Structure of the glomerular filtration barrier in the kidney

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INTRODUCTION
The University of Copenhagen's Core Facility for Integrated Microscopy (CFIM) offers a wide range of state-of-the-art light and electron microscopes for users of all levels of experience and from any discipline.

In the past, light and electron microscopy have been physically separated with little interaction between disciplines. At CFIM the two disciplines are combined, encouraging inter-disciplinary microscopy approaches to scientific questions. A number of the users are clinical pathologists from the two major hospitals in Copenhagen. In fact, the need of local pathologists for better quality at lower cost was an important driver for establishing a core facility. Approximately 20% of the current sample volume is clinical pathology.

CFIM's ability to maintain a suite of high-end instruments tuned for optimal performance, combined with a highly trained staff, provides local clinicians with timely, high-quality results at a lower cost than they could achieve with in-house facilities.

The overwhelming majority of clinical samples processed in our unit are kidney tissue. As a medical doctor I have a particular interest in the clinical aspects of this work and this interest lead to a recent publication. The study provides an excellent example of the power of integrating multiple microscopy techniques over a range of spatial scales to provide a more complete understanding of critical structures within the sample.

Filtration of fluid and solutes in the kidney occurs through the renal glomerulus (Figure 1), a structure in the renal cortex consisting of a knot of microvessels sitting between afferent and efferent arterioles. The glomerular filtration barrier (GFB) allows small solutes to pass out of the vessels into the urinary system where they are collected and ultimately excreted. At the same time, it retains most of the larger plasma proteins (e.g. albumin) within the vasculature, thus maintaining the colloid osmotic pressure of the plasma and enabling fluid retention in the rest of the body.

In classical descriptions, the GFB consists of three layers—the podocyte foot processes, the basement membrane and the fenestrated endothelium. In recent years however, two additional layers to the GFB have been described. On the vascular side, a fine meshwork of glycosaminoglycans covers the endothelial layer, including the fenestrations. This glyocalyx contributes to the permeability properties of the GFB. On the urinary side the fluid passes through the filtration slits into an area under a podocyte cell body called the subpodocyte space (SPS). This area is bounded by the podocyte cell body on one side and the podocyte foot processes on the other. Along with...
FIGURE 2 SBF-SEM contrast imaging of glomerular glyocalyx. A: Section sequence (every 75 μm) from raw data stack (71 μm x 71 μm x 84 μm). The LaDy GAGs stained components can be seen as dark regions adjacent to the lower contrast of a conventionally processed TEM resin block. B: An inverted contrast image sequence (every 40°) of a 3D surface projection of the same Image Stack as above. C: The 3D surface projection has been intensity thresholded to reveal the LaDy GAGs (glyocalyx) stained vessels and structures. Scale Bars = 20 μm in length.

the SPS there are two further types of urinary space: the interpodocyte space (IPS, between podocyte cell bodies) and the peripheral urinary space (PUS) between the glomerular edge and the walls of the glomerular capsule. Narrow exit pores between the SPS and IPS and the PUS restrict flow away from the GFB.

The current view on the shape and position of the urinary spaces inside the renal glomerulus assumes that the major resistance to fluid flow occurs at the classical three layered GFB. However, there is mounting evidence that podocyte cell bodies and processes which cover a substantial portion of the GFB make a SPS that restricts flow and changes in size depending on the pressure applied to the glomerular capillaries. Also, the SPS shows an ability to retain macromolecules and alter fluid flowing through it.

The SPS was first identified in 1950, re-described in 1965 and analyzed in detail more recently. Mathematical modeling has shown that the SPS can act as a resistance to fluid flow, and more recently that the increase in fluid exchange induced by vascular endothelial growth factor over-expression across the glomerulus can be explained, at least in part, by a reduction in the SPS coverage of the GFB that reveals a greater free surface area of the GFB for unrestricted fluid filtration.

A detailed analysis of the SPS from 6 glomeruli of 4 rats showed that 50-60% of the GFB was covered by SPS. The IPS conduits inside the glomerulus feed filtrate away from the 'free' GFB or from an SPS exit region, and the peripheral area that collects filtrate from the glomerular edge forms the PUS (though this has very narrow dimensions when the vascular oncocytic and hydrostatic pressures used at perfusion have physiological values). However, these definitions are only as good as the serial sectioning and reconstruction techniques will allow. Larger runs of serial sections allow a better appreciation of glomerular structural relationships.

To date only one complete SPS has been reconstructed with conventional transmission electron microscopy (TEM) sectioning and reconstruction, using 120 sections through parts of a rat glomerulus.

All other data have been reported on lower numbers of sections of incomplete SPS. Longer sectioning runs are needed to gain a better appreciation of the ultrastructure, but they are time consuming, labor intensive, technically difficult, and expensive.

Recent advances in EM instrumentation, particularly in the areas of three-dimensional microscopy and automated sample preparation, now offer a chance to address these limitations.

NEW METHODS FOR GREATER INSIGHT

Many of the same considerations apply to investigating the endothelial glyocalyx at much higher magnification. At the larger scale, single EM sections of the glyocalyx cannot provide details of the overall glyocalyx coverage of the endothelial cells. Nor can they show how the glyocalyx sits over the fenestrations, the relationship with the underlying foot processes, or whether the glyocalyx coverage is similar in different areas of the glomerulus.

At the finest scale, the detailed architecture of the glyocalyx requires three-dimensional imaging of the fiber matrix network that provides resistance to flow in both the horizontal (en face to the endothelial surface) and vertical (from the lumen through to the endothelial surface or pore) directions.

Three EM techniques hold promise for expanding our understanding of glomerular structure and function. Serial block face scanning electron microscopy (SBF-SEM - Teneo VolumeScope, FEI) images the surface of a resin embedded tissue block, then uses an inbuilt diamond knife to remove a thin layer (30-100 nm) from the block surface (Figure 2). By repeating the imaging/sectioning process, it creates a stack of images.
FIGURE 4 Tomographic reconstruction of rat glomerular capillary glycocalyx using LaDy GdCa staining on a 300 nm thick section. A-D: raw transmission electron microscopy at -60°, 30°, 30°, and 60° respectively. E-H: reconstructed images of glycocalyx in cross-section 30 nm apart. There is the appearance of a gap between the glycocalyx (G) and the endothelial cell (E). Small holes can be seen throughout G; these are gaps between the fibres. Boxes are examples of areas used for spacing analysis. I-L: are the reconstructed viewed perpendicular to the membrane. I: is at the top of the glycocalyx, J: is 70 nm from the top. K: 140 nm from the top and L: is within the endothelial layer (fenestrations can clearly be seen). M-O: are still from the 3D projection. A-L scale bar is 300 nm. M-O: scale bar is 150 nm. Labels: Cap = capillary lumen, Gly = glycocalyx, Fen = fenestration, GBM = glomerular basement membrane, SPS = subpodocyte space, Slt = slit diaphragm (the diaphragm is discernable). Pod = podocyte (foot process). En = endothelial cell.

from which it can reconstruct 3D structure.

Typically, the technique uses the backscattered electron signal for imaging, thus generating atomic number contrast while reducing the sensitivity to any residual topography on the sectioned surface. Inverting the backscattered grey-scale values results in image contrast that is similar to the mass thickness contrast revealed by imaging the transmitted amplitude in a TEM

Serial block face imaging in SEM can be compared to serial sectioning in a TEM, and though it is relatively low resolution (in TEM terms) it can be undertaken automatically and without operator interaction. Conventionally, the depth resolution of SFSEM has been limited by the minimum thickness of the layers removed between images. Recently, software algorithms have been developed that combine multiple images acquired over a range of accelerating voltages (penetration depths) at each layer to derive depth information within the layer.

Focused ion beam scanning electron microscopy (FIB-SEM - Quanta FEG 3D, FEI) is similar to SFSEM, except that the removal of the surface layer between images is performed by the sputtering action of a focused ion beam rather than the mechanical cutting of a microtome knife (Figure 3).

FIB can remove layers of precise thickness as thin as 10 nm, giving FIB-SEM a depth resolution that is roughly equivalent to its xy resolution. This improvement in depth resolution is further enhanced by the absence of artifacts associated with surface charging and mechanical sectioning in SFSEM. As with SFSEM, FIB-SEM can be completely automated and performed without operator intervention.

Finally, transmission electron tomography (Tom-TEM - Tecnai G2 20 TWIN, FEI) can be used to reconstruct a 3D model (Figure 4 and 5). The reconstruction process can be compared to the mental process we go through when we turn something over in our hand to get a better idea of its overall shape. In tomography, 2D images are acquired from different perspectives as the object is physically rotated in precisely determined increments. A computer combines the 2D images using a technique known as back projection to reconstruct the object's 3D structure. It is the same reconstruction technique used in medical imaging technologies such as X-ray CAT scans and MRI. Tom-TEM can create models of -300 nm thick tissue sections with nanometer scale resolution in all directions.

SUMMARY AND CONCLUSIONS

We compared three complementary techniques of 3D electron microscopy in kidneys derived from the same animal to investigate their potential for resolving the three-dimensional organization and structures of the glomerulus. Additionally, a human kidney is used to demonstrate SFSEM. The examples illustrate the potential power of integrating multiple microscopy techniques over a range of spatial scales to provide a more complete understanding of critical structures within the sample.

REFERENCES

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ABSTRACT

Understanding the structure and function of the glomerulus, the filtering element of the kidney, requires imaging and 3D modeling capability over a range of size scales. Conventional techniques can be difficult to execute and time-consuming. Recent advances in automated electron microscopy address the scale, difficulty and speed concerns, making large-scale, high resolution 3D modeling practical and leading to a better understanding of glomerular function in both a fundamental and clinical context.

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