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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 chronical 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
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