

THREE-DIMENSIONAL OBSERVATION OF SUBUROTHELIAL INTERSTITIAL CELL BY FIB/SEM TOMOGRAPHY

Hypothesis / aims of study

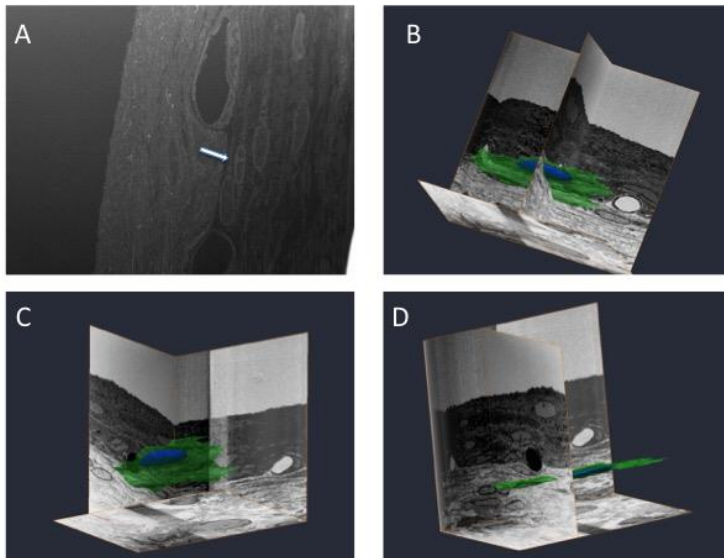
Several lines of anatomical and physiological evidences support suburothelial interstitial cells have crucial role to bladder function. Transmission electron microscopy (TEM) were standard method for the ultrastructural analysis of suburothelial interstitial cells, however, those findings are two-dimensional (2D). Recently, a focused ion beam/scanning electron microscope tomography (FIB/SEM tomography) has been developed, making it possible to reconstruct and analyze the three-dimensional (3D) ultrastructures. The purpose of this study was to observe the 3D ultrastructure of the suburothelial interstitial cells using new scanning method.

Study design, materials and methods

Hartley guinea pigs were deeply anesthetized and fixed by transcardially perfusion of *half Karnovsky* solution. After perfusion, urinary bladder was extirpated and further immersed in the same fixative. The specimens were cut and further fixed with ferrocyanide and 2% OsO₄. Subsequently, the specimens were treated with 1% thiocarbohydrazide and then immersed in a 2% OsO₄ solution for membrane enhancement. After *en bloc* staining, they were infiltrated with an epoxy resin mixture, and polymerized. Specimen was set on the stage of the FIB/SEM instrument. Serial images of the block face were acquired by repeated cycles of sample surface milling using a focused gallium ion beam and by image acquisition using SEM as a compositional contrast image. The reconstructed images included a whole detrusor bundle.

Results

In each block surface image, suburothelial interstitial cells were observed locality in the lamina propria (Figure: A). From this micrograph suggesting these cells shape spindle or stellate morphology. After reconstruction, the 3D image (Figure: B, C, D) showed that suburothelial interstitial cells were flat like a sheet structure and connected one another at their processes.



Interpretation of results

It has been difficult to image the actual 3D structure of suburothelial interstitial cell form previous TEM method. It has been reported suburothelial interstitial cells communicate and intermediate between urothelium and detrusor smooth muscle cells. Obtained 3D image could be observed freely from all directions and makes it possible to find the structural interaction between suburothelial interstitial cells and around other cells.

Concluding message

FIB/SEM tomography technique leads a new architectural interpretation of suburothelial interstitial cells.

Disclosures

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Clinical Trial: No **Subjects:** ANIMAL **Species:** Guinea pig **Ethics Committee:** Animal research committee of the Kurume University school of medicine