169

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HUMAN URINARY BLADDER ORGANOIDS: A NEW TOOL FOR UROTHELIUM STUDY

Hypothesis / aims of study
Limits for research on bladder diseases partly come from the lack of *in vitro* and *in vivo* models mimicking human pathophysiology. To overcome this problem, a new model is required that will improve preclinical target identification, pharmacological lead validation, and compound optimization. For this purpose, we started the development of the first three-dimensional (3D) urothelial organoid model.

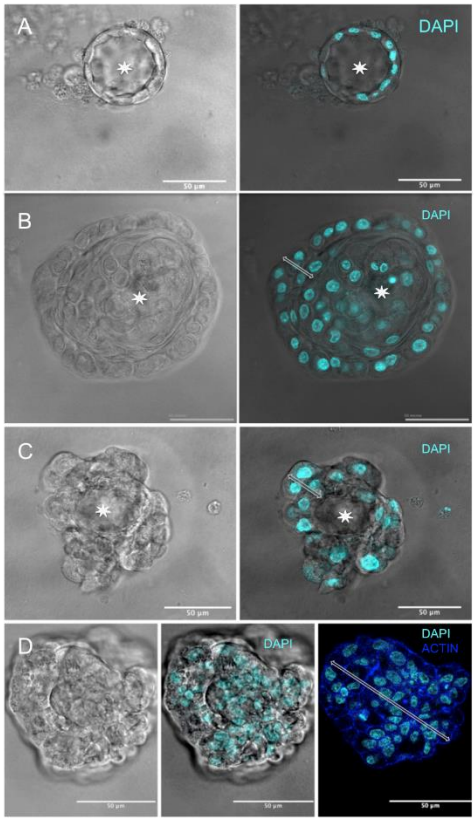
Study design, materials and methods
Tissues were dissected from macroscopically healthy area of bladder from patients treated by cystectomy. Urothelium was removed as a sheet and dissociated by enzymatic digestion. Urothelial cells were seeded in Matrigel®. The culture growth medium used was KSFM® (Keratinocyte serum free medium) complemented with human epithelial growth factor and bovine pituitary extract. The morphological monitoring of the structures was performed by confocal microscopy twice per week. The architecture and cell differentiation of organoids were assessed by immunofluorescence staining of the nucleus, actin, laminin bêta-1 (LB1), cytokeratins (CK) 17 and 20, uroplakin 3A (UPK3A) and alpha-occludin.

Results
Thirteen bladder organoid cultures were realized. A continuous growth of the structures was observed until 3 weeks of culture. After 2 weeks, the 3D structures evolved leading to four different phenotypes of organoids (figure 1): cystic monostratified organoids, cystic pluristratified organoids, “budding” organoids with central lumen and “budding” organoids with no visible lumen. The mean size of the organoids was 3025±1175 μm^2. Urothelial cell differentiation was observed in a few number of organoids (figure 2): basal cells expressing the CK17 antigen were detected on the outer border of organoids surrounded by a basal membrane expressing LB1 antigen, superficial cells expressing the CK20 antigen were located in the center of the organoids. The staining of the barrier function protein UPK3A and alpha-occludin was not conclusive.

Interpretation of results
At this stage of development, we obtained human urothelial organoids mimicking the architecture and cellular differentiation within urothelium. Optimization of the culture is necessary to obtain complete terminal differentiated organoids potentially expressing barrier function proteins.

Concluding message
Such a new model reproducing *in vitro* the main characteristics of human urothelium will be useful for studying pathophysiology leading from normal to dysfunctional urothelium.

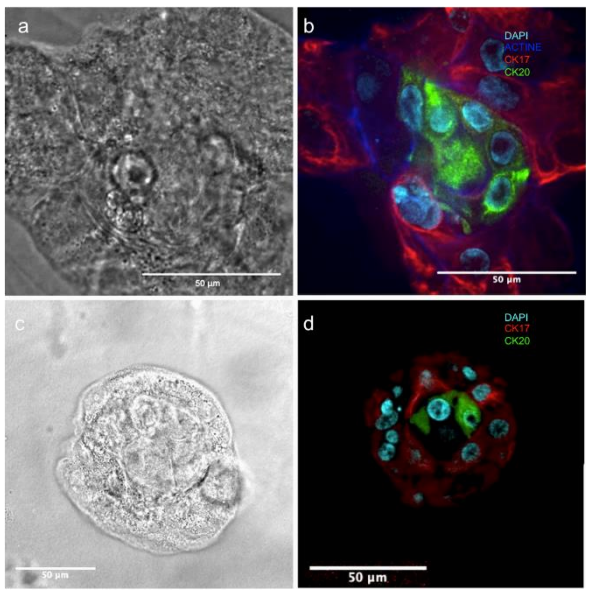
Figure 1 : human bladder organoids phenotypes



A) Cystic monostratified organoid
B) Cystic pluristratified organoid
C) "Budding" organoid with little central lumen
D) "Budding" organoid with no lumen

white stars represent the organoid lumen;
white arrows represent the cellular thickness (pluristratification)

Figure 2: Differentiated human urinary bladder organoids



Bladder organoids in brightfield (a et c) and immunostained (b et d). Nucleus in DAPI, Actin in blue, superficials differentiates CK20 positives urothelial cells in green, and basals CK17 positives urothelial cells in red.
Scale bar 50 micrometer

Disclosures
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