Imaging brain microenvironments of glial restricted precursor cells in injectable growth-factor supplemented hydrogels using CEST MRI

Kannie WY Chan[1,2], Antje Arnold[1,3], Ali Fatemi[4], Michael Porambo[4], Peter CM van Zjil[1,2], Jeff WM Bulte[1,3], Piotr Walczak[1,3], and Michael T McMahon[1,2]

[1]Radiology, Johns Hopkins University School of Medicine, Baltimore, MD, United States, [2]Kennedy Krieger Institute, Baltimore, MD, United States, [3]Institute for Cell Engineering, MD, United States, [4]Neurology and Developmental Medicine, Kennedy Krieger Institute, Baltimore, MD, United States

Target audience: Physicians and researchers who are interested in cell transplantation.

Purpose: Cell transplantation has shown promise for restoring lost brain function due to damage or degeneration, in disorders such as stroke, spinal cord injury and multiple sclerosis (1-3). Stem and precursor cells have been successfully used in animal models to replace the loss of specific cell populations and in initiating regeneration. Glial restricted precursor (GRP) cells were shown to engraft and replace astrocytes and oligodendrocytes (4). A variety of hydrogels have been used to facilitate the transplantation of therapeutic cells, however, the cell fate after transplantation is unknown and evaluation is typically based on postmortem histopathology. That in turn is a limitation to refinements in the treatment regimens. Here, we propose to use an injectable hydrogel modified with pH-nanosenors to monitor the transplanted cell microenvironment using CEST MRI (5). Moreover, this hydrogel formulation also allows a sustainable supply of growth factors (6) to support therapeutic cells *in vivo.*

Method: Hydrogel preparation: L-arginine (Arg) and barbituric acid (BA) liposomes were prepared by thin film hydration as described (5,7). In brief, PC, cholesterol and PEG-PE were mixed to form a thin film and hydrated with either 100 mg/ml Arg or 20 mg/ml of BA. The resulted liposomes were mixed with the genipin-crosslinked hydrogels as describe previous (8) with (20 ng/ml) and without basic fibroblast growth factor (bFGF). Glial restricted precursor cells: GRPs were isolated from a transgenic mouse model expressing firefly luciferase under a constitutive β-actin promoter and eGFP under the oligodendrocyte-specific proteolipid protein (PLP) promoter. Primary GRPs$^{(luc++/eGFP++)}$ were maintained in serum-free Dulbecco's modified Eagle's medium (DMEM)-F12 supplemented with N2, B27, bovine serum albumin, and bFGF, as described (9). CEST imaging: Phantoms positioned in a 500 MHz vertical bore Bruker Biospec scanner were imaged. CEST images were acquired through collection of two sets of saturation images, a water saturation shift referencing (WASSR) set for B_0 mapping, and a CEST data set for characterizing the contrast. For B_0 correction with WASSR images, the saturation parameters were t_{sat}=500 ms, B_1=0.5 uT, TR=1.5 sec with a saturation offset incremented from -1 to +1ppm with respect to water in 0.1ppm steps. For the CEST images: t_{sat}=4 sec, B_1=3.6 uT, offset incremented from -7 to +7 ppm (0.2 ppm steps). The acquisition parameters were: TR=6.0 sec, effective TE=5 ms, RARE factor=16. Data Analysis: images were processed using custom-written Matlab scripts with CEST contrast quantified by calculating the asymmetry in the magnetization transfer ratio (MTR_{asym}) using $MTR_{asym}=(S^{-\Delta\omega}-S^{\Delta\omega})/S_0$ for Arg and BA at $\Delta\omega = 2$ and 5 ppm form water, respectively.

Results and discussion: Liposome-labeled injectable hydrogels showed distinctive CEST contrast at 2 ppm for the Arg-labeled hydrogel and 5 ppm for the BA-labeled hydrogel (Fig. 1). These labeled hydrogels can potentially be used for delivery of various cell populations with the unique contrast at 2 and 5 ppm. Moreover, they exhibited a thermogelation at 37°C, facilitating their application for cell delivery to the CNS. The *in vitro* study with hydrogel supplemented with bFGF showed supportive effect for cell proliferation (Fig. 1c), and the hydrogel itself can be a carrier for the bFGF providing a sustainable supply of bFGF for GRPs. We are now investigating the proliferation and differentiation of GRPs in the liposome-labeled hydrogel using bioluminescence imaging and fluorescence microscopy.

Conclusion: We have showed the detection of the liposome-labeled hydrogels using CEST MRI, and that the hydrogel can potentially carry bFGF. This has potential as an injectable carrier for delivery of therapeutic cells to the brain.

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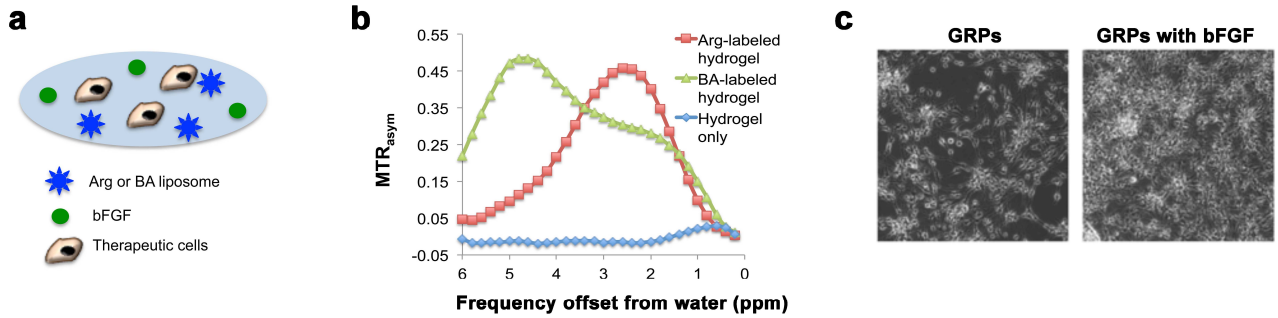

Fig. 1. a. Cartoon showing the labeling of hydrogels supplemented with growth factors. **b.** CEST contrast in terms of MTR_{asym} for the Arg-labeled and BA-labeled hydrogels and the hydrogel only, showing large effects at 2 ppm and 5 ppm from water (0 ppm). **c.** Unencapsulated mouse GRPs$^{(luc++/eGFP++)}$ cultured for 3 days *in vitro* without and with 20 ng/ml of bFGF, showing its supportive effect for cell proliferation.