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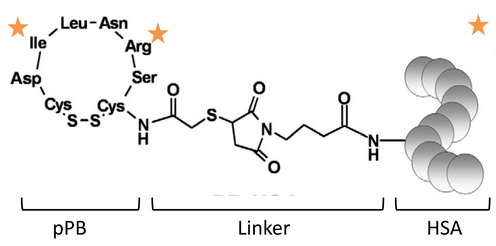
Introduction

Fibrosis

Platelet Derived Growth Factor (PDGF) plays a key role in the development of fibrotic processes in several tissues. Accordingly, the PDGFβ-receptor is abundantly present in these fibrotic tissues.

The cyclic peptide pPB

- pPB is a cyclic receptor binding peptide that contains the binding site of endogenous PDGF-BB.
- Coupling the cyclic peptide pPB to human serum albumin (HSA) prevents rapid renal excretion and allows for a better receptor presentation.
- The cyclic peptide can bind to the PDGFβ-receptor without eliciting a response.
- pPB-HSA can be used as a carrier to target therapeutic drugs, prolonging the $t_{1/2}$.



- Long term use of pPB-HSA requires a sophisticated formulation, such as polymeric microspheres for controlled and sustained release.

Aim

The aim of this research was to develop a solid formulation for the controlled and sustained release of pPB-HSA and assess the delivery and targeting of the intact protein construct *in vivo*.

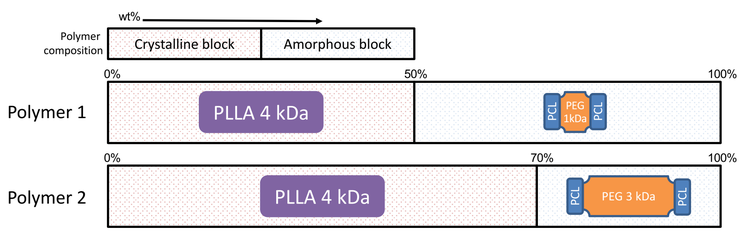
Methods – formulation development

Microsphere production - Water-in-oil-in-water process

- 1.0 g microsphere batches with 5% protein content were prepared using a double emulsion evaporation production process. After filtration and washing, the microspheres were freeze dried.

Polymers

- Combination of 2 polymers provides flexibility for release profile
- Semi-crystalline block copolymers developed by InnoCore Pharmaceuticals (Groningen, The Netherlands).



Microsphere characterization

- *Particle size assessment*: laser diffraction and scanning electron microscopy
- *Total protein content/encapsulation efficiency (EE)*: microspheres were dissolved in a mixture of DMSO and 0.05N NaOH, 0.5% SDS. The total protein content was determined using the BCA assay.
- *In vitro release*: microspheres were immersed in phosphate buffer (pH 7.4) and placed in a 37°C shaking water bath. Samples were taken at predetermined time points and replaced by fresh buffer. The total protein content was measured using BCA. The pPB-HSA content was determined using a sandwich ELISA.

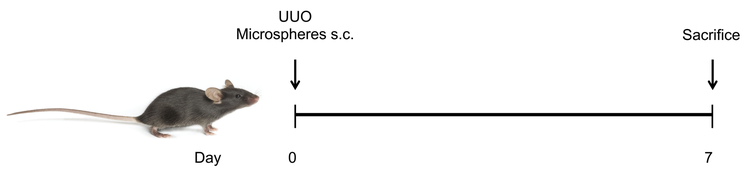
Methods – *in vivo* experiment

Unilateral ureteral obstruction (UUO) renal fibrosis model in C57bl6 mice

- Ligation of the left ureter causes the development of (renal) tubulointerstitial fibrosis in 7 days
- Renal fibrosis in the UUO kidney is associated with increased PDGFβ-receptor expression

Study design

- Day 0: UUO surgery
- Day 0: Subcutaneous administration of 31.5 mg microspheres
  (dispersed in 0.5 mL 0.4% carboxymethylcellulose solution)
  n=3: 5% HSA microsphere administration
  n=3: 3% pPB-HSA / 2% HSA microsphere administration
- Day 7: Sacrifice of animals
  Collection of kidneys and blood for analysis



Analysis

- ELISA and western blot for pPB-HSA in plasma
- Western blot for HSA in kidney tissue
- Immunohistochemical staining on pPB-HSA in kidney sections

Conclusions

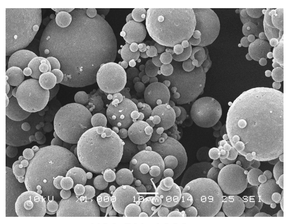
- pPB-HSA was successfully formulated in polymeric microspheres produced by a W/O/W method, which showed a first order release profile *in vitro* for 14 days.
- pPB-HSA was released from these microspheres *in vivo.*
- 7 days after adminstration, pPB-HSA was detectable in plasma and predominantly localized in fibrotic tissue with increased expression of the target, the PDGFβ-receptor.

The delivery and site specific targeting of pPB-HSA from polymeric microspheres is feasible and opens opportunities for developing controlled release formulations with therapeutic proteins targeted to fibrotic tissue.

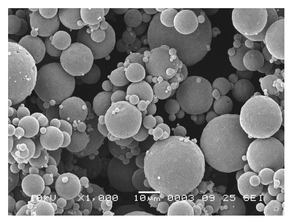
Results – formulation development

Microsphere size and appearance

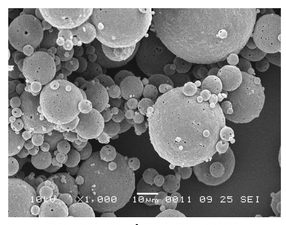
- After 7 days of *in vitro* release, small pores are formed, but no substantial degradation is visible.
- The particle size distributions of the two microsphere batches are comparable and confirm the SEM photographs.



HSA 5%, 1000x



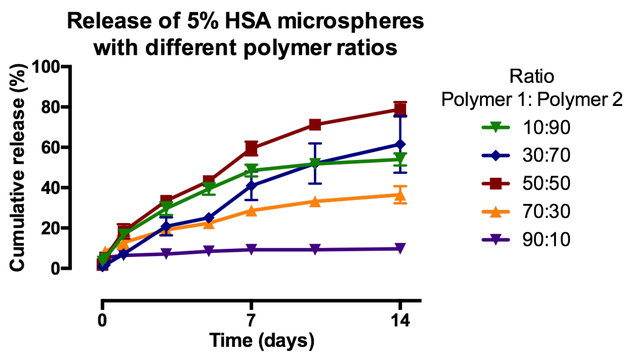
pPB-HSA 3% / HSA 2%, 1000x



pPB-HSA 3% / HSA 2%, 1000x, after 7 days *in vitro* release

Protein	Particle size distribution X_{10} (µm)	X_{50} (µm)	X_{90} (µm)	Encapsulation Efficiency	Recovery after 14d release
pPB-HSA/HSA	6.56	24.74	50.22	83%	95%
HSA	6.58	26.36	55.79	81%	105%

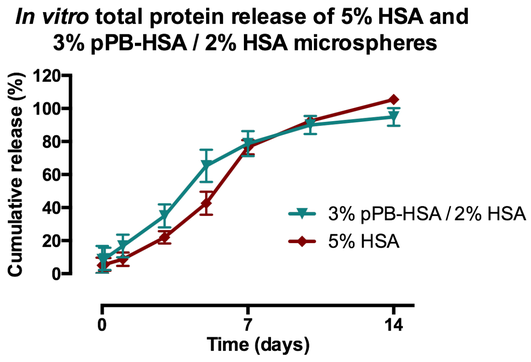
50:50 polymer composition shows most suitable release profile

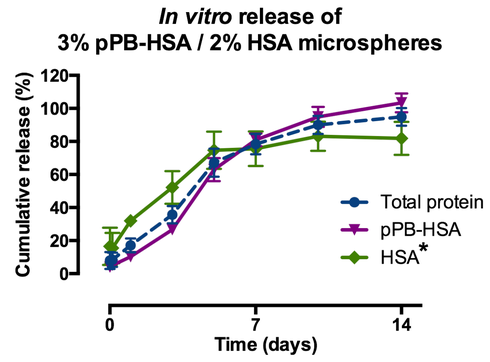


- Release accelerates with increasing content of polymer 2.
- 50:50 ratio is an exception: this formulation shows the fastest release.
- The burst release of all ratios is negligible.

In vitro release of microspheres for *in vivo* experiment

- The *in vitro* total protein release of the two batches is comparable. After 7 days, 80% has been released.
- The release of pPB-HSA from the microspheres shows the same profile as the total protein release.

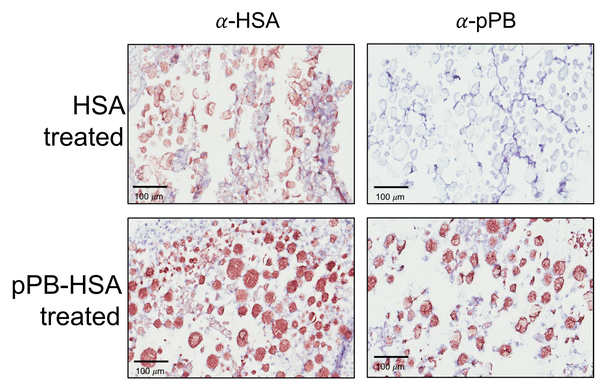




* HSA release data were calculated as: total protein released - pPB-HSA released.

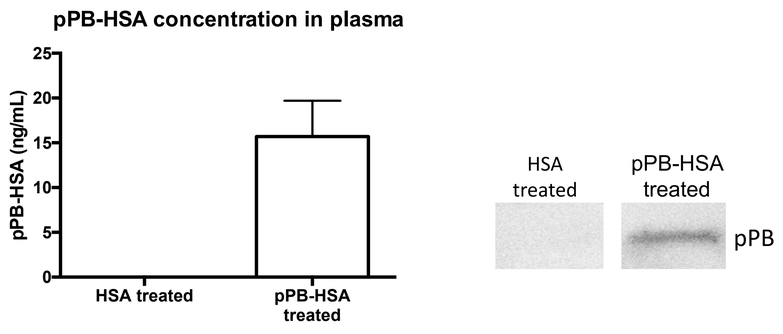
Results – *in vivo* experiment

Microspheres after subcutaneous administration



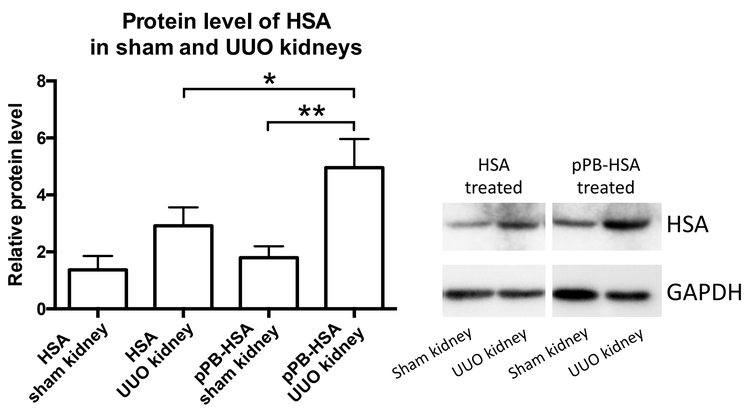
7 days after administration, HSA and pPB-HSA were still present in microspheres that were localized subcutaneously, indicating that the release was still ongoing.

pPB-HSA in plasma of UUO mice

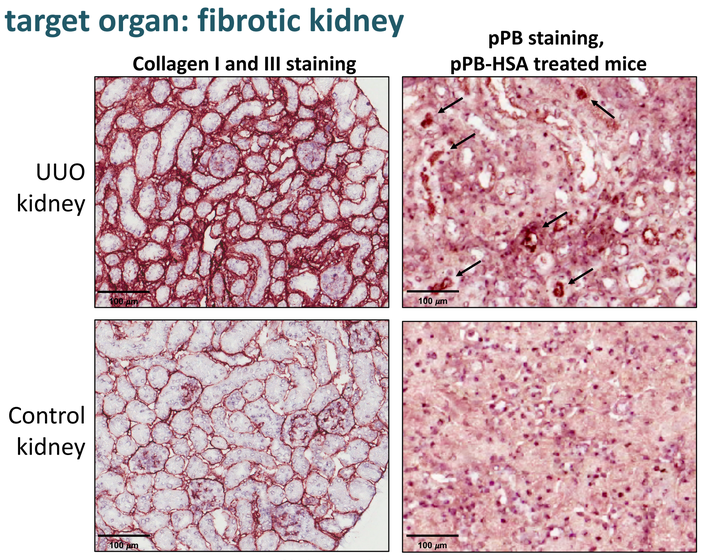


7 days after administration, pPB-HSA was present in plasma of pPB-HSA treated mice, as shown by ELISA and western blot.

pPB-HSA detection in the target organ: fibrotic kidney



Western blot analysis convincingly shows specific targeting of released pPB-HSA to the fibrotic kidney. Moreover, the leakage of HSA to the fibrotic kidney is not significant.



Representative pictures of kidney medulla. Arrows indicate pPB-rich areas.

Collagen deposition is increased in UUO kidneys 7 days after ligation. pPB-HSA is present in UUO kidney tissue of pPB-HSA treated mice.

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In collaboration with InnoCore Pharmaceuticals

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