Supplementary material

Noninvasive visualization of *in vivo* release and intratumoral distribution of surrogate MR contrast agent using the dual MR contrast technique

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Legends for Supplemental Figures

Fig. S1. Relation between factors for preparation of PLGA nanospheres (NS-GdDTPA/SPIO/5-FU) and characteristics of the resultant NS-GdDTPA/SPIO/5-FU to obtain the optimal PLGA nanospheres. Model formulations were designed, based on the L8 orthogonal experimental design [1], and variables were the amount of Span 80 (5 or 20 mg), the volume of dichloromethane (2 or 5 ml), the molecular weight of PLGA (5-15 or 40-75 kDa), and the volume of the outer aqueous phase (5 or 10 ml). The particle size and the drug loading were analyzed by analysis of valiance (ANOVA) using STATISTICA (StatSoft Inc., Tulsa, OK, U.S.A.) to calculate contribution ratios of the formulation factors to the various characteristics. Based on the experiment about the optimization of NS-GdDTPA/SPIO/5-FU, the volume of the outer aqueous phase was a critical factor for both the particle size and the drug loading. The particle size decreased with increasing volume of the outer aqueous phase, whereas an increase in the volume of the outer aqueous phase resulted in a decrease in the drug loading. Based on this result, we further optimized the volume of the outer aqueous phase, and the optimal NS-GdDTPA/SPIO/5-FU (≤200 nm with relatively high drug/contrast agent loadings) were successfully obtained by using 7.5 ml of the outer aqueous phase.

Fig. S2. ^{19}F-MRS spectra of 5-FU at a concentration of **(a)** 500 μg/ml, **(b)** 100 μg/ml, and **(c)** 50 μg/ml. Acquisition parameters for ^{19}F-MRS are as follows: number of acquisitions = 512 and 1,024; number of points = 2,048; sweep width = 10,000; and repetition time = 1,000 ms. One hundred μg/ml of 5-FU was detectable as a signal-to-noise ratio of 10 with 1,024 acquisitions, whereas no peak was apparent for 5-FU at a concentration of 50 μg/ml.

Fig. S3. MR images of a MatLyLu prostate cancer xenograft before and 2 hr after intratumoral administration of 10 μl of GdDTPA/SPIO-loaded liposome suspension at a dose of 14.2 nmol equivalent to GdDTPA. MatLyLu rat prostate cancer cells were maintained in RPMI-1640 medium (Sigma-Aldrich Co.) with 1% penicillin, streptomycin, and 10% fetal bovine serum at 37°C with 5% CO_2. MatLyLu cells (3×10^6 in 50 μl of Hanks' balanced salt solution) were inoculated subcutaneously into male mice. Liposomal dual contrast agent was prepared by the sonication method, followed by extrusion, as reported previously [2], except that 500 μl of GdDTPA was used instead of 100 μl of GdDTPA. Post-contrast images were co-registered to the pre-contrast images for comparison. Negative contrast in the tumor was generated by extravasated liposomes (red arrow). Orange areas on the right panel represent leakage of low molecular weight GdDTPA released from spontaneously disrupted liposomes. Images are representative of three mice, and one representative pre-contrast and post-contrast slice is shown.

References

[1] Onuki Y, Hoshi M, Okabe H, Fujikawa M, Morishita M, Takayama K. Formulation optimization of photocrosslinked polyacrylic acid modified with 2-hydroxyethyl methacrylate hydrogel as an adhesive for a dermatological patch. J Control Release 2005;108:331-340.

[2] Kato Y, Artemov D. Monitoring of release of cargo from nanocarriers by MRI/MRSI: Significance of T_2/T_2* effect of iron particles. Magn Reson Med 2009;61:1059-1065.