Supporting Information for:

Stabilized porous liposomes with encapsulated Gd-labeled dextran as highly efficient MRI contrast agents

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Materials

1,2‘-bis[10-(2‘,4‘-hexadienoyloxy)decanoyl]-*sn*-glycero-3-phosphocholine (bis-SorbPC) was prepared and purified as previously described.[1] Lipid purity was evaluated using thin-layer chromatography. 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) was obtained from Avanti Polar Lipids (Alabaster, AL). Dextran (Amino, 10,000 Da) was purchased from Invitrogen. FITC-dextran (dextran; ~10, 000 MW) was obtained from Sigma-Aldrich. 1,4,7,10-Tetraazacyclododecane-1,4,7,10-tetraacetic acid mono(*N*-hydroxysuccinimide ester) (DOTA–NHS ester) was obtained from Macrocyclics (Dallas, TX, U.S.A.). All other chemical were used as received. All of the buffer solutions were prepared with DI water.

Synthesis of Gd-DOTA-dextran

A 350 mg portion of dextan (Amino, MW 10,000 Da) was dissolved with 5 mL of sodium bicarbonate buffer (0.1 M, pH 9.5) and reacted with 100 mg of DOTA–NHS–ester (Macrocyclics, Dallas, TX, USA). The reaction solutions were maintained at pH 9.5 with NaOH over the reaction time of overnight. The DOTA-dextran was purified by centrifugal filter devices (Amicon Ultra-4, 5000 MWCO, Millipore Corp.). The purified DOTA-dextran conjugates were mixed with 200 mg of $GdCl_3$ in 0.1 M citrate buffer (pH 5.6) for two days at 42 °C. The unreacted Gd^{3+} was removed by centrifugal filter devices (Amicon Ultra-4, 5000 MWCO) while simultaneously changing the buffer to 0.1 M PBS buffer. To ensure complete removal of unreacted Gd^{3+}, the Gd content in the eluent was checked after each centrifugation by performing a Xylenol Orange assay[2] or measuring the T1 relaxation time until no Gd^{3+} was detectable. The purified Gd-DOTA-dextran conjugates were used for liposome encapsulation.

Preparation of nanometer-sized liposomes

Unilamellar liposomes were prepared using the film hydration method. Briefly, chloroform (DPPC) or benzene (bis-SorbPC) was removed from the lipid stock solution using a direct stream of nitrogen prior to vacuum desiccation for a minimum of 4 h. The resultant dried lipid films were rehydrated with aqueous buffer for 30 min. Samples were subjected to 10 freeze−thaw−vortex cycles in liquid nitrogen and warm H_2O (45 °C), followed by extrusion 21 times through two stacked 100 nm Nuclepore polycarbonate filters using a stainless steel extruder (Avanti Polar Lipids).

For dye encapsulation, 1 mL of FITC-dextran (10 mg/mL, dextran MW 10,000 Da) in 0.1 M sodium phosphate (pH 7.4) was added to the dried lipid film (2 mg lipid) and freeze−thaw and extrusion were performed as described above. Nonentrapped FITC-dextran was removed via size exclusion chromatography using Sepharose CL-4B (Sigma-Aldrich) and rehydration buffer as the eluent.

For Gd-DOTA-dextran, 1 mL of Gd-DOTA-dextran (10 mg/mL) was added to the dried lipid film (2 mg of lipid) and freeze−thaw and extrusion were performed as described above. Nonentrapped Gd-DOTA-dextran was removed through repeated washing on centrifugal filter devices (Amicon Ultra-4, 100K MWCO, Millipore Corp.). To ensure complete removal of Gd-DOTA-dextran, the T1 relaxation time of the eluent was checked after each centrifugation until no Gd was detectable, i.e. until the T1 relaxation time was equivalent to that of sodium phosphate buffer.

Quantification the number of Gd on polymer

To determine the number of Gd per dextran, Gd-DOTA-dextran was lyophilized. The residual solid was weighed and resuspended in pure water. The amount of Gd in the sample was measured by ICP-OES. The polymer weight was dry weight minus Gd weight. The number of Gd per polymer was then calculated based on their respective molecular weights. It was found that, on average, there were 3 Gd ions per dextran.

Liposome polymerization

The redox initiator was prepared from $K_2S_2O_8$ and $NaHSO_3$. An aliquot (10 μL) was added to the Gd-DOTA-dextran encapsulated bis-SorbPC liposome suspension, giving a [lipid]/[initiator] molar ratio of 5. The sample was sealed in an ampoule and flushed with nitrogen for 30 minutes. Polymerization was performed at 60 ± 2 °C in a water-circulating bath for 24 hours.

Instrumentation

Dynamic light scattering (DLS) measurements were performed on a Zetasizer Nano from Malvern Instruments. The scattering angle was held constant at 90°. Fluorescence spectra measurements were done on a SPEX FluoroMax-3 spectrofluorometer (Horiba Jobin Yvon). T1 relaxation times were determined using a Bruker mq60 MR relaxometer operating at 1.41 T (60 MHz). Gadolinium concentration in samples was determined by ICP-OES analysis using a Genesis ICP-OES (Spectro Analytical Instruments GMBH; Kleve, Germany). MR Images were taken on a 9.4-T magnet interfaced to a Varian INOVA console using a 70 mm inner diameter volume coil for radiofrequency transmission and reception. T1-weighted images were collected using a multi-slice spin-echo sequence with the following parameters: repetition time (TR) = 500 ms, echo time (TE) = 16.98 ms, flip angle = 10°, slice thickness = 0.5 mm, number of acquisitions = 6.

1. I. W. Jones and H. K. Hall, *Tetrahedron Lett.*, 2011, **52**, 3699-3701.
2. N. Kamaly, T. Kalber, A. Ahmad, M. H. Oliver, P. W. So, A. H. Herlihy, J. D. Bell, M. R. Jorgensen and A. D. Miller, *Bioconjugate Chem*, 2008, **19**, 118-129.