Scanning electron microscopy of thin sections: A technique to overcome limitations in kidney ultrastructural research

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Introduction: Changes in kidney function are often related with changes in tissue ultrastructure. Here, transmission electron microscopy (TEM) is still an important technique in research and diagnosis, permitting superior resolution and an "open view" analysis [1]. Access to TEM, however, is limited by expensive equipment and time-consuming steps in preparation, documentation and examination. As a result, ultrastructural studies in kidney research often suffer from poor image quality and limited reproducibility. New techniques in scanning electron microscopy (SEM) offer advantages in preparation and examination of thin sections. We studied the potential of SEM to overcome these limitations in kidney ultrastructural research.

Methods: Mice and rats were fixed by retrograde infusion of formaldehyde containing fixatives, allowing tissue post processing for immunohistochemistry (IHC) and – after postfixation with glutaraldehyde containing fixatives – epon embedding. Improved ultrastructural preservation of podocytes and proximal tubules was achieved by adaption of vehicle osmolarity to 200 mOsmol. Membrane contrast was improved by reduced osmication. Thin sections were placed on conventional grids for TEM or on conductive substrate (carbon coated glass slides or silicon wafers) for SEM and stained with heavy metals. For TEM, imaging was done using a "Zeiss 906". For SEM, imaging was done using a "Zeiss Sigma" (single frames) and a "Zeiss Ultra" (large-scale stitched datasets).

Results: Thin sections were collected onto glass or silicon substrate resulting in drastically reduced formation of artifacts such as wrinkles and precipitates, permitting digitization of large structures such as entire glomeruli. Large-scale stitched datasets offered a coherent "Google earth"-like view from low to high magnification, preserving renal micro-ultra correlation. Cortical cells were well resolved in overview and showed distinct membranes at high resolution detail. Compared to TEM, image quality in SEM was equal or superior across a wide range of magnification. At very high magnification, image acquisition by SEM was limited by scanning artifacts due to vibration.
Such datasets may be utilized for virtual observation of ultrastructure – independent of an EM.

Conclusions: We have produced high quality thin sections of kidney tissue for SEM and performed imaging for single frames and large-scale stitched datasets. Limitations of TEM, especially artifacts caused by instable preparation and small fields of view, were mostly overcome. We produced high quality images with low cost and effort as compared to TEM. This kind of preparation and digitization could further be accelerated using high throughput workflows and multibeam technology [3], allowing turnaround times (TAT) of 6-24 hours for up to 200 samples. As a result of this approach, EM – facultatively combined with light microscopy (CLEM) – should be re-evaluated as a method in biomedical applications, especially in nephrology.

References

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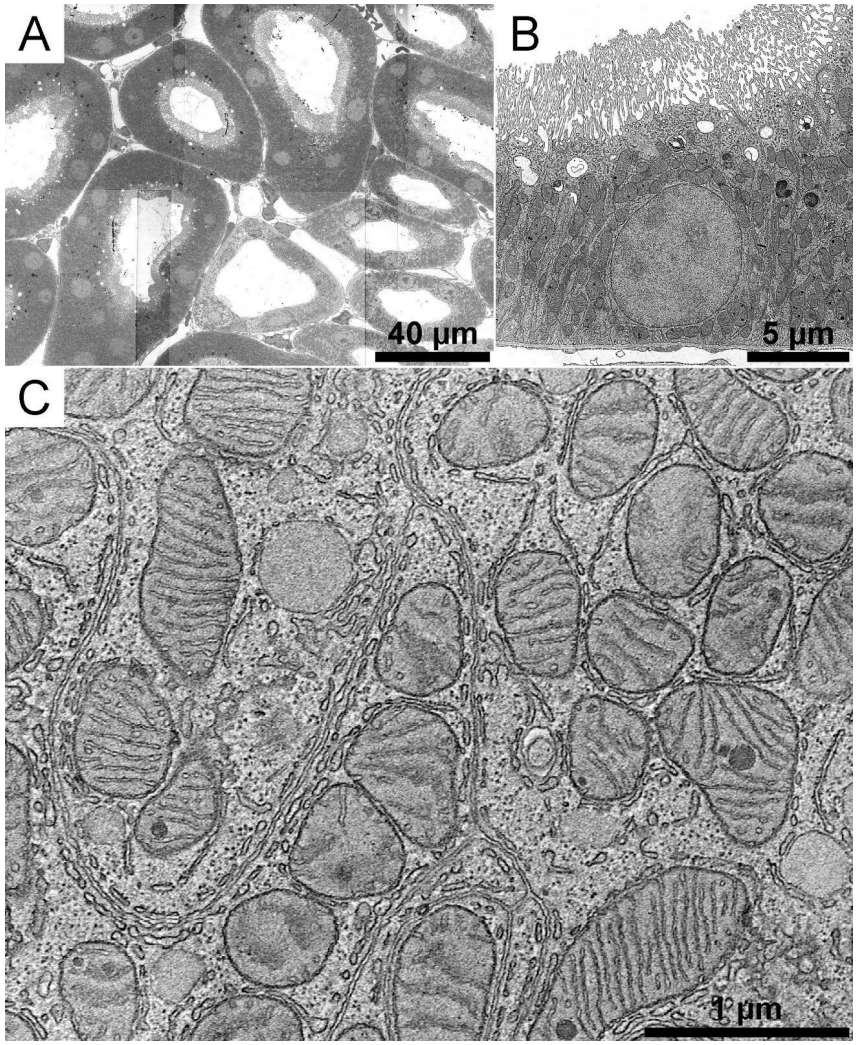

Figure 1. SEM-images, recorded from a 50 nm thin section presenting large-scale stitched dataset (A,B) and single frame (C). Overview of kidney cortex, 33350 x 24649 pixel covering an area of about 170 x 125 µm (A). Detail from that dataset showing one proximal tubular epithelium cell (B). Cellular detail from a proximal tubule (C): Morphology appears similar to TEM with well-resolved membraneous organelles such as mitochondria and lysosomes. Cellular membranes are well defined and show high contrast over electron lucent cytoplasm.