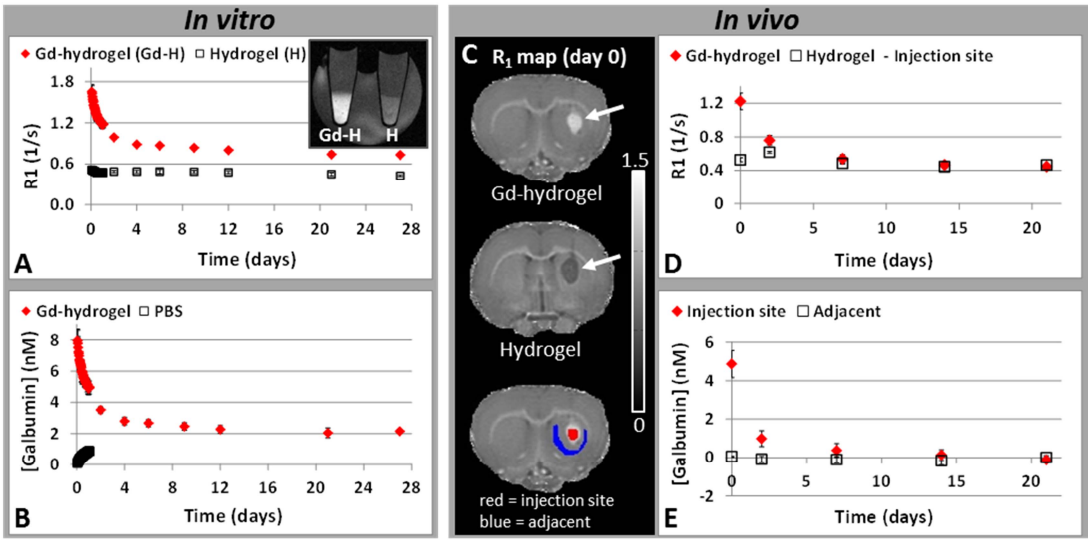
Thermosensitive biodegradable hydrogels for sustained delivery of therapeutic proteins: MRI-based monitoring of in vitro and in vivo protein release

Geralda A.F. van Tilborg[1], Pavel Yanev[1], Kristel W.M. Boere[2], Tina Vermonden[2], Annette van der Toorn[1], Wim E. Hennink[2], and Rick M. Dijkhuizen[1]
[1]*Biomedical MR Imaging and Spectroscopy Group, Image Sciences Institute, University Medical Center Utrecht, Utrecht, Netherlands,* [2]*Department of Pharmaceutics, Utrecht Institute for Pharmaceutical Sciences, University Utrecht, Utrecht, Netherlands*

INTRODUCTION: Hydrogels have been suggested as novel drug carrier system for sustained release of therapeutic proteins in several disorders, including stroke [1]. The main advantage of such a system involves prolonged exposure to a therapeutic dose of proteins after only one single intracerebral injection, which can never be obtained by systemic administration or a single intracerebral bolus injection of the protein. In the present study we developed a biodegradable hydrogel composed of thermosensitive biodegradable polymers and thiolated hyaluronic acid. The hydrogel is fluid at room temperature, gelates upon injection at 37 ºC and easily allows the encapsulation of neuroprotective and/or neuroregenerative proteins, such as growth factors. For MRI-based monitoring of protein release from the hydrogel we included an MR contrast agent with a molecular weight comparable to many growth factors, i.e. gadolinium-labeled albumin (74 kDa), with the aim to assess its release kinetics first in vitro and subsequently in the brain of living rats.

MATERIALS AND METHODS: Thermosensitive triblock polymers (triblock) and thiolated hyaluronic acid (HA-SH) were synthesized as described earlier [2]. Release properties of 15wt% triblock/4 wt% HA-SH hydrogel were studied by additional incorporation of 10 nM gadolinium-labeled albumin (galbumin; BioPAL, Inc.). **Relaxivities:** T_1 values of increasing concentrations of galbumin in PBS or hydrogel at 37ºC were measured at 4.7T (Agilent), using a Look Locker inversion recovery sequence (TR_{total}/ TR_{image}/TE=10000/25/4.2ms, α=5º, 100 images per inversion pulse, 2 averages, 3 slices, 0.188mm x 0.188mm x 1mm). **In vitro release:** PCR tubes were filled with 40 µl of the hydrogel, covered by 160 µl PBS. Sample temperature was maintained at 37ºC up to 27 days post preparation, and PBS was refreshed 24 h before each MRI session. MRI of non-labeled hydrogel (n=3) and galbumin-labeled hydrogel (n=3) samples was performed at 4.7T (Agilent). Serial T_1 maps were acquired using a Look Locker Inversion Recovery sequence (parameters similar to relaxivity measurements) from 1.5 to 24 h post preparation. Additional T_1 maps were acquired at 2, 4, 6, 9, 12, 21 and 27 days post preparation. At each time point, spin echo images were also acquired (TR/TE=500/15.54ms, 2 averages, 3 slices, 0.188mm x 0.188mm x 1mm), with slice positioning equivalent to the T_1 maps. In each sample, two regions of interest (ROI), containing hydrogel (ROI 1) or PBS (ROI 2), were manually outlined in the spin echo images. The average R_1 ($1/T_1$) value in each ROI was calculated as a function of time. Galbumin concentrations in hydrogel and PBS were calculated from the average differences in R_1 values between the non-labeled and galbumin-labeled samples, and their corresponding relaxivities. **In vivo release:** 5 µl of non-labeled hydrogel (n=2) or galbumin-labeled hydrogel (n=3) was stereotaxically injected in the right striatum of healthy male Wistar rats. Serial MRI was performed directly after, and at 2, 7, 14 and 21 days after injection. At the onset of each MRI session, multi-slice gradient-echo images were acquired to localize the injected hydrogel, and correctly position the slices for T_1 mapping. T_1 maps were acquired using a Look Locker Inversion Recovery sequence with EPI read-out (TR/TE=6000/8.75ms, α=5º, 28 images per inversion pulse, 8 shots, 24 averages, 5 slices, 0.150mm x 0.150mm x 1mm). Two ipsilateral ROI's were manually outlined in the R_1 ($1/T_1$) maps at the injection site (Fig C; red) and an adjacent area in the striatum (Fig C; blue). Average R_1 values and galbumin concentrations in the different ROI's were determined as described for the in vitro release study.



RESULTS AND DISCUSSION: **Relaxivities** of galbumin in PBS and hydrogel were 95.5 ± 5.6 and 142.9 ± 7.3 $mM^{-1}s^{-1}$, respectively. **In vitro release:** Non-labeled hydrogel showed a small and gradual decrease in R_1 over time (Fig A), most probably caused by gradual degradation of the hydrogel. In contrast, galbumin-labeled hydrogel showed a larger and faster decrease in R_1 as a function of time (Fig A; Gd-hydrogel), which could be explained by release of galbumin from the hydrogel into the PBS. The concentration of galbumin in the hydrogel was calculated to progressively decrease over the first 2 days, after which it gradually stabilized at 2.2 ± 0.1 nM (i.e. 22% of its initial concentration) (Fig B). The concentration of galbumin in PBS increased accordingly over the first 24 hours (PBS was refreshed thereafter). **In vivo release:** After stereotaxic injection of galbumin-labeled and non-labeled hydrogel in the rat brain, its accumulation in the striatum could be clearly appreciated in R_1 maps (Fig C; arrows). Within 2 days after injection of both hydrogels, R_1values normalized and became similar to values in unaffected striatal tissue (0.73 ± 0.01 s^{-1}) (Fig D), presumably due to hydrogel degradation and, in case of galbumin-labeled hydrogel, galbumin release. The concentration of galbumin at the injection site was calculated to progressively decrease to approximately 10% of its initial concentration within the first 2 days after injection (day 1: 4.9 ± 0.7 nM, day 2: 1.0 ± 0.4 nM), and became negligible (0.4 ± 0.4 nM) at day 7 (Fig E). In ipsilateral adjacent striatal tissue, galbumin concentrations remained below detection limits.

CONCLUSION: Our study shows that biodegradable hydrogels, in their current composition, release ≥ 80% of their gadolinium-labeled albumin load within 2 days, under in vitro and in vivo conditions. The presented experimental setup provides a unique opportunity to non-invasively monitor protein delivery to the brain through implanted hydrogels in living animals, which could aid in the development and assessment of such innovative drug carrier systems for prolonged drug delivery to injured brain tissue.

REFERENCES: [1] Emerich et al., Cell transplantation 2010, 1063-1071(9), [2] Vermonden et al., Macromolecules 2010, *43*, 5771-5778