A New and Straight Foreward In Vitro Bladder Model for Experimental Research

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Introduction

Experimental research on the urinary bladder often requires the use of laboratory animals. In general, the use of animals for research comes with ethical drawbacks and high costs. Organotypic culturing with the use of tissue engineering techniques may generate alternatives for this. The current knowledge on cell and organ culture techniques, make it possible to keep organ tissue viable for longer periods. The aim of this study is to develop a straight foreword bladder model that can be used for experimental research on functionality, toxicity and regeneration of this organ.

Materials and Methods

We mechanically isolated the bladder mucosa from freshly dissected (intact) pig bladders that were collected from the abattoir. Twenty sterile biopsy punches (0.5 mm Ø) were taken from the mucosa and were cultured on type I collagen scaffold (Fig. 1A), PET membrane (0.4 µm pore size) and metal raster, and placed in a CO₂-incubator at 37°C. Secondly, five different culture media were tested (KSFM®, SMCM®, DMEM®, RPMI®, Epilife®). Media was refreshed every 2 days. The biopsies with its outgrowth were evaluated after different time points (0, 2d, 1wk, 3wk), fixed and embedded in paraffin. Cultured tissues were evaluated with standard HE, scanning electron microscopy (SEM) and immunohistochemical staining, i.e. apoptosis (TUNEL), proliferation (Ki67) and cell type (urothelial cells (CK’s), smooth muscle cells (αSMA, Desmin) and myofibroblasts (Vimentin)).

Results

On type I collagen scaffolds the mucosa remained viable for more than 2 weeks, compared to PET membrane (<1wk) or metal raster (<1wk). The outgrowth on the type I collagen scaffold were mainly urothelial cells, although smooth muscle cells and myofibroblast were also found in the scaffolds. Urothelial cells proliferated and covered the collagen scaffold within 1 week. Of the five media used, three (SMCM®, DMEM®, RPMI®) were able to sustain the mucosa with normal morphology, proliferation (Ki67), and hardly any apoptosis (TUNEL-assay) for at least 1 week (Fig. 1B).

Discussion & Conclusions

The collagen scaffold was necessary to support the bladder mucosa during culture and to facilitate the outgrowth of cells. The immediate outgrowth of urothelial cells made it possible to generate a two compartment system (1 wk), enabling the possibility to test the effect of a component of interest on bladder mucosa tissue (Fig. 1A).

This bladder model is a straight forward tool which can be useful to investigate bladder functionality (physiology) and regeneration, or to screen toxicity (oncology). For tissue engineering purposes it may be helpful to identify the key-regulators (growth factors, cytokines) that are responsible for the outgrowth of specific bladder cells. In general, this model can be an alternative for the currently used laboratory animal models.

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