Polyethylene glycol (PEG) labeled liposomal drug delivery systems as a source for dynamic absolute MR thermometry

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Introduction Most systemic liposomal drug delivery systems, such as liposomal doxorubicin HCl (Doxil) and liposomal daunorubicin citrate (DaunoXome), target tumors by extravasation from leaky tumor vessels followed by passive drug release into the tumor interstitium over the course of several days. In contrast, temperature sensitive liposomal drug delivery systems achieve complete (intravascular) release of their contents within 20 seconds when exposed to mild local hyperthermia (40 – 42 °C), thereby reducing systemic toxicity and improving therapeutic efficacy. There is a growing interest for using MR guided high intensity focused ultrasound as non-invasive method for applying local hyperthermia, which requires the precise and accurate measurement and control of the temperature.
MR-thermometry methods have been developed to verify that the heating is correctly localized with regard to the targeted volume and that the temperature increase is sufficient to release the contents and low enough to prevent tissue damage at the same time. For this purpose absolute MR thermometry would be preferred over techniques that can only be used to measure temperature changes, like proton resonance frequency shift (PRFS)-based thermometry. Several absolute MR thermometry techniques have been developed, such as liposomal thermometry [1], MR spectroscopic imaging [2] and MR thermometry using a multi-gradient echo sequence [3,4]. With liposomal thermometry, the release of (paramagnetic) contrast agent is achieved when the liposomal phase transition temperature (T_m) is reached. Thus, signal enhancement is seen when the absolute temperature rises to at least liposomal T_m. Paramagnetic thermosensitive liposomes only indicate if the T_m is reached and cannot be used for continuous MR temperature mapping. The latter two methods allows for dynamic temperature measurements, but require the presence of two components of which only one has a temperature dependent resonance frequency, e.g. tissue containing water and fat.
In this study we evaluated the feasibility of performing dynamic absolute MR thermometry using an mFFE sequence in combination with pegylated liposomes. Liposomes consist of bilayer-forming lipids and often polyethylene glycol (PEG) labeled lipids are incorporated in the bilayer to increase circulation half-lives *in vivo* and to reduce interactions of the liposomes with plasma proteins [5]. We hypothesized that these PEG labeled lipids may provide a temperature insensitive proton resonance frequency (PRF) component than can serve as a reference for performing dynamic absolute MR thermometry aimed at the guidance of temperature-controlled drug release. The proton spins in water molecules inside the tissue in which release will take place will provide a temperature dependent resonance frequency by means of the PRFS.
Materials & Methods *Liposomes:* In this study 100-nm sized liposomes (DPPC:DSPE-PEG:Cholesterol 2-x:x:1) with a phospholipid concentration of 100 mM in phosphate buffered saline(PBS) were used with varying molecular ratios of DSPE-PEG (x = 0, 0.075 and 0.15).
Spectroscopy study: 1D ^{1}H-NMR spectra were recorded on a Bruker Ultrashield 600 MHz spectrometer with water resonance suppression by a WATERGATE sequence at 25, 35 and 45° C. The liposome samples were in 90% (v/v) PBS solution and 10% D_2O. DSS (2 mM) was added as internal chemical shift reference.
Imaging study: MR imaging experiments were performed on a 3T whole body MR scanner (Achieva, Philips Healthcare, Best, The Netherlands) using a phantom consisting of a 25 ml boiling flask filled with liposome solution suspended in a 2000 ml beaker filled with manganese doped water. Important scan parameters were: TR=300 ms, $TE_1/\Delta TE$=1.0/1.5 ms, 32 echoes, α=50°, acquisition voxel size 2x2x8 mm^3, dynamic scan duration= 39.6 s. SPIR was used to achieve partial water suppression. For all experiments, the frequency difference (Δf_{eh}) between the PRF of the ethylene oxide group in PEG (f_e) and the PRF of the hydroxyl group in water (f_h) was found by peak fitting in spectra constructed from the complex mFFE signal. Δf_{eh} was measured at 5 temperatures (20, 26, 29.4, 35.1 and 39.7° C), which were verified with a calibrated thermometer.

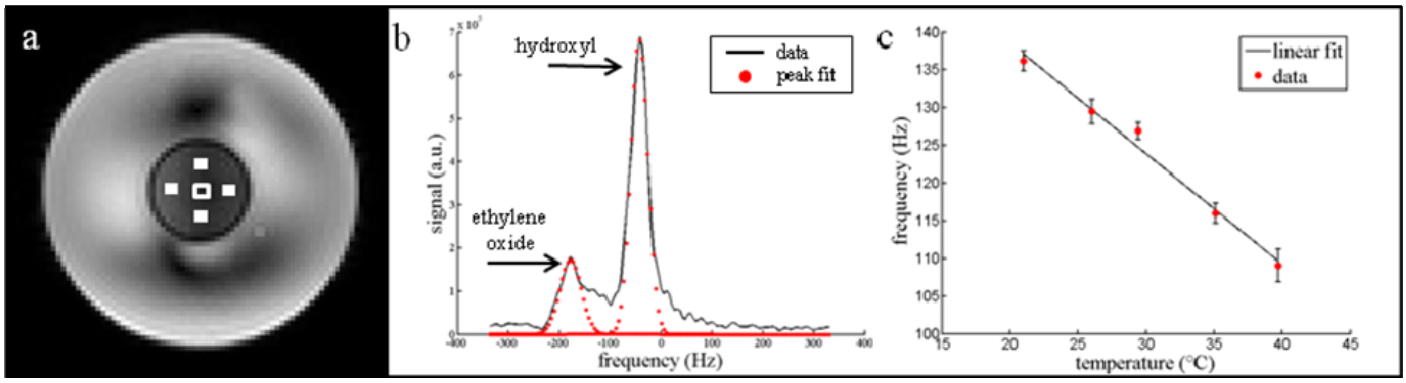

Figure 1*: Phantom containing pegylated liposome solution (7.5%) in the middle surrounded by water (a). Frequency spectrum of averaged signal in ROI (5x5 pixels) placed in the middle of the liposome solution (open white square in a) with applied peak fitting (b). Frequency difference between the PRF of the ethylene oxide group in PEG and the PRF of the hydroxyl group in water as function of temperature in liposome solution (average value and standard deviation of 5 ROIs (white squares in a)) (c).*

Results *Spectroscopy study:* The PRF of the ethylene oxide group in PEG and the PRF of the hydroxyl group in water were found (with respect to DSS) at 3.7 ppm and 4.7 ppm (at 25° C), respectively. Furthermore, the amplitude of ethylene oxide peak scaled with the percentage of pegylated phospholipids in the liposome. The PRF of ethylene oxide in PEG was confirmed to be temperature independent, though the PRF of water shifted with 0.014 ppm/°C.
Imaging study: When an ROI was placed in the liposome solution the PRF of the hydroxyl group as well as the PRF of the ethylene oxide group could be detected in the frequency spectrum from the signal evolution over 32 echoes acquired in time (figure 1b). As expected, the ethylene oxide peak was not observed when a similar analysis was done for a ROI placed in the surrounding water. In figure 1c the frequency difference between the ethylene oxide and hydroxyl peaks is plotted as function of temperature. The slope of the linear fit of these data points is 0.011 ppm/°C.
Discussion & conclusion The NMR spectroscopy study showed that the PRF of ethylene oxide in PEG is temperature independent and may serve as reference for determining the frequency shift of the temperature sensitive PRF of hydroxyl in water. Furthermore, we showed that we can detect both frequency components in a PEG labeled liposome solution with an mFFE imaging sequence. Finally, we found comparable values for the temperature dependent frequency shift of the hydroxyl group in both studies. Our preliminary findings indicate that it may be possible to use PEG labeled liposomes as drug delivery system and at the same time as a probe for dynamic absolute thermometry during hyperthermia induced local drug delivery. In future work we would like to optimize the MR sequence for optimal peak detection and to investigate the lower detection limit for pegylated liposomes with mFFE, which is important for translation to *in vivo* applications.
References
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