A novel iron oxide contrast agent: Nano in size and functioning as micron-sized particles

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INTRODUCTION

MR cellular imaging using iron-oxide-based contrast agents is an attractive technique in cell tracking and monitoring cell therapies. However, one major obstacle of the widespread application of this technique in clinics is the low sensitivity inherently associated with MRI. Therefore, there are a lot of efforts aimed at enhancing the susceptibility effect induced by the iron-oxide-labeled cells and thus the signal sensitivity of MRI either through using transfection agent or electroporation to increase the cell labeling efficiency [1] or through using new contrast agent with higher relaxivity or larger size [2]. Though large micron-sized iron oxide particles enhance the signal sensitivity, it might not be biodegradable. In this study, we investigated the labeling efficiency and signal sensitivity of a newly synthesized iron oxide particle with extremely higher relaxivity, compared with a couple of commercially available iron oxide particles by labeling macrophage *ex vivo*.

METHODS

Description of iron oxide particles: The novel biocompatible ultrasmalll super-paramagnetic iron oxide (USPIO) particle (ITRI-IOP) consists of an iron oxide core and is coated with a monolayer of polyethyleneglycol (PEG). The PEG coating prevents the iron-oxide from aggregation, and also makes the particles highly hydrophilic. Transmission electron microscopy shows the size of iron core is around 10 nm. This particle has much higher relaxivity than currently available particles: the longitudinal and transverse relaxivities are 52.4 and 304.7 $s^{-1}mM^{-1}$, respectively. Due to its high transverse relaxivity, ITRI-IOP is particularly suitable for T_2*-weighted images and might provide higher sensitivity for cellular MRI. In addition, *in vitro* results show that ITRI-IOP has good biocompatibility and is not cytotoxic. Two other commercially available and commonly used iron oxide particles with different sizes were tested in this study: micron-sized super-paramagnetic iron oxide (MPIO) particles are styrene-divinyl benzene polymer microspheres (catalog No. MC05F, Bangs Laboratories, Fisher, IN) and Feridex (Berlex, Montvale, NJ). The sizes of these particles were measured again with dynamic light scatting (Malvern Instruments Ltd, Worcestershire, UK).

Cell culture and *ex vivo* iron labeling protocol: Macrophages were chosen as the cell type to label because of its ease of handling, ability of phagocytosis and its important role in immune response. Macrophages were isolated from spleen of Brown Norway rat (Harlan Labs, IN). All types of particles were incubated in macrophages for 12 hrs with an iron concentration of 25 μg/ml. The cell viability was examined by Trypan blue. Perl's Prussian blue staining was used to confirm the presence of iron inside the cells after labeling.

MRI protocol: 2×10^5 iron labeled macrophages were diluted in 2% agarose gel and were then imaged at 11.7T (Bruker AVANCE, gradient Micro 2.5) with the following parameters: TR= 600 ms, TE= 8.1 ms, FOV=10 mm, resolution= 78×78×130 μm, NA=8.

RESULTS

The sizes of all iron oxide particles were measured as follows: MPIO of 1 μm, Feridex of 128 nm, and ITRI-IOP of 65 nm. The Pussian's blue staining shows that macrophages labeled with ITRI-IOP have a very large iron-staining in the cytoplasm comparable with MPIO, which is approximately 18 times large in particle size (Fig.1). MRI shows the hypointensity spots induced by the macrophages labeled with different types of iron oxide particles (Fig. 2). Similarly, we found that ITRI-IOP-labeled microphages exhibit more effective area of signal hypointensity than Feridex, and no aggregation was found in the ITRI-IOP-labeled macrophages.

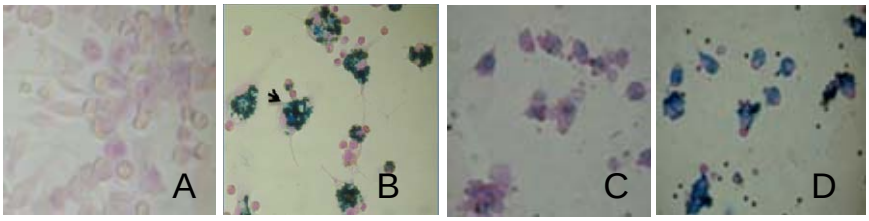

Fig. 1: Perl's Prussian blue staining of macrophages labeled with different particles at a concentration of 25 μg/ml iron. (A) unlabeled macrophage; (B) with MPIO; (C) with Feridex;(D) with ITRI-IOP.

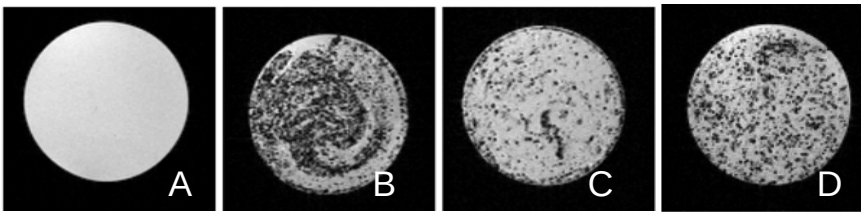

Fig. 2: MRI at 11.7 T. (A) agarose +unlabeled Macrophage; (B) macrophage + MPIO; (C): macrophage + Feridex; (D) macrophage + ITRI-IOP

DISCUSSION

The iron oxide labeled cells resulting in hypointensity in T_2*-weighted MR images and the detection of labeled cells can be challenging at low signal to noise ratio. We have previously shown that in situ MPIO-labeled macrophages accumulate at the rejecting site after organ transplantation and can be detected as punctuated hypointensity spots with *in vivo* MRI [3]. However, the MPIO particle is not degradable in live animal and might not be used for clinical studies. Instead, we found that the novel nano-sized iron oxide particles, ITRI-IOP, tested in this study shows comparable sensitivity in MRI as the micron-sized particles, probably because of their high relaxivity and its hydrophilic characteristics which make it easier to be incorporated by cells. Thus, the introduction of the novel ITRI-IOP might offer a powerful tool for cellular MRI and open doors for its future clinical applications.

REFERENCES (1)Frank et al., Radiology,228:480-7 (2003) (2)Shapiro et al., *PNAS* ,101:110901-6(2004) (3)Wu et al. *PNAS*,103(6):1852-7(2006).