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A NOVEL IN VITRO PIG BLADDER MODEL FOR EXPERIMENTAL RESEARCH

Hypothesis / aims of study

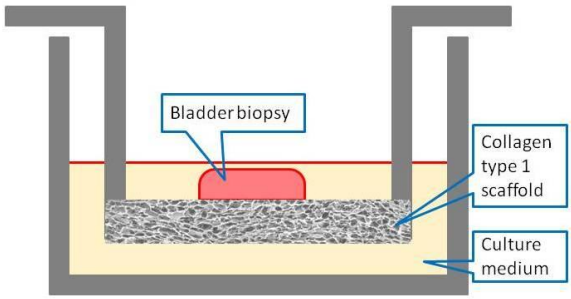
Experimental research on the urinary bladder often requires the use of laboratory animals. In general, the use of animals for experimental research is time consuming and has ethical drawbacks. From the different species, the pig bladder is the best translational model. However, because of practical and financial reasons, researchers often choose for a lesser comparable animal species, e.g. rats or rabbits. Organ tissue (organotypic) culturing could be an alternative for *in vivo* experiments. With current cell and organ culture technologies (tissue engineering), it is possible to keep organ tissue viable under *in vitro* conditions for longer periods. The aim of this study is to develop an experimental bladder model that can be used to investigate functionality (physiology), toxicity and regeneration of this organ.

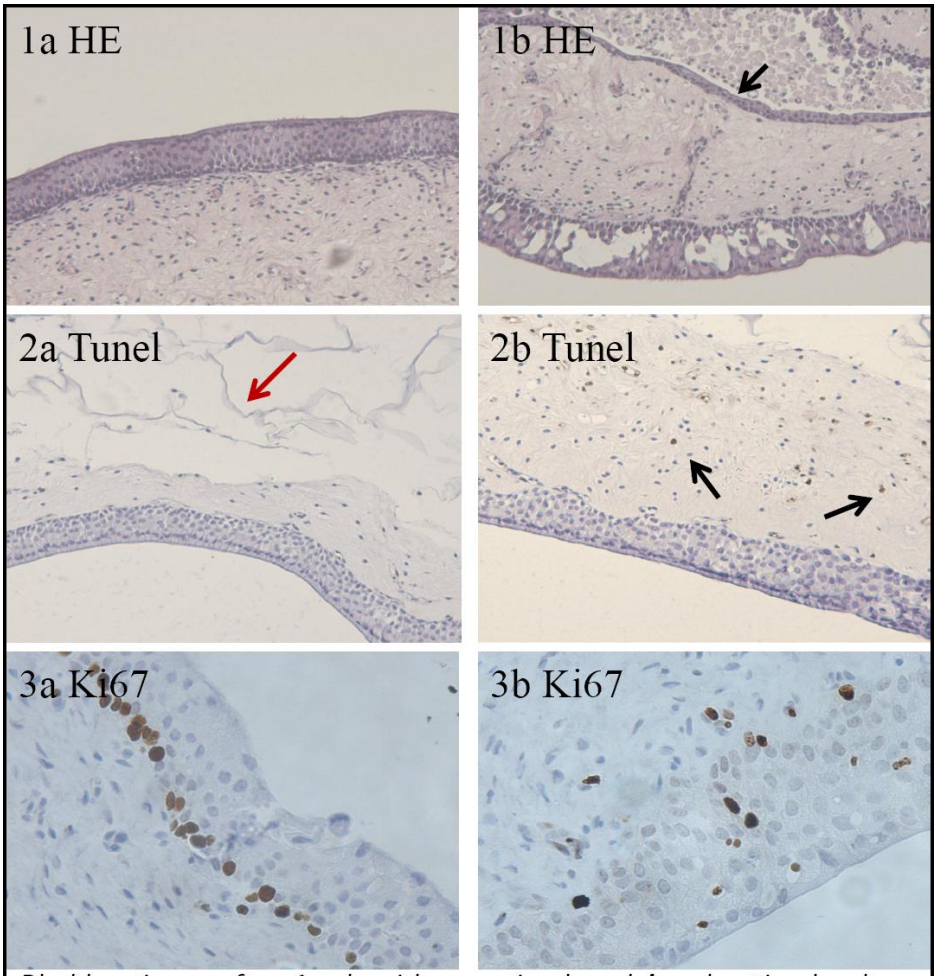
Study design, materials and methods

We mechanically isolated the mucosa from freshly dissected (intact) pig bladders that were collected from the abattoir and from the university animal laboratory. Twenty sterile punch biopsies (0.5 mm Ø) were taken from the mucosa. First, we cultured the biopsies on 3 different substrates, i.e. type I collagen scaffold, PET membrane (0.4 µm pore size) and metal roster), and placed in a CO_2-incubator at 37°C. Secondly, five different culture media were evaluated (Keratinocyte, Smooth muscle cell, DMEM®, RPMI®, Epilife®). Media was refreshed every 2 days. Biopsies were evaluated at different times (0, 2d, 1wk, 3wk, 6wk), fixated and embedded in paraffin. Tissue was histologically evaluated with standard HE and immunohistochemical staining, i.e. apoptosis (TUNEL-assay), proliferation (Ki67) and cell type (urothelial cells (UC), smooth muscle cells (SMC) and myofibroblasts).

Results

On type I collagen scaffolds the mucosa remained viable for more than 3 weeks, compared to PET membrane (<1wk) or metal rostar (<1wk). No differences were observed between bladders obtained from the abattoir or animal laboratory. There was outgrowth of mostly UC on the type I collagen scaffolds, but also SMC and myofibroblast were found in the scaffolds. UC proliferated and covered the cutting edges within 2 days. Of the 5 media used, 3 (SMC, DMEM, RPMI) were able to sustain the tissue in good condition with normal morphology of the mucosa, proliferation of cells (Ki67), and hardly any apoptosis (TUNEL-assay) for at least 1 week (see figure). Results were reproducible.





*Bladder tissue after 1 wk with **a** optimal and **b** suboptimal culture conditions:* **1a**) HE: normal morphology urothelium **1b**) HE: bad morphology + necrosis. Arrow shows sealing of necrotic area with proliferatory urothelial cells **2a-b**) Tunel: brown nuclei (arrows) represent apoptotic cells. Note the collagen type 1 scaffold (red arrow) **3a)** Ki67: proliferative basal urothial cells **3b**) Ki67:reduced proliferation of urothelial cells**.**

Interpretation of results

Results indicate that by systematically assessing the different components that influence viability of tissue in culture conditions, the quality of this experimental *in vitro* model can be improved. The type I collagen scaffold supported the mucosa tissue during culture and enables outgrowth of UC and ingrowth of SMC and fibroblasts. The use of abattoir pig bladders seems qualitatively similar and more cost efficient compared to ones from the university animal laboratory. A one week timeframe is sufficient for many bladder experiments, but further improvements on the culturing techniques can possibly extend this timeframe to broaden the applications of this experimental model.

Concluding message

Bladder mucosa cultured on type I collagen scaffolds under optimal circumstances, can be used as a biological experimental model for the bladder. This new *in vitro* bladder model may be a possible alternative for currently used laboratory animal models.

Specify source of funding or grant	**Pohl Boskamp** **Astellas award**
Is this a clinical trial?	**No**
What were the subjects in the study?	**ANIMAL**
Were guidelines for care and use of laboratory animals followed or ethical committee approval obtained?	**Yes**
Name of ethics committee	**Animal Ethical Committee Radboud University Nijmegen medical Centre**