SPIO-loaded unilamellar polyion complex vesicles (SPIO-Cy5-PICsomes) as a high relaxivity contrast agent for tumor detection

Daisuke Kokuryo[1], Yasutaka Anraku[2], Akihiro Kishimura[2], Sayaka Tanaka[3], Mitsunobu R Kano[4], Nobuhiro Nishiyama[3], Tsuneo Saga[1], Ichio Aoki[1], and Kazunori Kataoka[2,3]

[1]*Molecular Imaging Center, National Institute of Radiological Sciences, Chiba, Chiba, Japan,* [2]*Graduate School of Engineering, The University of Tokyo, Tokyo, Tokyo, Japan,* [3]*Graduate School of Medicine, The University of Tokyo, Tokyo, Tokyo, Japan,* [4]*Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama University, Okayama, Okayama, Japan*

Introduction The detection of early-stage tumors, especially in the metastatic case, is important for improving treatment efficacy and prolonging patient survival. Super paramagnetic iron-oxide (SPIO) nanoparticles are a highly sensitive MRI contrast agent and have the potential to be a powerful tool for a wide range of clinical and pre-clinical cancer studies.[1] However, after intravenous administration conventional SPIO nanoparticles (eg ferucarbotran) in the bloodstream are rapidly captured by the reticuloendothelial system (RES), predominantly in the liver. Therefore, to avoid recognition by the RES and effectively target tumour tissue, it is necessary to equip the SPIO nanoparticles with a "stealth" capability. Previously, it has been reported that polyion complex vesicles (Nano-PICsomes), which are composed of biocompatible poly(ethylene glycol) (PEG) and poly(amino acid)s, can be easily engineered for size and are capable of prolonged circulation in the bloodstream.[2, 3] In this paper, a novel MR and fluorescence contrast nanocarrier (named "SPIO-Cy5-PICsome"), that is specific for targeted tumor imaging and is based on the encapsulation of FDA-approved SPIO nanoparticles inside Nano-PICsomes, was synthesized and evaluated *in vitro* and *in vivo* for its ability to detect small tumors with high-field MRI.

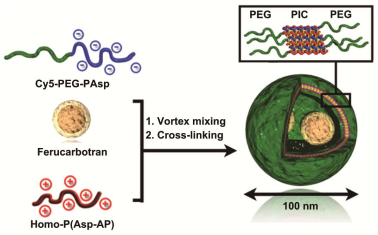

Figure 1: Schematic representation of SPIO-Cy5-PICsome preparation.

Materials and Methods The SPIO-Cy5-PICsomes were composed of ferucarbotran (Resovist®, Fujifilm RI Pharma) and two oppositely charged block copolymers: block-aniomer, fluorescence (Cy5)-labeled PEG-*b*-poly(α,β-aspartic acid) (Cy5-PEG-PAsp) and homo-catiomer, poly([5-aminopentyl]-α,β-aspartamide) (Homo-P(Asp-AP)) (Figure 1). A solution of Cy5-PEG-PAsp and ferucarbotran was prepared and then mixed with the Homo-P(Asp-AP) solution in an equal unit ratio of charged polymers, and stirred with a vortex mixer. The SPIO-Cy5-PICsome solution was then added to the 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) solution for cross-linking. The size of the SPIO-Cy5-PICsomes was controlled to be around 100 nm.

In vitro evaluation: To evaluate the relaxivity of the SPIO-Cy5-PICsomes, the relaxation rate (R_2) of the SPIO-Cy5-PICsomes was measured with a multi-echo spin-echo sequence and a 35-mm diameter volume coil (Rapid Biomedical) on a 7.0 Tesla, 40 cm bore MRI system (Magnet: Kobelco and Jastec, System: Bruker-Biospin). The imaging parameters were as follows: TR / TE = 3,000 / 10-100 ms, FOV = 48.0 × 48.0 mm^2; matrix = 256 × 256; slice thickness = 2.0 mm; and NEX = 1.

In vivo evaluation: Female Balb/c nude mice (Japan SLC Inc.) were used. Colon 26 murine cancer cells (1.0×10^6 cells / 50 μl) were subcutaneously inoculated into the mice. The accumulation of SPIO-Cy5-PICsomes in *in vivo* tumor 7 to 9 days after the tumor cell transplantation was evaluated with T_2-weighted MRI (TR / TE = 3,000 / 10-100 ms; FOV = 38.4 × 19.2 mm^2; matrix = 256 × 128; slice thickness = 1.0 mm and NEX = 1) and T_2-mapping. Images were acquired before and at 1, 3, 6 and 24 hours after the administration of a 0.45 mg/kg Fe dose of SPIO-Cy5-PICsomes to five tumor-bearing mice (17.9 g ± 1.0 g). After MR acquisition, *in vivo* fluorescence images were acquired using Maestro EX (Caliper LifeScience) to confirm the accumulation of SPIO-Cy5-PICsomes in the tumors. As a control, ferucarbotran (0.45 mg/kg Fe) was injected via the tail vein of another five mice (19.0 g ± 1.8 g).

To test whether small tumors can be detected with SPIO-Cy5-PICsomes, we performed an *in vivo* 7.0-T MRI experiment on another mouse at 2 days after tumor transplantation when the tumor volume was only ~4 mm^3. T_2-weighted MR images were acquired using a 2-ch high-sensitivity RF coil (CryoProbe™) before and 24 hours after SPIO-Cy5-PICsome administration. The imaging parameters were: TR / TE = 3,000 / 30 ms; FOV = 25.6 × 12.8 mm^2; matrix = 256 × 128; slice thickness = 0.75 mm and NEX = 2. Fluorescence images were obtained after the MR acquisition to confirm the accumulation in the small tumor.

Results and Discussion Figure 2 (A) shows the *in vitro* dependence of R_2 on iron concentration. The transverse relaxivity (r_2) of the SPIO-Cy5-PICsomes calculated from R_2 was 2.54 times higher than that of ferucarbotran (SPIO-Cy5-PICsomes: 663 ± 28 mM^{-1}s^{-1} and ferucarbotran: 261 ± 27 mM^{-1}s^{-1}). Two factors probably influence the increase of SPIO-Cy5-PICsome r_2 over that of free SPIO: (i) multiple ferucarbotrans (Fig. 2 (B), orange arrows) can aggregate within a single SPIO-Cy5-PICsome (Fig. 2 (B), white arrows),[4-6] and (ii) the diffusion of water molecules is constrained by the vesicle membrane.

MR and fluorescence images of the tumor region before and after SPIO-Cy5-PICsome administration (~24 hours) are shown in Figure 3 (A). The signal from the tumor at 3 hours after administration decreased in comparison to that obtained before administration, and the area of signal alteration expanded over the next 21 hours (arrows). Figure 3 (B) depicts changes to R_2 after SPIO-Cy5-PICsome and ferucarbotran administration. The R_2s at 6 and 24 hours were significantly higher (~20-30%) than the values after ferucarbotran administration. This result is illustrates the advantages of SPIO-Cy5-PICsomes, including enhanced permeability and retention (EPR) promoting longer circulation in bloodstream[7] and the larger relaxivity effects (Fig. 2). Figure 4 presents T_2-weighted images of a small and early-stage tumor before and 24 hours after SPIO-Cy5-PICsome administration. The MR signal from the small-sized tumor at 24 hours after administration was different from the signal obtained before administration, indicating that accumulation of SPIO-Cy5-PICsomes in the colon cancer model occurred as early as 3 days after inoculation of cancer cells. The accumulation is thought to be predominantly facilitated by the EPR effect.

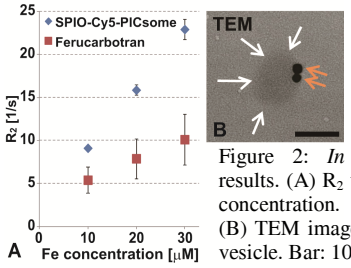

Figure 2: *In vitro* results. (A) R_2 vs. Fe concentration. (B) TEM image of a vesicle. Bar: 100 nm.

Conclusion: SPIO-Cy5-PICsomes have the potential to detect small and early-stage tumors for early diagnosis. In the future, we believe that SPIO-Cy5-PICsomes loaded with anti-cancer-drugs have many potential theranostic applications.

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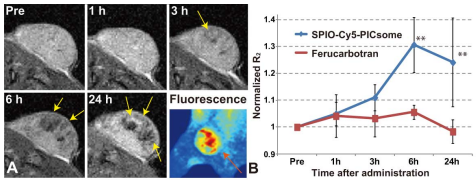

Figure 3: *In vivo* results. (A) T_2-weighted MR and fluorescence images obtained during the 24 hours after SPIO-Cy5-PICsome administration. (B) R_2 normalized by its value before administration. **: $p < 0.001$ for two-way ANOVA.

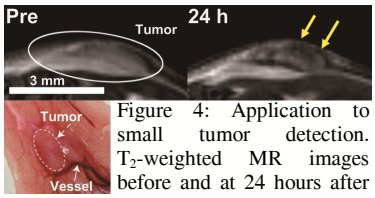

Figure 4: Application to small tumor detection. T_2-weighted MR images before and at 24 hours after SPIO-Cy5-PICsome administration are shown. The signal from the tumor (arrows) decreased at 24 hours. (photo) The vessels were connected to the tumor.