Preparation and MR imaging of giant vesicles containing superparamagnetic iron oxide for cell-tracking MRI probe

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Target audience

Our results will contribute to the provision of a noninvasive cell-tracking MRI system for *in vivo* events such as development, tumor formation, or tissue reconstruction.

Purpose

Superparamagnetic iron oxide (SPIO) is a promising candidate for the development of smart and functional probes for cell-tracking systems. However, larger SPIO particles (>1 μm)[1] are not degraded but remain permanently in the cells and may induce long-term toxicity or change the function of the labeled cells. The use of smaller SPIO particles, especially of the nanometer-sized SPIO particles that are currently used for cell tracking, results in loss of signal detectability,[2] as well as accumulation of the particles to a high density within cells, which may also be toxic to the cells. Therefore, we focused on encapsulation of SPIO nanoparticles at high number density into giant vesicles (GVs; diameter, >1 μm) providing SPIO-containing GVs (SPIO-GVs). The concept will be also applicable to be "switched off" contrast agents by means of various vesicle-destroying stimuli.

Methods

The SPIO suspension (ϕ_{ave}; 93 nm) containing carboxydextran was mixed into a buffered saline solution. After the SPIO suspension was dispersed in liquid paraffin containing egg lecithin, phospholipid, and cholesterol, the resulting water-in-oil (W/O) emulsion was layered on a buffered saline solution containing sucrose. After incubation, the W/O emulsion was centrifuged and the precipitated SPIO-GVs were collected. The diameter of SPIO-GV was determined by the differential interference contrast (DIC) microscopy and confocal laser scanning fluorescence microscopy using a lipophilic fluorescent dye. The SPIO-GVs dispersion or normal saline solution (approximately 0.5 nL) was injected into one cell of the 4-cell stage embryo of medaka fish (*Oryzias latipes*) with a glass micropipette (i.d. < 10 μm). For MR imaging, the medaka embryos were fixed and placed on a silicon sample plate. The MR images were acquired in a 7.0-T, 400-mm horizontal bore magnet (Kobelco and Jastec, Tokyo, Japan) interfaced to a Bruker Avance-I console (Bruker Biospin, Tokyo, Japan).

Results

By means of the DIC and confocal laser scanning fluorescence microscopy, we confirmed that the formed SPIO-GVs were composed of almost single lamellar membrane and had a diameter of 4.7 ± 2.2 μm. The gradient-echo MR image of the SPIO-GVs showed dark spots that corresponded to the positions of SPIO-GVs as indicated by DIC microscopy. For *in vivo* MR imaging of SPIO-GVs, we first examined a non-injected embryo that had been fixed in paraformaldehyde and embedded in a pectin-calcium gel, using both DIC and MRI imaging. We observed no dark spots at the location of the blastomeres in this MR image (Figure 1A). Next, we acquired an MR image of an embryo into which approximately 0.5 nL of the SPIO-GV dispersion was injected into one cell of a 4-cell stage embryo with a glass capillary and the embryo was then fixed with paraformaldehyde. Dark spots with a typical diameter of 200 μm were observed at the location of the blastomeres in the MR images of these injected cells (Figure 1B). In contrast, cells injected with SPIO-GVs that were destroyed by surfactant treatment showed no dark areas in the MRI (Figure 1C). The cellular distribution of the intact SPIO-GVs that was detected using MRI was consistent with the distribution of fluorescent-labeled SPIO-GVs that was determined using fluorescence microscopy (Figure 1D). Note that only a few SPIO-GVs were detected in the blastomeres using fluorescence microscopy (Figure 1D). There was no statistically significant difference between the viability of the medaka embryos after microinjection of the SPIO-GV dispersion and that of the embryos injected with the saline solution 6 or 48 h after microinjection.

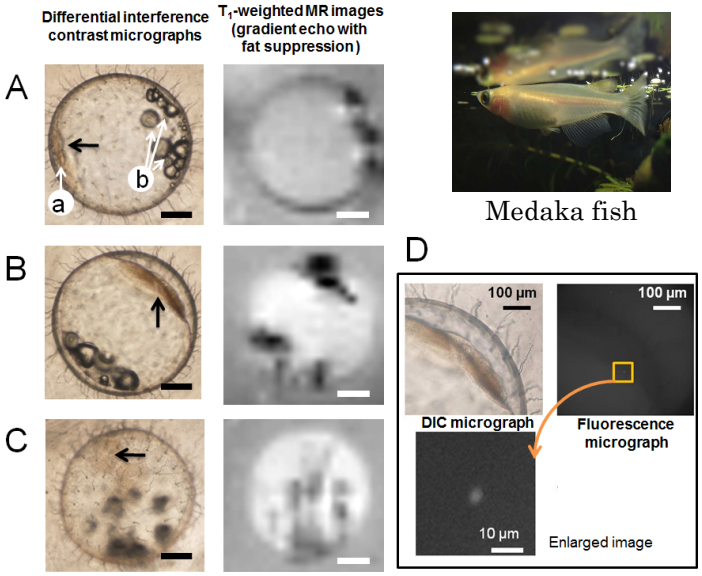

Figure 1. Differential interference contrast micrographs and MR images (gradient echo with fat suppression) of fixed medaka embryos at the 4-cell stage. (A-C) The black arrows indicate (A) untreated cells, (B) cells microinjected with approximately 0.5 nL of the SPIO-GV dispersion and (C) cells treated with the SPIO dispersion in which the GV membranes had been destroyed using a surfactant. Scale bars = 200 μm. As shown in (A), the cells in blastomeres (a) of the untreated cells displayed positive contrast while oil droplets in the embryo (b) displayed negative contrast due to the chemical shift of the fat molecules. (D) Differential interference contrast and fluorescence micrographs of a medaka embryo in which some cells had been microinjected with a fluorescent SPIO-GV dispersion.

Discussion

These SPIO-GVs were stable during MRI detection in the presence of a high magnetic field. The stability of the SPIO-GVs is probably due to the W/O emulsion centrifugation method used for their formation, which affords GVs with membranes that contain liquid paraffin, an oil-phase compound. Incorporation of liquid paraffin into the GV membrane can enhance SPIO-GV stability by inserting between the intramembrane phospholipids[3] under a high magnetic field. Moreover, even though the diameter of the SPIO-GV that is shown in the DIC micrograph was 20 μm, the expanded diameter of the corresponding dark spot in the MR image was 200 μm due to the susceptibility effect.[4] This effect results in good detectability of SPIO-GVs in cell-tracking using MRI.

References

[1] Shapiro, E. M. et al. *Proc. Natl. Acad. Sci. USA* **2004**, *101*, 10901-10906. [2] Bulte, J.W. et al. *Meth. Enzymol.* **2004**, *386*, 275–299. [3] Matsubara, H.et al. *Langmuir* **2003**, *19*, 2249-2253. [4] Atlas, S.W. et al *Am. J. Roentgenology* **1988**, *150*, 1383-1389.