defined by \( r = a \). It was assumed that fluid can enter the SPS by crossing the GFB over the entire circular area. Accordingly, the flow rate into the SPS equals the area of the circle times the average filtrate velocity (or volume flux). 

\[
Q = \frac{\pi a^2 h}{128 \mu b} \left( P_{\text{SPS}} - P_{\text{BS}} \right)
\]  

(8)

and the total resistance of the exit pores is

\[
R_{\text{SEP}} = \frac{128 \mu \delta}{\pi n b^4}.
\]  

(9)

The resistance of the SPS including the SEP is then

\[
R_{\text{SPS}} + R_{\text{SEP}} = \frac{6 \mu h}{\pi h} \frac{I_0(G)}{I_1(G)} - \frac{1}{\pi a^2 k} + \frac{128 \mu \delta}{\pi n b^4}.
\]  

(10)
If the GBM has thickness \( l \) and Darcy permeability \( \kappa \), and its surface is fully exposed (i.e., not partly blocked by cells), then its hydraulic permeability is \( \kappa \mu l \). Accordingly, by analogy with Eq. 4, the resistance of bare GBM is

\[
R_{\text{GBM}} = \frac{\mu}{\pi \alpha' \kappa}
\]  

(11)

This provides another convenient scale for judging the resistances of the SEP and exit pores.

**Relative Resistances**

From Eqs. 4, 6, and 7, the resistance of the SPS relative to that of the underlying portion of the GFB is

\[
\frac{R_{\text{SPS}}}{R_{\text{GFB}}} = \frac{G_2(G)}{2l_1(G)} - 1.
\]  

(12)

From Eqs. 4 and 9, the relative resistance of the SEP is

\[
\frac{R_{\text{SEP}}}{R_{\text{GFB}}} = \frac{128\mu \delta \kappa a^2}{nb^4}.
\]  

(13)

This resistance refers only to the part of the GFB that underlies the SPS under consideration and assumes that the downstream resistances of the IPS and PUS are negligible. Thus the extent to which a given SPS increases the resistance already provided by the GFB is found by adding Eqs. 12 and 13, which gives

\[
\frac{R_{\text{SPS}} + R_{\text{SEP}}}{R_{\text{GFB}}} = \frac{G_1(G)}{2l_1(G)} - 1 + \frac{128\mu \delta \kappa a^2}{nb^4} = B
\]  

(14)

Certain subsequent equations are shortened by denoting this resistance ratio as \( B \), as shown by the last equality. Resistances were calculated in \( \text{Pa} \cdot \text{s} \cdot \text{m}^{-1} \). Estimates of all these quantities are provided in Results.

Areas of the capillary wall that are either covered or not covered by the SPS provide two parallel pathways for filtration. To assess the effect of partial SPS coverage on the overall hydraulic permeability (\( \kappa_{\text{TOT}} \)), let \( Q_1, k_1, \) and \( A_1 \) be the filtration rate, hydraulic permeability, and area of the uncovered part and \( Q_2, k_2, \) and \( A_2 \) the corresponding quantities for the covered part. Adding the flows through the two pathways gives

\[
Q_{\text{TOT}} = (k_1A_1 + k_2A_2)(P_{\text{CAP}} - P_{\text{BS}}) = k_{\text{TOT}}A(P_{\text{CAP}} - P_{\text{BS}})
\]  

(15)

where \( Q_{\text{TOT}} = Q_1 + Q_2 \). It follows that

\[
k_{\text{TOT}} = \frac{(1 - \varepsilon)k_1 + \varepsilon k_2}{1 - \varepsilon}
\]  

(16)

where \( \varepsilon = A_2/A_1 \) is the fractional area covered by subpodocyte spaces. Because hydraulic permeabilities are inversely proportional to resistances, \( k_2/k_1 = R_{\text{GFB}}/(R_{\text{GFB}} + R_{\text{SPS}} + R_{\text{SEP}}) \). A rearrangement of Eq. 16 then gives

\[
k_{\text{TOT}} = k_1 - \frac{\varepsilon B}{1 + B}.
\]  

(17)

Thus the effect of partial SPS coverage on the overall hydraulic permeability can be estimated from the area fraction (\( \varepsilon \)) and the resistance ratio given in Eq. 14.

**SPS Height**

The true height of the SPS for resistance purposes, presumably is the cumulative sum of the resistances all the way along its length, so as resistance relating to \( h^2 \)

\[
R = \sum 1/h_1^3 = (1/h_1^3 + 1/h_2^3 + 1/h_3^3 + \ldots + 1/h_n^3)
\]

Therefore, \( h \) was calculated as the sum of the reciprocals of the measured height cubed from locations along the SPS. The resistance of the exit pores of the SPS is in series to that of the SPS itself and therefore can be calculated by addition to the resistance of the SPS.

**\( P_{\text{SPS}} \)**

The pressure in the SPS can be calculated from

\[
P_{\text{SPS}} = \frac{BP_{\text{CAP}} + P_{\text{BS}}}{B + 1}
\]  

(18a)

which is obtained by adding Eqs. 2b and 2c and rearranging.

The relative flow between the SPS and the free GFB \( (Q_{\text{SPS}}/Q_{\text{GFB}}) \) were calculated according to Darcy’s law

\[
\frac{Q_{\text{SPS}}}{Q_{\text{GFB}}} = \frac{(P_{\text{CAP}} - P_{\text{BS}})(P_{\text{CAP}} - P_{\text{BS}})}{(R_{\text{SPS}} + R_{\text{SEP}} + R_{\text{PB}})R_{\text{GFB}}}
\]  

(18b)

**Calculation of Distance to Perimeter of SPS**

The fluid pathlength to the exit pore was calculated by random sampling along the GBM underneath the SPS. For the model, the SPS was assumed to be circular in the top view. To calculate the distance from the center of the SPS to the exit pore (\( a \)), the probability of choosing a point with a radial position between \( r \) and \( r + dr \) on a circle equals the differential area between those positions \( (dA = 2\pi r dr) \) divided by the total area of the circle \( (A = \pi R^2) \). That probability times the distance to the perimeter \( (R - r) \), integrated over \( r \), gives \( R/3 \) as the expected value of the pathlength. Thus for the purposes of the model the SPS radius (\( a \)) is simply three times the randomly derived mean pathlength.

**APPENDIX B: MODEL FOR FLOW IN SPS**

This derivation of the pressure-flow relationships in the SPS is based on the geometric idealization in Fig. 2. Employing the lubrication approximation to the Navier-Stokes equation (2), the fluid pathlength to the exit pore was calculated by random sampling along the GBM underneath the SPS (13). For the model, the SPS was assumed to be circular in the top view. To calculate the distance from the center of the SPS to the exit pore (\( a \)), the probability of choosing a point with a radial position between \( r \) and \( r + dr \) on a circle equals the differential area between those positions \( (dA = 2\pi r dr) \) divided by the total area of the circle \( (A = \pi R^2) \). That probability times the distance to the perimeter \( (R - r) \), integrated over \( r \), gives \( R/3 \) as the expected value of the pathlength. Thus for the purposes of the model the SPS radius (\( a \)) is simply three times the randomly derived mean pathlength.
or, using Eq. 5 to evaluate \( v_r(r) \),

\[
\frac{d}{dr}(rU) = \frac{rv_v}{h}
\]  

(23)

This is the second of the two differential equations that are needed to determine \( U(r) \) and \( P(r) \).

Equations 21 and 24 can be uncoupled and solved sequentially as follows. Rearranging Eq. 21 to provide an expression for \( dP/dr \), multiplying by \( r \), and differentiating gives

\[
\frac{d}{dr}\left( rU \right) = -\frac{12\mu r^2}{h^2}(P_{CAP} - P).
\]  

(25)

This can be rearranged as

\[
\frac{1}{r} \frac{d}{dr}\left( rU \right) - \gamma (P - P_{CAP}) = 0
\]  

(26)

which is known as the modified Bessel equation of order 0. The coefficient in Eq. 26 is defined as

\[
\gamma = \left( \frac{12\mu k}{h^2} \right)^{1/2}.
\]  

(27)

The solution to Eq. 26 that gives a finite pressure at \( r = 0 \) and a pressure at the outer radial boundary of \( P(a) \), i.e., at the entrance to the SEP = \( P_{SEP} \) is

\[
P(r) - P_{CAP} = \frac{I_0(\gamma r)}{P_{SEP} - P_{CAP}} \frac{I_9(\gamma a)}{I_0(\gamma a)}
\]  

(28)

where \( I_0 \) is the modified Bessel function of the first kind of order 0.

Having solved for \( P \), the radial pressure gradient, local mean velocity, and total flow rate are evaluated as

\[
\frac{dP}{dr}(r) = -\frac{\gamma I_1(\gamma r)}{I_0(\gamma r)}(P_{CAP} - P_{SEP})
\]  

(29)

\[
U(r) = -\frac{h^2}{12\mu} \frac{\gamma I_1(\gamma r)}{I_0(\gamma r)}(P_{CAP} - P_{SEP})
\]  

(30)

\[
Q = 2\pi h U(a) = \frac{\pi a h^3}{6\mu} \frac{I_0(\gamma a)}{I_1(\gamma a)}(P_{CAP} - P_{SEP})
\]  

(31)

where \( I_1 \) is the modified Bessel function of the first kind of order 1. Finally, the combined resistance of the capillary wall and SPS is found from Eq. 31 to be

\[
R_{GFB} + R_{SEP} = \frac{6\mu I_0(\gamma a)}{\pi h^2 G_1(\gamma a)}
\]  

(32)

where \( G = \gamma a \). Subtracting \( R_{GFB} \) (from Eq. 4) leads to Eq. 6.

GRANTS

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