Glioma cells transfected with mms6 enhance cellular iron oxide nanoparticles uptake and retention in vitro

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Introduction
Iron oxide nanoparticles (IONPs) and magnetic resonance (MR) reporter genes are tools used for cell labeling and tracking using MRI [1]. Because IONPs are metabolized and diluted with cell growth and division, the IONP-induced MR contrast may be lost over time. On the other hand, MRI endogenous iron [2]. mms6 is a gene expressed in magnetotactic bacteria known to bind iron [3]. We have shown previously that glioma cells transfected with mms6 produce a strong increase in transverse relaxivity in vitro and thus have the potential to be an MR reporter gene [4]. In this work, we hypothesized that expressing mms6 with IONPs could enhance cellular IONPs uptake and retention.

Materials and Methods
Rat glioma cells (9L) were transfected with AMB-1 mms6. Expression of mms6 was confirmed with RT-PCR and Western blot analysis. A single mms6 positive clone (9L4S) was selected for in vitro studies.

Cellular labeling of IONPs: After 24 hours of incubation, both parental 9L and 9L4S cells were labeled with PEG coated IONPs with a core size of 10 nm (Ocean Nanotech, Springdale, AR) at concentrations of 0, 1, 2.5, 5, 10, and 20 μg Fe/mL for 24 hours.

Prussian blue staining: After incubation with IONPs for 24 hours, both 9L and 9L4S cells were stained using a Prussian blue staining kit (Ocean Nanotech) and examined under an Olympus BX51 inverted microscope.

Results and Discussion
Intracellular iron measurement: After incubation with IONPs for 24 hours, both 9L and 9L4S cells were collected, counted and measured. The colorimetric method described by Gupta et al. was used to measure iron concentrations [5]. Absorption measurements were read using a microplate reader (Promega, Madison, WI) at a wavelength of 490 nm.

Relaxivity measurement: Both 9L and 9L4S cells were labeled with different concentrations (0, 1, 2.5, 5, 10 μg Fe/mL) of IONPs for 24 hours. The cells were then collected in a 1.5 ml eppendorf tube. After an hour of settling by gravity at room temperature, the transverse relaxation time (T2) of the cell pellets was measured using a 3T MR scanner (Siemens Medical Solutions, Malvern, PA) (TR: 2 sec, TE: 7.7 msec, echoes: 20).

To determine whether mms6 would increase the retention of iron in cells, intracellular iron was quantified at different passages after the incubation with IONPs. Both the 9L and 9L4S cells labeled with a high concentration of IONPs, 10 μg Fe/mL, were passaged three times successively, and the intracellular iron was measured after each passage. As shown in figure 3, no significant difference in intracellular iron between 9L and 9L4S was found at passage 0, however, 9L4S cells contained significantly more iron than 9L cells from passage 1 to passage 3 (P < 0.01, n=3).

Conclusion
Our results show that expression of mms6 can potentially enhance cellular uptake and retention of IONPs. This capability of mms6 may allow us to develop a mean for targeted uptake and retention of IONPs for MR molecular imaging.

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References