In vivo MR CEST imaging of the Viability of Microencapsulated Cells

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Introduction

Alginate microcapsules have been employed to immunoprotect therapeutic transplanted cells such as hepatocytes for the treatment of acute liver disease and insulin-secreting cells for treatment of diabetes[1,2]. Both the location and the viability of the transplanted cells are important pieces of information for understanding and improving the therapeutic outcome. We have shown previously that chemical exchange saturation transfer (CEST) contrast produced by peptides can be used to monitor pH, and were interested in determining whether immunoprotective capsules producing CEST contrast could allow us to monitor the viability of encapsulated transplanted cells *in vivo*. In order to test this, we performed CEST MRI both in-vitro and in-vivo with luciferase-tranduced hepatocytes.

Method

CEST microcapsules: Capsules were prepared as described previously[3,4] with the addition of L-arginine containing liposomes and using protamine sulfate[5] as the cross-linking agent in order to generate CEST contrast. We suspended 1×10^7 luciferase-expressing HepG2 cells/ml in Protanal-HF alginate with the cell line transduced by CMV lentivirus using firefly luciferase as the reporter gene. A mixture of alginate/cells and liposomes (50% vol/vol) was passed through a needle at 100 μl/min using a nanoinjector pump. Droplets were collected in 20 mM $BaCl_2$, washed and suspended 0.1% PS for 5 min for crosslinking and then washed again, resuspended in 0.15% Keltone HVCR alginate for 5 min followed by one more wash step. Animal Preparation: Hepatocyte-containing microcapsules (2000-2500 capsules or 2×10^6 cells) were transplanted subcutaneously into the lower abdomen of Balb/c mice (20 g; n=4). Both bioluminescence and CEST images were acquired at day 0 and 1 after transplantation. CEST imaging: Mice were anesthetized using isoflurane and positioned in a 9.4T horizontal bore Bruker Biospec scanner. CEST images were acquired through collection of two sets of saturation images, a water saturation shift referencing (WASSR)[6] set for B_0 mapping and a CEST set for characterizing contrast. For the WASSR images, the saturation parameters were t_{sat}=250 ms, B_1=1.0 μT, TR=1.5 sec with saturation offset incremented from -1 to +1ppm with respect to water in 0.1ppm steps, while for the CEST images: t_{sat}=3.5 sec, B_1=3.6 μT, TR=5 sec, with offset incremented from -4 to +4ppm (0.2 ppm steps) with a fat suppression pulse. The acquisition parameters were: TR=5.0 sec, effective TE= 21.6 ms, RARE factor=8. Data Analysis: MR spectra were processed using custom-written Matlab scripts and the CEST contrast was quantified by calculating the asymmetry in the magnetization transfer ratio (MTR_{asym}) using $MTR_{asym}=(S^{-\Delta\omega}-S^{\Delta\omega})/S^{-\Delta\omega}$. Bioluminescence imaging: 150 mg/kg D-luciferin was administered intraperitoneally before imaging. Images were obtained 15 min post-injection and the exposure time was 10 sec.

Results and discussion We were first interested in determining whether or not CEST contrast could be used to determine cell viability in vitro. Representative images displaying the individual capsules are shown in Fig 1a. The *in vitro* CEST contrast decreased by 41% after the addition of staurosporine (STS) to induce cell death (Fig. 1a), i.e. the MTR_{asym} dropped from 0.273 to 0.163 (Fig. 1b) at 12 hrs, with a 64% drop in viability as measured by luciferase expression (Fig. 1c). This indicates that CEST contrast decreases for dying cells, as we hypothesized, with the encapsulated cells in close proximity to the capsule wall that contains the protamine sulfate as CEST pH reporter probe. Fig. 2a shows the MTw and CEST in vivo images of a representative mouse at day 0 and 1. Individual microcapsules could be visualized in MTw images (yellow arrows) *in vivo*. The average MTR_{asym} of the region of interest was 0.243 at 2 ppm, which decreased to 0.215 on day 1 (Fig. 2b). The viability of the cells was also determined using bioluminescence imaging, with the luminescence decreasing on day 1 (Fig. 2c), indicating a drop in the viability, presumably resulting from limited assess to nutrients in the subcutaneous pouch site of injection.

Conclusion

CEST microcapsules can be produced which are suitable for encapsulating therapeutic cells, with the contrast being dependent on the local pH. We have tested this technology *in vivo* and demonstrated that the CEST contrast drops when cells are dying as validated by luciferase expression.

References [1] Strom SC et al. Semin Liver Dis 1999;19(1):39-48. [2] Duvivier-Kali VF et al. Diabetes 2001;50(8):1698-1705. [3] Barnett BP et al. Nat Med 2007;13(8):986-991. [4] Chan KC et al. ISMRM 2010;Abstract 1889. [5] McMahon MT et al. Magn Reson Med 2008;60(4):803-812. [6] Kim M et al. Magn Reson Med 2009;61(6):1441-1450.

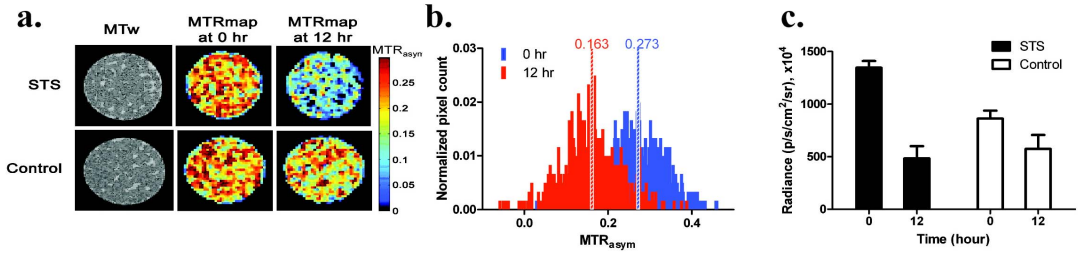

Fig. 1 Luciferase expressing hepatocytes in CEST microcapsules (a) MT-weighted images, MTRasym map at 2.4 ppm of 100 μM staurosporine (STS) treated-cells and untreated control cells at 0 hr and 12 hrs, respectively; (b) histogram showing the pixel count of phantoms after STS treatment (mean values are shown in dash lines); (c) average BLI signal (in radiance).

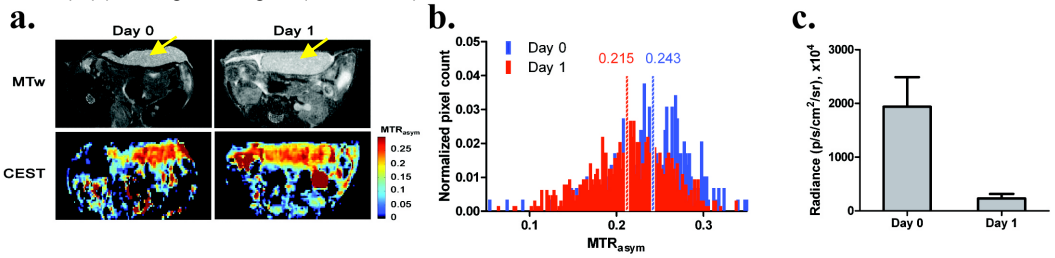

Fig 2. (a, Top) MT-weighted in vivo images of a representative animal showing the location of each individual hepatocyte-containing CEST microcapsule; (a, Bottom) MTR_{asym} map at 2 ppm of the corresponding axial slice on day 0 and day 1 of transplantation, indicating a decrease in CEST contrast; (b) corresponding histogram shows the pixel count of region of interest (mean values are shown in dash lines); (c) In vivo bioluminescence imaging.