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TWO-PHOTON LASER SCANNING MICROSCOPY OF MURINE BLADDER TISSUE – A NEW STUDY OF MORPHOLOGY AND INNERVATION.

Hypothesis / aims of study

The aim of our study was to develop a two-photon laser scanning microscopy (TPLSM) analysis of the murine bladder wall using both label-free and labeled bladder tissue, with a special emphasis on bladder innervation.

Study design, materials and methods

Freshly dissected urinary bladder tissue from C57bl6J mice was examined using TPLSM autofluorescence (AF) imaging [1] and Second Harmonic Generation (SHG). Our newly established experimental set-up allowed ex-vivo imaging of a stretched bladder piece during several hours.

Results

Autofluorescence of the urothelium, nerve and muscle structures were seen in the green spectral channel, while collagen was visible in the blue channel. Also, necessary structural characteristics were confirmed by appropriate staining (eg elastin and nerves). Using imaging from the urothelial side, the urothelium layer with its typically shaped urothelial cells was clearly discernible at a depth of 0-30 µm. The autofluorescence of these cells is due to the high NAD(P)H content, which could be detected based on its fluorescence lifetime. Below this cellular layer the network-like collagen layer was visualized at a depth up to approximately 50 µm. This layer also contains vessel structures with a diameter of 15-20 µm and nerve structures with a diameter of 1-4 µm. The various nerve structures are seen as curly or rope-like fibres that split and branch out into a syncytium. The serosal side contains a radiant layer of collagen at the surface, covered with a mesh-like structure of nerves and large cells, either ganglion cells or macrophages at a depth of 0-20 µm. Below this layer we visualized a layer with muscle fibres, elastin, and ganglion cells or macrophages, at a depth of approximately 20-25 µm. This layer can have a thickness of at least 130 µm.

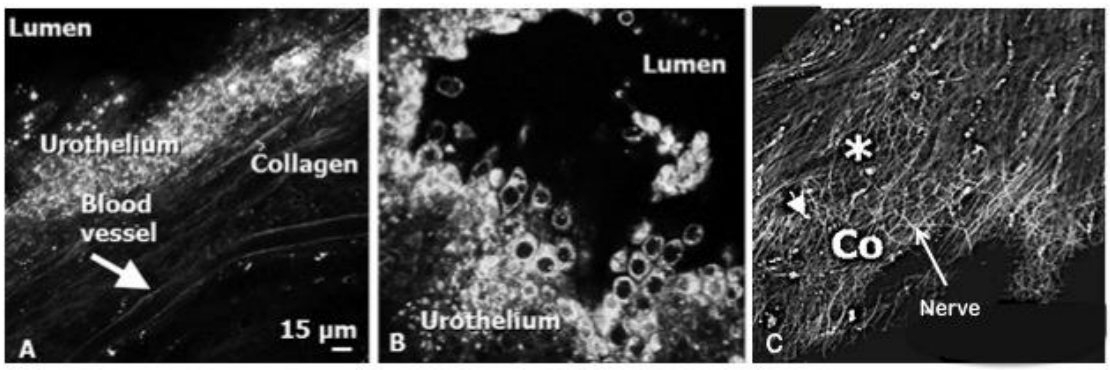

Figure 1. TPLSM images of the murine bladder wall. A) Overview of various layers, urothelial side. B) Image of urothelial layer with higher magnification, urothelial side. C) Visualisation of muscles, collagen and nerve structures, lamina propria-muscle layer transition

Interpretation of results

Through imaging from both sides of the murine bladder, we could detect different bladder wall layers and structures. These layers and structures therein could be analysed using differences in colour, size, shape, morphology and fluorescence lifetime. The identity of some structures was confirmed using specific staining. Also, the findings were confirmed in 3D-reconstructions, which provided more information about relative localisation and orientation of layers and structures, like muscle bundles and nerves.

Concluding message

TPLSM imaging is a powerful tool to obtain new insight in structures and morphology of the complete murine bladder wall, also in label-free tissue [2]. This promising technique opens avenues for identifying structural changes in the bladder wall in relation to pathological bladder function, like overactive bladder syndrome and bladder conditions associated with neurological pathology such as Alzheimer´s disease [3].

References

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