Multi-Color in Vivo MR Imaging of Lymph Nodes using DIACEST Liposomes

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INTRODUCTION
MR contrast agents are used for visualizing a number of processes, including liposomal delivery of anticancer drugs to their target. However, relaxation-based MR contrast agents are limited as compared to fluorescent contrast agents in that they are not distinguishable from each other. Magnetically labeled liposomes have long been used as MR contrast agents, and are known to accumulate in lymph nodes upon subcutaneous injection. In this study, we demonstrate that we can load liposomes with diagnostically Chemical Exchange Saturation Transfer (DIACEST) contrast agents and visualize their accumulation in regional lymph nodes in mice. In addition, we demonstrate that multiple types of DIACEST liposomes can be simultaneously detected in-vivo with CEST MRI, dependent on the saturation frequency. As a result, we are able to produce multi-frequency (multi-color) MR images, which has previously been demonstrated only in vitro.

METHODS AND MATERIALS
DIACEST liposomes containing L-arginine (Larg), poly-L-lysine (PLL), and glycogen (Glyc), were prepared as described previously, using a starting solution containing 574 mM Larg, 25 mg/ml PLL (MW=15k-30k) and 100mg/ml Glyc (MW=25k-100k) respectively. Liposomes were rendered fluorescent using rhodamine. The final particle concentration was 30-100 nM and the lipoimage size ranged from 100-400 nm. C57Bl6 mice were injected with GM-CSF-expressing cancer cell vaccine to produce an immune-responsive enlargement of popliteal lymph nodes 1 week prior to liposome injection. Delivery of DIACEST liposomes through lymphatic drainage was monitored by subcutaneously injecting 30 µl of liposome solution in the hind footpads of mice. Size-dependent pharmacokinetics of radioisotope labeled liposomes (containing 111InCl3 in the interior) were determined using small animal µSPECT at four different time points (5, 24 and 48 hours). In-vivo MR imaging was performed approximately 24 hours after injection of 100-200 nm liposomes. After completion of MRI, popliteal lymph nodes were removed for histological staining.

All in-vivo MR images were acquired on a 9.4T Bruker Avance system equipped with a 25 mm sawtooth RF coil. A modified RARE sequence was used (TR=5.0 sec, effective TE=21.6 ms, RARE factor =6, slice thickness=0.7 mm, and NA=2) including fat suppression and magnetization transfer (MT) modules. In order to generate multi-color CEST contrast images in this inhomogeneous region around the knee, we developed a new saturation image collection scheme consisting of two parts. The first set of saturation images were used to generate a B0 map, using the WAter Saturation Shift Reference (WASSR) method modified for use at high field. In brief, a set of images employ a short, weak saturation pulse (t sat = 500 ms, e 0 = 0.5 µT (21.3 Hz)), sweeping from -2ppm to 2ppm (0.1ppm step), with a reduced TR to keep the imaging time short. A second set of images with the saturation frequency stepped from -6ppm to 6ppm (step=0.3ppm) around the water resonance (0ppm) was acquired with saturation pulse e 0 = 3.6 µT (150 Hz), t sat = 3sec for CEST weighted images and corrected according to our B0 map map. This was used to compute MTR asym for our multi-color CEST contrast images. All data processing was performed using custom-written scripts in Matlab. Saturation spectra (x-scalata) were drawn and compared based on the mean water signal of the entire right and left lymph nodes. MTR asym was computed by MTR asym = (S 150 Hz - S 0Hz) / S 0Hz. A contrast-noise-ratio based threshold was used to reduce ‘artificial’ contrast due to random noise, and background endogenous asymmetric MT effects.

RESULTS AND DISCUSSION
Liposomes encapsulating three different DIACEST agents displayed different frequency dependent CEST contrast, thus enabling the assignment of artificial MR colors, i.e. blue for Glyc liposomes at 0.8ppm, red for Larg liposomes at 1.8ppm and green for PLL liposomes at 3.6ppm (Fig. 1A). Using the optimized condition (i.e. isoinisoe size=100-200nm and post-injection time=24hours) for detecting liposome accumulation in the popliteal lymph nodes, we tested the in-vivo contrast of these DIACEST liposomes. The mice were injected in two different ways, either in one foot with one “color” of liposome, or in both feet with each foot injected with a different “color” of liposome. One-foot injection allows the direct comparison of MRI signals in both nodes because no significant liposome accumulation was found in the nodes on the non-injected side as confirmed by a SPECT/CT study. In studies on multiple mice (n=5 for PLL and Larg, and n=3 for Glyc), all three types of DIACEST liposomes displayed the ability to increase CEST contrast in the nodes on the injection side, with a mean increase of 11% in ΔMTR asym for PLL liposomes at 3.6ppm, 8% in ΔMTR asym for Larg liposomes at 1.8ppm, and 4% in ΔMTR asym for Glyc liposomes at 0.8ppm, respectively. In Figs. 1B-1D, artificial colors were assigned to these in vivo CEST images based on the frequency of the MTR asym map. (3.6ppm (green), 1.8 ppm(red), 0.8ppm(blue)). To demonstrate the ability of simultaneously multi-color imaging on the same subject, a two-foot injection of Large and PLL liposomes was also performed on multiple mice (n=3). We were able to detect different CEST contrast frequency dependencies (Figures 2C). The CEST image (Fig. 2B) clearly shows that the right popliteal lymph node as a red color (representing Large type CEST, at 1.8ppm), while the left side shows a green color (representing PLL type CEST, CEST at 3.6ppm). We are currently developing a substitute for PLL, as these liposomes appear to cause damage to the nearby tissues resulting in changes to both CEST contrast (Figs. 1D and 2B and T2 contrast (Fig. 2A)). The majority of these liposomes were found to be extra-cellular as revealed by fluorescence immunohistological staining for anti-CD45 (lymphocytes and macrophages) and rhodamine (i.e. liposomes). A good correlation with the CEST signal (MTR asym) was found, confirming that CEST signal came from the presence of liposomes.

CONCLUSION
We acquired the first in-vivo multi-color DIACEST MR images for visualizing lymphatic accumulation of liposomes. Three bio-organic molecules, Large, PLL and Glyc, were adapted to make highly sensitive DIACEST liposomes with distinguishable CEST contrast. We expect this to have applications in MR imaging of liposomal (drug) delivery, as well as a scale of other biological applications where multi-color imaging is beneficial.

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