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LS2-O-3 Volume electron microscopy of intracellular compartments in the urinary bladder epithelium

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Introduction: Volume electron microscopy (vEM) provides crucial information about the distribution and spatial interactions of intracellular compartments within the cells, which helps us to better understand their functions in health and disease. Several vEM methods (e.g., electron tomography – ET, focused-ion beam—scanning electron microscopy – FIB-SEM, serial block-face—scanning electron microscopy – SBF-SEM) are available that differ in mechanical requirements, sample preparation, and in volume-to-resolution aspects.
Urinary bladder epithelium (urothelium) is a highly specialized 3-layered tissue that provides blood-urine permeability barrier and is frequently used as a research model to study polarized membrane trafficking [1]. During urothelial differentiation, membrane compartments (e.g. Golgi apparatus – GA) in superficial urothelial cells are reorganized, which leads to the assembly of ER-derived transmembrane proteins termed uroplakins into urothelial particles, and hundreds of particles into urothelial plaques [2]. Two urothelial plaques line a urothelium characteristic fusiform vesicle, which transport plaques to the apical plasma membrane, where they provide a molecular basis for the permeability barrier.
Our aim was to define spatial relationships between intracellular compartments involved in the formation and transport of urothelial plaques using vEM methods.

Methods: Urinary bladders of adult mice were cryo-fixed with an EM CPC metal mirror or EM ICE high-pressure device (both Leica), freeze-substituted in AFS (Leica) with 2% OsO_4 in acetone, and embedded in Epon. ET was performed on 300 nm thick serial sections with a Tecnai 20 (FEI), running at 200 kV. Tomograms covered angles from +65˚ to -65˚ in 1˚ steps. FIB-SEM analysis was done in Helios NanoLab 650 (FEI). The dimensions of pixels were x=5.49 nm, y=5.49 nm, z=15.0 nm. For SBF-SEM samples were fixed in 4% FA + 2% GA in 0.1 cacodylate buffer, followed by incubations in ferrocyanide reduced OsO_4, thiocarbohydrazide-OsO_4 liganding and subsequent uranyl acetate and *en bloc* lead aspartate staining following the protocol of Deerinck *et al* [3]. Manual segmentation and modelling were done with IMOD software. For the purpose of developing automatic segmentation pipelines and their evaluation, intracellular compartments were manually annotated in 5 FIB-SEM sub-volumes of size 256×256×256 voxels. Convolutional neural network-based protocols for automatic segmentation of mitochondria, GA, FVs, and endolysosomes from FIB-SEM data and the annotated training dataset (UroCell) we published on Github [4].

Results: Results of ET showed that GA contains an extensive tubule-vesicular cis- and trans-GA network surrounding large GA cisternae [5]. Fusiform vesicles were flattened and positioned individually or in stacks [6]. Direct tubular contacts between GA cisternae and FVs were not observed. Using FIB-SEM, larger volumes of tissue were analysed, and connections between the GA stacks were observed. However, manual segmentation of organelles in volumetric data becomes unfeasible within a reasonable time due to the high number of sections and numerous compartments present in each one. To overcome this problem, protocols for the automatic segmentation of multiple organelles were developed and compared with other publicly available protocols [4, 7]. Segmentation metric for mitochondria and endolysosomes showed Dice similarity coefficient 0.942 and 0.882, respectively. SBF-SEM was used to provide ultrastructural overview of the urothelium ranging from basal to

superficial cell layer. First, we had to adapt the protocol of sample preparation by additionally introducing heavy metals to the sample [3] to reduce the charging and to increase the contrast during imaging. Segmentation revealed that the number of mitochondria is the lowest in the basal cells and the highest in the superficial urothelial cells. Mitochondria in intermediate cells were frequently seen in proximity to the endoplasmic reticulum. In superficial cells, mitochondria often contained lipid-resembling inclusions in the matrix.

Discussion and conclusions: In this study, we implemented three vEM methods to investigate the urinary bladder's epithelium. The data revealed that the number of intracellular compartments – such as GA, FVs, mitochondria, and endolysosomes – that contribute to the formation of urothelial plaques, as well as the complexity of their interactions, increases as cells differentiate from less-differentiated basal cells to the terminally differentiated superficial cells. However, after obtaining the raw vEM data, the biggest challenge is finding reliable, user-friendly software for automatically segmenting large volumetric datasets. There is a strong need for such software that would allow researchers to efficiently analyse large amounts of volumetric data both qualitatively and quantitatively in a short time.

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References:

1. M.E. Kreft, S Hudoklin, *Protoplasma* **2010**, 246(1-4)
2. X.R. Wu, X.P. Kong, *Kidney Int* **2009**, 75(11)
3. T.J. Deerinck, E.A. Bushong, *NCMIR methods for 3D EM **2010***
4. M. Žerovnik Mekuč, C. Bohak, *Comput Biol Med* **2020**, 119
5. S. Hudoklin, K. Jezernik, *Plos One* **2011**, 6(8):e23636.
6. S. Hudoklin, K. Jezernik, *Plos One* **2012**, 7(3):e32935
7. M. Žerovnik Mekuč, C. Bohak, *Comput Methods Programs Biomed* **2022**, 223:106959