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EX VIVO TWO-PHOTON MICROSCOPY USING AUTOFLUORESCENCE OF THE PIG URINARY BLADDER WALL.

Hypothesis / aims of study

Two-photon laser scanning microscopy offers the opportunity to image living, unfixed tissue. Imaging of fixed and unfixed tissue using autofluorescence in murine bladders showed insight into the morphology and innervation of the murine urinary bladder in real time due to signals of endogenous fluorochromes such as NAD(P)H, elastin and collagen. Imaging of the whole bladder wall thickness of approx.300 µm was achieved in an *ex vivo* set up. and the urothelium, lamina propria, and the muscle layers were visualized, including blood vessels and nerves.
In addition, two-photon laser scanning microscopy was performed on living mice under anaesthesia. The complete bladder wall could be imaged and connective tissue, muscle bundles and nerve fibres were detected by autofluorescence (Data not published).
In order to use this imaging technique for diagnosis in patients, it is of utmost importance to make use of other species, better resembling the human urinary bladder. Therefore, we aimed to image porcine bladder as size and wall thickness are very similar to the human situation. In addition, we aimed for imaging based on autofluorescence, as staining based on immunohistochemical techniques would not be feasible in patients.

Study design, materials and methods

Bladders from five pigs were obtained from the local abattoir. A strip of the complete bladder wall was dissected from the lateral wall and stretched with 300%. The stretched tissue was fixated on a silicone plate using 23 gauge needles on a silicone plate (I.D.E.E., UM). In order to keep the tissue moist, it was covered with 0.5 M phosphate buffered saline. For imaging of the tissue, a two-photon laser scanning microscope, Leica TCS SP5 MP (Leica Mikrosysteme Vertrieb GmbH, Wetzlar, Germany), equipped with a HXC APO L 20x / 1.00 W water immersion objective was used. The working distance of the objective was 2mm, while the excitation source was a 140 fs-pulsed Ti:sapphire laser (Chameleon Ultra II, Coherent Inc., Santa Clara, CA, USA), mode locked at 780-820 nm. To avoid photo bleaching and tissue damage, laser power was kept as low as possible. Imaging was started from the urothelial side of the bladder.

Results

The urothelium could be visualized by using a laser excitation wavelength of 780 nm (figure 1A and B). In the suburothelial layer, collagen could be detected in blue with an excitation wavelength of 820 nm (figure 1C). Using the same excitation wavelength of 820 nm, spindle shaped cells with mostly multiple protuberances resembling earlier described interstitial cells could be visualized in yellow/orange and blood vessels in green. The interstitial cell like structures were seen in both a dense network in the suburothelium, and located directly surrounding the blood vessels (figure 1D).
Within the muscle layer, muscle fibres (green) and collagen (blue) were detected with an excitation wavelength of 800 nm (figure 1E), while thin fibres were detected with an excitation wavelength of 780 nm in green (figure 1F).

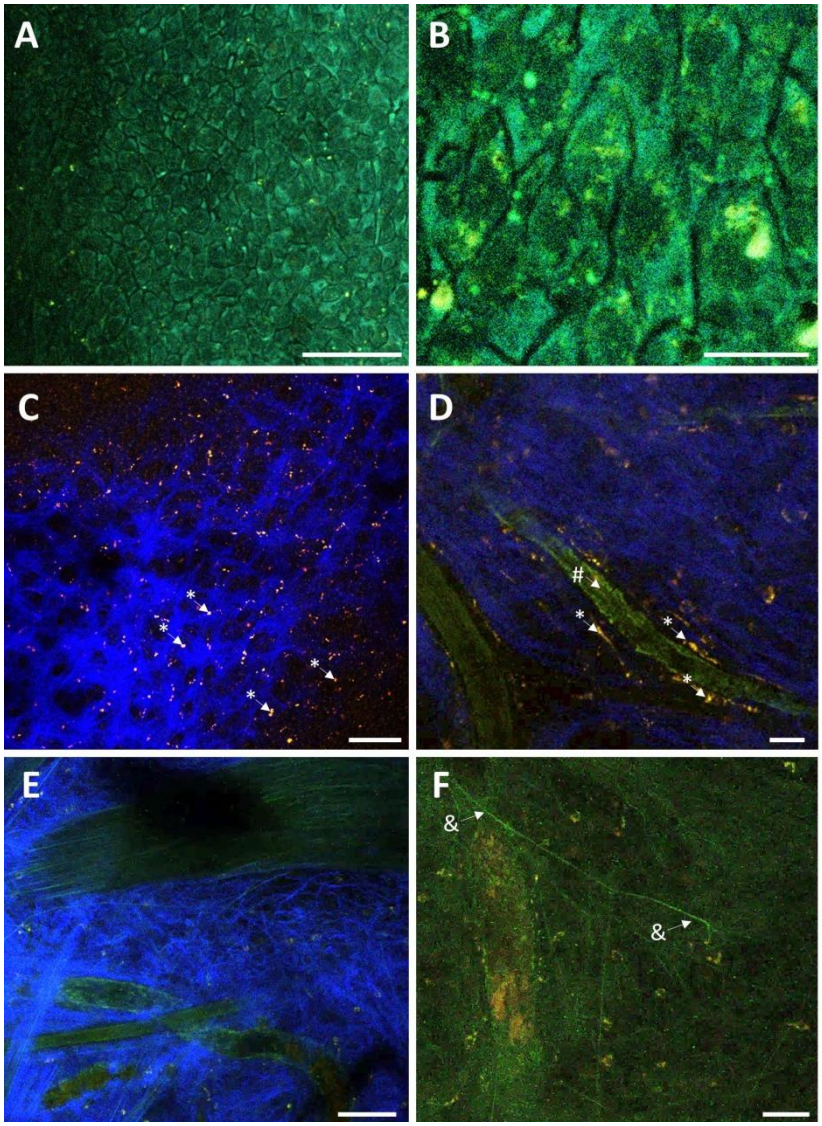

Figure 1. Two-photon laser scanning microscopy imaging of the pig urinary bladder. Panel A shows an image of the urothelium, excited with an excitation wavelength of 780 nm. Panel B shows an enlarged image of the urothelial layer, excited with the same wavelength. In panel C and D, structures of the suburothelial layer are shown, excited with 820 nm. A dense layer of collagen containing interstitial cells (marked with *) is shown in panel C, while interstitial cells (marked with *) surrounding a blood vessels (marked with #) are shown in panel D. The muscle layer is shown in panel E and F. In E, collagen is shown in blue, while muscle bundles are shown in green after excitation with 800 nm. In F, thin fibres (marked with &) are shown in green after excitation with 780 nm.

Interpretation of results

Urothelium, suburothelial interstitial cells, blood vessels, muscle fibres and collagen were detected and characterized based on their morphological characteristics. Furthermore, thin fibres were detected which could be both, elastin fibres or nerve fibres. To identify nerve fibres, immunohistochemical staining techniques would be needed. However, based on branches which are detected in these structures, it is likely that these fibres are neuronal structures.

Concluding message

It is possible to image all layers of the porcine urinary bladder, to identify urothelium, interstitial cells, blood vessels, muscle bundles and collagen based on morphological characteristics. To identify nerve fibres, immunohistochemical confirmation will be necessary. These data make a translational step towards the human bladder more feasible using this technique.

Disclosures

Funding: This study has been funded by the FP7 Marie Curie TRUST grant **Clinical Trial:** No **Subjects:** ANIMAL **Species:** pig **Ethics not Req'd:** The pig bladders were obtained from a local abattoir.