Using magnetization transfer contrast as a surrogate marker for the occurrence of a foreign body reaction in hydrogel-based cell therapy

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Target audience: Clinicians and researchers who are interested in using MRI for monitoring cell therapy and implanted biomaterials.

Purpose: Hydrogels have been used to immunoprotect therapeutic cells and are applied in a number of clinical trials in the USA (NCT00790257;NCT00940173). However, transplantation of these materials can lead to foreign body reactions (FBR). FBR often result in fibrosis due to the leakage of cytokines from transplanted cells, and are one of the major causes of graft failure (1). An early detection method for the occurrence of FBR with monitoring of immune cell infiltration prior to fibrosis, would allow tailoring of the immunosuppression regimen to improve therapeutic outcome. Recently, we prepared alginate hydrogels loaded with DIACEST agents to allow their detection through CEST MRI (2). Here, we studied the Magnetization Transfer Ratio (MTR) imaging properties of regions containing alginate hydrogel-encapsulated hepatocytes, knowing that MTR imaging has previously been used to detect fibrosis (3).

Methods: Animal studies - Encapsulated HepG2 hepatocytes (2000-3000 capsules with a total of ~0.5 million cells) were transplanted subcutaneously into the lower abdomen of Balb/c mice (20-25 g). Animals were divided into three groups: 1) immunosuppressed mice transplanted with microcapsules containing viable hepatocytes (Group 1, n=3); mice without immunosuppression transplanted with 2) microcapsules containing apoptotic hepatocytes (Group 2, n=3) and 3) microcapsules containing viable hepatocytes (Group 3, n=3). Alginate microcapsules were prepared by mixing alginate and L-arginine loaded liposomes (v:v ratio 1:1) and hepatocytes, followed by electrospraying the mixture into a 20 mM $BaCl_2$ bath to form the gelled beads. After gelation, the microcapsules were crosslinked with 0.1 wt% protamine sulfate and then coated with a second layer of alginate. MRI – Mice were anesthetized using isofluorane and positioned in a 9.4T horizontal bore Bruker Biospec scanner. MT images were acquired two weeks after transplantation at -50, -25, -12.5, -5 and -2.5 ppm offsets using a continuous-wave (CW) saturation pulse of (B1=4.2 μT, 3 sec). Imaging parameters: 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=$(M_0-M_{SAT})/M_0$, where M_0 and M_{SAT} are the signal amplitude measured without and with the saturation pulse, resp. (4). Histology - Mice were sacrificed for histology and stained with Haematoxylin and Eosin (H&E).

Results and Discussion: On day 0, the calculated MTR values at -12.5 ppm for the region containing alginate hydrogels were similar among the three groups, but by day 14 the MTR was significantly higher for the two groups of animals without immunosuppression (Fig. 1; P<0.01). The capsule regions for these groups contained a higher number of infiltrating cells (Fig. 1), representing the extent of the FBR. As the capsules prevent direct cell-cell contact, the cell infiltration is most likely caused by soluble cytokine production (1). The largest number of infiltrating cells was seen in Group 3, which contained live hepatocytes (Fig. 1c), and showed the highest MTR. A large number of infiltrating cells was expected in the presence of highly immunogenic xenografts, i.e. human hepatocytes. The apoptotic cells in Group 2 (Fig. 1b) exhibited a milder cell infiltration and intermediate MTR values. The immunosuppression regimen applied in Group 1 (Fig. 1a) resulted in the lowest number of infiltrated cells (Fig. 1), with the lowest MTR values (Fig. 1d; P<0.01).

Conclusions: We are showing the potential of quantitative MT imaging for monitoring the occurrence of an FBR in hydrogel-based cell therapy. MT imaging may be used for an early detection of cell infiltration prior to fibrosis and adjustment of the given immunosuppressive drug regimen.

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References: 1. Strand BL, Ryan L, In't Veld P, et al. Poly-L-Lysine induces fibrosis on alginate microcapsules via the induction of cytokines. Cell Transplantation 2001;10:263-275. 2. K. W. Chan, X. Song, G. Liu, et al. In vivo MR CEST imaging of the Viability of Microencapsulated Cells. In Proceedings of the 18th Annual Meeting of ISMRM, 2011. p. 314. 3. Adler J, Swanson SD, Schmiedlin-Ren P, et al. Magnetization transfer helps detect intestinal fibrosis in an animal model of Crohn disease. Radiology 2011;259(1):127-135. 4. Stanisz GJ, Webb S, Munro CA, et al. MR properties of excised neural tissue following experimentally induced inflammation. Magn. Reson. Med. 2004;51:473-479.

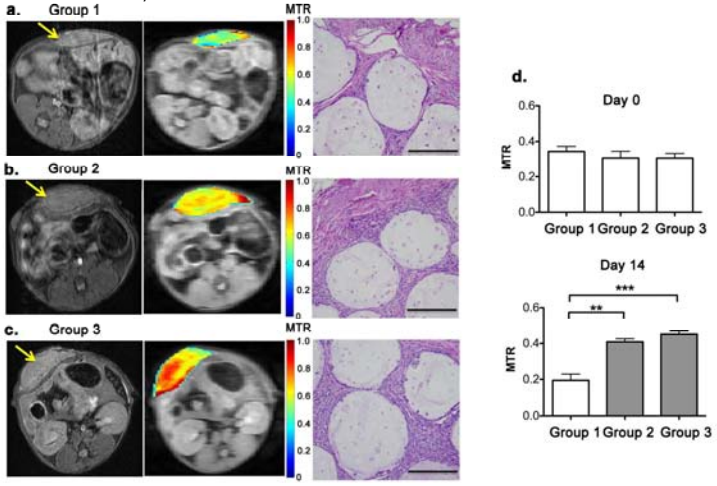

Fig. 1 (**a-c**) MT-weighted images (left panels) showing the capsule regions (yellow arrows), MTR map at -12.5 ppm (middle panels) and H&E staining (right panels) for the representative animals in (**a**) Group 1 (capsules with live cells and animals receiving immunosuppression), (**b**) Group 2 (capsules with apoptotic cells and animals without immunosuppression) and (**c**) Group 3 (capsules with live cells and animals without immunosuppression). (**d**) While the MTR values were similar for all the groups on day 0, they increased significantly for groups 2 and 3 on day 14 (P<0.01). H&E staining of the animal in (a), (b) and (c) shows the differences in the number of infiltrating cells within the graft (scale bar = 200 μm).