Supplemental Digital Content 1

TSL preparation

The lipids were dissolved in chloroform:methanol (4:1 v:v) in a DPPC/DSPC/chol/DPPE-PEG2000 molar ratio of 53:22:15:3. Liposomes were prepared by film hydration with 125 mM $(NH_4)_2SO_4$ buffer, pH 5.4, containing 250 mM gadoteridol, and subsequent extrusion at 60 °C using a thermobarrel extruder. Samples were extruded 2 times through polycarbonate filters of 200 nm pore size and 6 times through filters of 100 nm pore size. The external buffer was removed by loading the sample onto a PD-10 column and elution with HEPES buffered saline (HBS), pH 7.4. Doxorubicin loading was achieved by incubating the liposomes with doxorubicin overnight at 37°C at a doxorubicin : lipid ratio of 0.06 : 1 in HBS, pH 7.4. Residual doxorubicin was removed by exchanging the external buffer for fresh HBS, pH 7.4 using a PD-10 column.

TSL characterisation

The hydrodynamic radius of the TSL was determined by dynamic light scattering (DLS, ALV/CGS-3 Compact Goniometer System, ALV-GmbH, Langen, Germany). Doxorubicin concentrations were determined by fluorescence measurements on a fluorimeter (Perkin Elmer LS55, λ_{ex}=485 nm and λ_{em}=590 nm, Perkin Elmer, Waltham, Massachusetts, United States).

Gadolinium (Gd) concentrations were determined by inductively coupled plasma – optical emission spectroscopy (ICP-OES, Perkin Elmer Optima 4300DV) and phosphorus concentrations by a Rouser assay.[1]

The stability of the doxorubicin and gadoteridol encapsulation in TSL at 37 °C and their release from the TSL at 42 °C were measured in 90% Fetal Bovine Serum (FBS). Doxorubicin release was measured using fluorimetric assays while gadoteridol release was measured at 1.41 T using a 60 MHz Bruker Minispec (Bruker, Billerica, Massachusetts, United States) as described previously by Kneepkens *et al*.[2] Full release of both doxorubicin and gadoteridol was ensured by addition of 10% Triton-X 100.

The longitudinal relaxivity (r_1) of the TSL before and after release of the gadoteridol was determined at 3 T using a Philips 3 T Achieva® MR scanner (Philips Healthcare, Best, The Netherlands) at 37 °C in HBS by measuring a series of samples with gadoteridol concentrations in the range 0.25 to 2 mM. For this experiment, release was ensured by incubating the TSLs at 42 °C for 1 hour. The heated and unheated TSL samples were placed in a heated Eppendorf holder filled with 0.77 g/L $CuSO_4$ and 2 g/L NaCl and scans were acquired using a whole body SENSE rat coil (Rapid Biomedical, Rimpar, Germany). The temperature was kept at 37 °C by a water bath (Julabo GmbH, Seelbach, Germany) and was verified using a fiber-optic temperature sensor (Neoptix inc., Québec City, Québec, Canada) inside the Eppendorf holder. The R_1 data were obtained using an inversion recovery (IR) Look Locker sequence (inversion time (TI) : 8.4 s, repetition time (TR) / echo time (TE) : 14/5.3 ms, flip angle (FA) : 5°, 60 phases, sample time 8.4 s, field of view (FOV) : 80 x 60 mm^2, 1 slice, slice thickness : 4 mm, echo planar imaging (EPI) factor : 5, number of signal averages (NSA) : 3). The r_1 was determined from the slope of a linear fit through the R_1 values measured at different concentrations.

References

(1) Rouser, G.; Fleischer, S.; Yamamoto, A. *Lipids* **1970**, *5* (5), 494–496.

(2) Kneepkens, E.; Fernandes, A.; Nicolay, K.; Grüll, H. *Invest. Radiol.* **2016**, *0* (0), 1.