CYTOTOXICITY OF ARTIFICIAL URINE IN THE DEVELOPMENT OF A BIODEGRADABLE MPEG CARRIER POLYMER FOR INTRAVESICAL APPLICATION

Hypothesis / aims of study
To date there is no degradable drug delivery system (DDS) for personalised, intravesical treatment of chronic bladder disorders, which can release active substances over a long period of time. Following current medical product laws, new medical devices have to be tested for cytotoxicity in relevant media and relevant application time for long-term use.

For assessing the influence of artificial urine (AU) over emission qualities and cytotoxicity of a DDS, we first determined short- and long-term cytotoxicity of the most physiological AU according to (1) for benign urothelial UROtsa cells in vitro and, for future in vivo experiments, in porcine urinary bladder (PUB) urothelium under organ bath conditions.

Then, we characterised cytotoxicity to urothelial cells and the degradation behaviour of PLGA-b-mPEG, [Poly(D, L-lactide-co-glycolide-co-poly(ethylene glycol))] -polymer-carriers (mPPC) in AU and pig urine (PU), resulting in a higher translation potential to in vivo experiments.

Study design, materials and methods
Different concentrations of AU in fetal bovine serum-containing RPMI 1640 medium were used to incubate SV40 immortalized, benign UROtsa cells for 24 hours and 7 days. The following media were changed at day 1-7 after pH-value measurements: RPMI; RPMI + 30% AU and RPMI + 30% PBS. Quantitative and qualitative cell viability was determined by XTT cell assay and by live/dead staining.

PUBs were filled with 100% AU and every hour, contractions were induced via extravesical Carbachol (8 µM) for 8 hours. After 1, 2, 6 and 8 hours of AU instillation, urothelial sections were histologically stained (H&E) to assess tissue damage.

The toxicity of mPPC, manufactured by controlled expansion of saturated polymers (CESP), was examined according to (2) after 24 hours and 7 d in the same setting.

For degradation studies, mPPC were incubated rotating each in 1 ml AU or 1 ml PU for 4 weeks at 37°C, with daily urine exchange and pH-value measurements. On day 7, 14, 21 and 28 the mPPC were characterised, dried and weighed.

All data were tested for Gaussian distribution and analysed either via unpaired Student’s t-Test or Mann-Whitney-U-Test, if not normal distributed. Level of significance was p < 0.05.

Results
After 24 hours, significant cytotoxicity was measured for 50% AU (negative control) and after 7 days for 30% AU, while 30% PBS did not influence UROtsa cell viability. Live/Dead staining confirmed proliferation results. No pH-changes were observed. In contrast, urothelium showed severe detachment by histology at least after 8 hours in PUBs filled with 100% AU.

mPPC were non-toxic for UROtsa cells. Native mPPC changed its starting weight by liquid and salt accumulation after week 1, 2, 3 and 4 from 3.50 mg ± 0.48 mg to 5.38 mg ± 0.37 mg to 6.02 mg ± 1.54 mg to 4.82 mg ± 3.16 mg and 3.88 mg ± 1.07 mg, respectively (Mean ± SD). After 28 days, all mPPC disintegrated. The pH-value proceeded stable and physiological at 6.0 and dropped after 10 days to constant 5.75.

Interpretation of results
After 7 days, only 30% AU has, per se, significant cytotoxic effects on UROtsa cells. In addition, this study indicates, that intact urothelial mucosa in PUB can protect against cytotoxicity effects of 100% AU at least for 6 hours in organ bath while in cell culture on short- and long-term 50% and 30% AU induce significant cytotoxicity after 24 hours and 7 days.

mPEG-polymer-carriers promise to be biodegradable for long-term intravesical application as fire-and-forget devices.

Concluding message
To follow medical product law, we propose in general to consider the use of a correct test environment with the physiological AU according to (1) in the development and application of medical devices.

The cytocompatibility of mPEG-polymer-carriers, the constant pH-value and morphological stability within the first 3 weeks encourage subsequent in vivo studies for drug delivery systems and their long-term intravesical applications.

References
1. Griffith et al., 1976
2. DIN EN ISO 10993-5

Disclosures