# A molecular, ultrastructural and functional differentiated urothelium *in vitro*

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**Introduction:** The primary function of bladder urothelium is to provide one of the tightest and most impermeable barriers in the body. This function is dependent on expression, supramolecular organization and subcellular distribution of the molecular assemblies that are acquired during urothelial cell differentiation. Unique membrane proteins, uroplakins [1], which are packed into the asymmetric unit membranes (AUMs) forming the fusiform vesicles (FVs) in the cytoplasm of highly differentiated urothelial cells and the rigid-looking urothelial apical surface, are together with specific claudin proteins in the tight junctions [2], the main contributors to transcellular and paracellular permeability barrier functions, respectively. The significant contribution to the transcellular barrier has also minimal apical endocytosis in highly differentiated bladder urothelial cells [3]. Various factors, such as intravesical cystitis, chemical or mechanical wounding (urinary stones formation), bladder outlet obstruction and tumor formation, can compromise the bladder barrier function. A long-term goal of our tissue engineering project is therefore to generate an urothelium *in vitro* that is highly differentiated on molecular, ultrastructural and functional levels and to understand the factors and mechanisms of these processes.

**Materials and Methods:** The urothelial cells were isolated from normal mouse and porcine bladders and propagated in the culture for 2 to 4 months. Effects of lamina propria, 2.5% fetal bovine serum (FBS) and exogenous calcium were investigated. Molecular differentiation was assessed by fluorescence and/or cryo-immunolabelling for urothelial differentiation-associated proteins (uroplakins, CK20, claudin-8). Ultrastructural differentiation was determined by transmission and scanning electron microscopy. The barrier function was assessed by quantitative analysis of internalized endocytotic markers (WGA-FITC, dextran-FITC) and by measurement of transepithelial electrical resistance (TER).

**Results:** Mouse and porcine urothelial cells growing with or without lamina propria in serum-free medium, supplemented with physiological calcium, developed typical threelayered urothelia with the basal, intermediate and superficial cells. The supplementation with 2.5% FBS, irrespective to calcium, resulted in development of hyperplastic urothelia. Both, mouse and porcine superficial urothelial cells expressed urothelial differentiation-associated markers (uroplakins, CK20 and claudin-8) irrespective of the medium used. Further investigation of cell differentiation status at the ultrastructural level demonstrated, that only in long-term mouse urothelial cultures influenced by paracrine action of lamina propria, the superficial urothelial cells developed ultrastructural characteristics (FVs and AUMs) of highly differentiated superficial urothelial cells *in vivo* (Figure 1). Endocytotic activity of mouse and porcine superficial urothelial cells was low and inversely related to their differentiation stage in all growth media tested. TER values were ranging from 500 Ω $cm^2$ to 3000 Ω $cm^2$ in threelayered urothelia and up to 14 000 Ω $cm^2$ in hyperplastic urothelia.

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**Discussion:** We have shown that expression of differentiation-associated molecules *per se* and morphological stratification alone cannot predict the differentiated or functional status of the urothelium. Unexpected finding of this study was that the number of cell layers has a significant influence on the TER value and that extremely high TERs were found in hyperplastic urothelia, although the ultrastructural differentiation of those urothelia was not recognised as final. Further ultrastructural (electron microscopy) and functional analyses (measurement of endocytotic activity) are therefore crucial for proper evaluating of the urothelial differentiation status.

**Conclusions:** We report the development of a mouse urothelium *in vitro* with the molecular, ultrastructural, and functional properties of native urothelium. Such *in vitro* established mouse and also porcine urothelium have application in developing tissue-engineered bladders with permeability barrier properties.

1. C-C. Hu et al., Mol. Biol. Cell. **16** (2005) 3937-3950.
2. M.E. Kreft et al., Histochem. Cell Biol. **125** (2006) 475-485.
3. M.E. Kreft et al., Differentiation **77** (2009) 48-59.

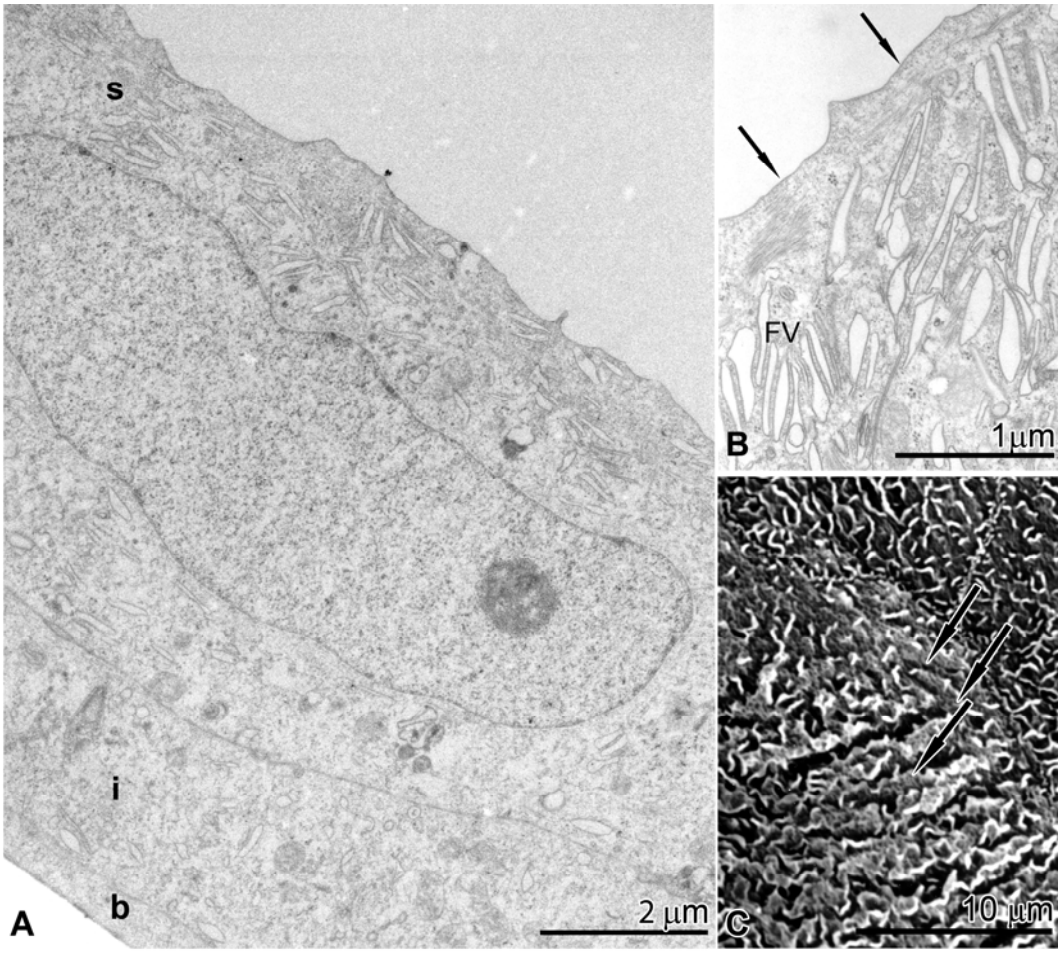


**Figure 1.** Mouse urothelium *in vitro* with the ultrastructural properties of native urothelium. (**A**) The mouse urothelial cells are organized into a threelayered urothelium with the basal (b), intermediate (i) and superficial (s) cells. (**B**) Superficial urothelial cell *in vitro* with the fusiform vesicles (FV) and the apical plasma membrane with thicker AUMs (arrows). (**C**) AUMs as are seen by scanning electron microscopy (only three of the AUMs are denoted by arrows).

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