Cardiac CEST MRI of paraCEST labeled Cells in Cell Therapy

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Target Audience Cell Tracking or Chemical Exchange Saturation Transfer (CEST) researchers. **Purpose** To image paraCEST labeled cells following myocardial transplantation using a novel pre-clinical cardiac CEST method. **Introduction** MRI cell tracking has typically relied upon labeling of cells with T1 or T2/T2* shortening agents that alter underlying image contrast and restrict additional measurements. Labeling of cells with paraCEST agents enables selective visualization of cell populations without persistent signal voids, and has been used to track cells in stationary organs[1,2] and tumors[3], but never in the mouse heart. We sought to utilize our recently developed cardio-respiratory gated CEST-encoded steady state cine pulse sequence (termed cardioCEST[4]) to image paraCEST labeled cells following intramyocardial transplantation in mice. **Methods** ***Pulse Sequence***: CEST encoding used a 2s train of Gaussian saturation pulses (1080°, bandwidth = 200Hz, duration = 8.8ms, number of pulses = 196) prior to a constant repetition time cine gradient echo readout that was cardiorespiratory gated (TR/TE = 7.1/3.1 ms, flip angle = 10°). After each saturation period 4 averages of one phase-encode step were acquired for each cardiac phase, and dummy pulses were played out to maintain steady state magnetization during respiratory motion. Additional parameters were FOV=2.56x2.56cm, Matrix = 256x128, and slice thickness = 1mm. All imaging was performed on a 7T Bruker Clinscan (Bruker, Ettlingen, Germany) scanner using a cylindrical volume coil for excitation and a 4-channel phased-array surface coil for reception. ***Phantom Imaging***: CEST phantoms containing either saline or 20mM solutions of Eu-HPDO3A or Yb-HPDO3A were prepared in 5mm borosilicate glass tubes. Imaging of phantoms used a gradient-echo CEST sequence with identical preparation times and readout durations. Images were obtained with saturation offsets of -100ppm to +100ppm in increments of 1ppm. ***Cell Labeling***: C2C12 (murine myoblast) cells were labeled with either Eu-HPDO3A, Yb-HPDO3A, or saline using a hypotonic swelling technique as described by Di Gregorio et al[5]. Briefly, cells were isolated suspended in 1.5 ml falcon tubes (3.0 x 10^6 cells/tube) containing 500 µl of 160 mOsm hypotonic solution (100 µl Eu-HPDO3A/Yb-HPDO3A, 107µl PBSx1, 293µl distilled H_2O) and incubated at 37°C in a dry bath for 30 min, with agitation every 10 min. Afterwards cells were incubated in a hypertonic/restoring solution (PBS at 400 mOsm/L) for 30 min and washed 3x in PBS. A fraction of labeled cells were freeze dried and labeling was assessed using inductively-coupled plasma mass spectrometry (ICPMS). ***Cell Transplantation and Imaging*:** C57B6 male mice underwent cardiac implantation of approximately 1x10^6 cells either labeled with Eu-HPDO3A (n=4) or saline (n=2, control). Cell implantation was accomplished using the "pop-out" technique as described by Gao et al[6]. In vivo cardioCEST imaging was performed 24 hours after cell implantation using the sequence described above. Anesthesia was maintained using 1.5% isoflurane in oxygen and core body temperature was maintained using circulating thermostated water. ECG and respiratory gating used a Small Animal Instruments (Stonybrook, NY) system. Once core temperature stabilized at 37°C, pairs of cardioCEST images were acquired with saturation offsets of ±15ppm in 2 slices: 1 at the site of injection and one as an internal control apical to the injection site. ***Analysis*:** CEST contrast was quantified via the asymmetric magnetization transfer ratio (MTR_{asym}). Maps of MTR_{asym} were calculated as $(S_{OFF} - S_{ON})/S_{OFF}*100$ (%), where S_{OFF} and S_{ON} represent the signal intensity when saturation is applied at the conjugate or resonant frequency of the CEST agent, respectively. **Results** ICP-MS revealed significant labeling of cells with both Eu-HPDO3A (6.14 ng/mg cell mass in control vs. 22355.67 ng/mg in labeled cells) and Yb-HPDO3A (6.14 ng/mg control vs. 21656.25 ng/mg in labeled cells). Phantom results are shown in Figure A. The mean MTR_{asym} for Eu-HPDO3A solution (E) at 19 ppm was 40.3±1.5%, and was 5.4±1.0% and 4.2 ± 0.9% for Yb-HPDO3A (Y) at 66 and 95 ppm respectively. Implantation of cells labeled with Eu-HPDO3A induced a significant increase in MTR_{asym} at ±15 ppm compared to saline labeled control cells (Fig B). The average MTR_{asym} in regions containing Eu-HPDO3A labeled cells was 29.45 ± 13.9%. The fraction of voxels demonstrating MTR_{asym} above threshold values of 5%, 15%, and 30% were all significantly higher in slices with Eu-HPDO3A labeled cells compared to slices from the same animal that did not contain labeled cells or saline control cells (Fig C). **Discussion/Conclusion**: Use of our novel cardioCEST sequence enabled in vivo imaging of Eu-HPDO3A labeled cells following implantation into the mouse heart for the first time. The high fraction of voxels with MTR_{asym}>15% in slices containing Eu-HPDO3A labeled cells likely reflect ongoing apoptosis of labeled cells with steady and low level redistribution of Eu-HPDO3A, consistent with our prior findings[4]. Compared to labeling of cells with conventional agents, the contrast generated by paraCEST agents can essentially be "turned on" at the exchangeable proton's resonant frequency. With the ability to perform this imaging in the heart, cardioCEST imaging of multiple contrast agents can allow for multiplex imaging of cell tracking alongside important functional parameters of cardiac function and perfusion without causing disruptive signal voids. **Acknowledgments**: This work was supported by the National Center for Advancing Translational Sciences, National Institutes of Health through grant number KL2TR000116. **References**: (1) Gilad et al. Nat. Biotech. 2007; 25: 217-19. (2) Bar-Shir et al. JACS. 2013; 135(4): 1617-24. (3) Ferrauto et al. 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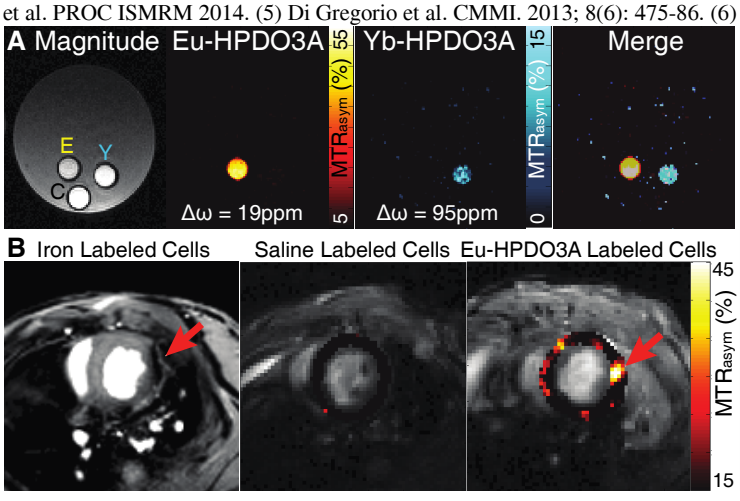

Figure. A. CEST-phantom experiments demonstrate selective visualization of Eu/Yb-HPDO3A complexes with cardioCEST. **B.** Implantation of iron labeled cells generates signal voids (arrow), whereas Eu-HPDO3A labeled cells can be selectively visualized (arrow). **C.** Images containing Eu-labeled cells demonstrate higher fractions of voxels with elevated MTR_{asym} across multiple threshold (-$p<0.05$)