pH-nanosensors for monitoring the cell fate after transplantation into diabetic mice using CEST MRI

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Target audience: Clinicians and researchers who are interested in using MRI for monitoring the function of cells during cell therapy.
Purpose: Insulin-secreting cells have shown promise as therapeutic cells for glycemic control in type I diabetes mellitus (1), with demonstrated therapeutic activity for greater than one year in patients (2). However, long-term survival and therapeutic effects for transplanted islets are poor without the use of immunosuppressants, which can be quite toxic. Alginate has been used to immunoprotect therapeutic cells and has been applied in a number of clinical trials in the USA (NCT00790257; NCT00940173). Hence, it is desirable to have an imaging approach that can monitor the insulin secretion, providing information as to when adjustments in therapeutic regiment would be appropriate. Insulin secretion is accompanied by a decrease in pH (3) due to the production of H^+. Previously we have shown that monitoring pH through chemical exchange saturation transfer (CEST) MRI can help determine when there are changes in the viability of transplanted cells (4). Here, we investigate the use of LipoCEST microcapsules as pH nanosensors (4) to monitor changes in pH during insulin secretion. We hypothesized that the CEST contrast would decrease with insulin release due to a reduction in proton exchange rate with pH. **Methods:** Animal studies - Encapsulated luciferase-mouse ßTC6 insulinoma cells ($16X10^3$ capsules, 1000 cells per capsule) were transplanted intraperitoneally (i.p.) into diabetic NOD/Shiltj mice (blood glucose>300 mg/dL). Alginate microcapsules were prepared by mixing alginate and L-arginine loaded liposomes (v:v ratio 1:1) and cells, followed by electrospraying the mixture into a 20 mM $BaCl_2$ bath to form the gelled beads. After gelation, the microcapsules were cross-linked with 0.1% protamine sulfate and then coated with a second layer of alginate (4). MRI and bioluminescence imaging (BLI) – Mice were anesthetized using isofluorane and positioned in a 11.7T horizontal bore Bruker Biospec scanner. Images were acquired on days 1, 7 and 14 post-transplantation. WASSR (5) was used for B0 inhomogeneity correction and CEST Z-spectra were acquired using a continuous-wave (CW) saturation pulse of (B_1=3.6 µT, 3 sec) with the saturation frequency between -4 ppm and +4 ppm with 0.2 ppm steps. The other imaging parameters were TR=5 sec, RARE factor=10, effective TE=5 ms. T2-w images were acquired using a multi-slice multi-echo sequence. Data Analysis: Images were processed using custom-written Matlab scripts with MTR=(S_0-S_{SAT})/S_0, where S_0 and S_{SAT} are the signal amplitude measured without and with the saturation pulse, respectively. The cell viability in the implanted capsules was measured using BLI prior to MRI. C-peptide secretion – Blood was collected prior to each imaging session and the amount of c-peptide secreted by the cells was measured using a c-peptide ELISA kit. For statistical significance, a Student's t-test with $p<0.05$ was used. **Results and Discussion:** The anatomical images showed LipoCEST microcapsules surrounding the bladder. Representative CEST images are displayed (Fig. 1). The CEST contrast as calculated by MTR_{asym} was 27% on day 1 in i.p. cavity (Fig. 1a,b), which resembled the contrast described previously (4). A decrease of the average MTR_{asym} of the capsules regions to 21% was observed on day 7 (n=3, Fig. 1c), which corresponded to an increase in the c-peptide level from 100 to 150 pM (Fig. 1d). MTR_{asym} value increased again to 27% on day 14 with a reduction in c-peptide level to 120 pM. To exclude the pH effects of decreasing cell viability, we conducted BLI to interrogate the cell viability independently, and found that the cell viability steadily decreased between day 1 and 14. Therefore, further studies are required to separate the effect of cell death from insulin release. **Conclusions:** We investigated the sensitivity of pH-nanosensors to monitoring the *in vivo* insulin secretion of cells encapsulated in LipoCEST microcapsules. Our CEST MRI approach is pH-sensitive for detecting a decrease in pH accompanying insulin secretion and/or a decrease in cell viability. Since the changes in CEST contrast corresponded to the insulin secretion as measured by the c-peptide levels, the contrast displayed by our pH-nanosensors appears to be influenced by changes in insulin secretion. Further work characterizing this influence is ongoing. This study was supported by NIH R01 EB012590, EB015031, EB015032 EB007825, and MSCRFII-0161-00. **References:** 1. Calafiore R. Expert Opin Biol Ther 2003;3(2):201-205. 2. Elliott RB, et al. Xenotransplantation 2007;14(2):157-161. 3. Malaisse WJ, et al. Biochem Soc Trans 1990;18(1):107-108. 4. Chan KW, et al. Nat Mater 2013;12(3):268-275. 5. Kim M, et al. Magn Reson Med 2009;61(6):1441-1450.

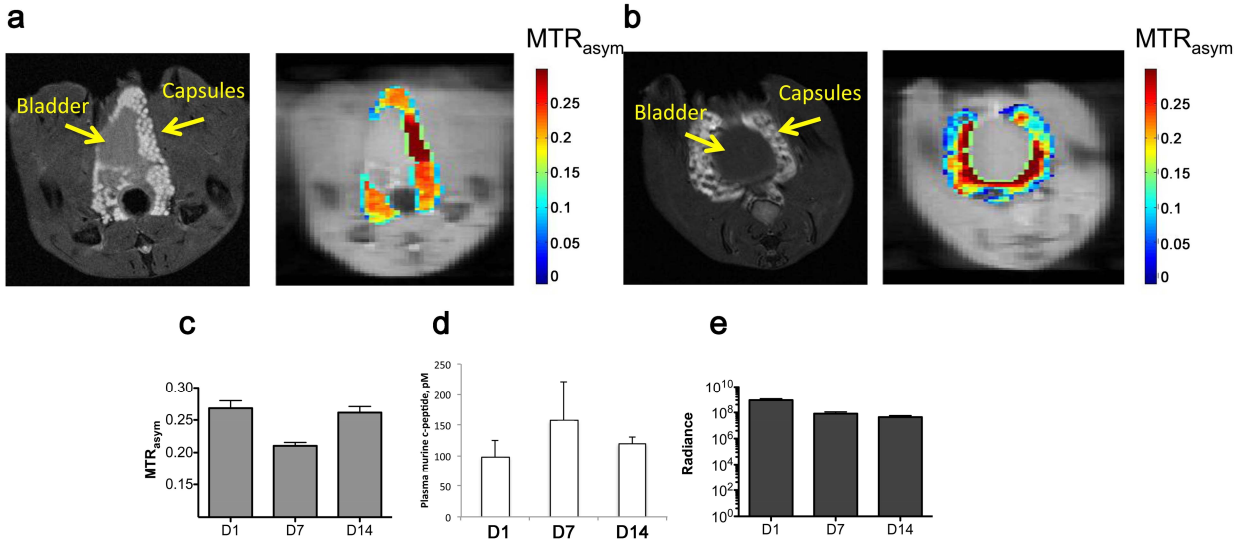

Fig. 1. LipoCEST microcapsules containing mouse insulinoma cells transplanted i.p. (n=3), **a.** MT-weighted anatomical image showing the location of capsules and the CEST contrast map at 2 ppm of capsules on day 1 and **b.** day 7, respectively. **c.** MTR_{asym} values of capsules on day 1, 7 and 14 post-transplantation. **d.** C-peptide levels for the corresponding time points indicating the amount of insulin secreted. **e.** BLI signal measured in radiance indicating the number of viable insulinoma cells.