MRI Contrast and Functional Properties of Novel Amphiphilic Triblock Polymer Coated Size Uniform and Tunable Magnetic Iron Oxide Nanoparticles

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INTRODUCTION

Magnetic iron oxide (IO) nanoparticles are increasingly attractive in the field of MRI for developing new applications, such as magnetic cell labeling for in vivo cell tracking, target specific molecular imaging and imaging-guided drug delivery. Those novel applications often need specific chemical and physical properties of the materials and require the surface functionalization to provide the affinity to the biomarkers. We have developed a class of amphiphilic triblock polymer coated, size uniform and tunable magnetic iron oxide nanoparticles (magIO) that exhibit high T2 relaxitivity for MRI contrast enhancement and can be readily used for further surface modifications in molecular MRI applications. The chemical, physical and MRI properties of this material are reported here.

MATERIAL AND METHODS

Iron Oxide Nanoparticle Preparation Size uniform and tunable magIO nanoparticles were formed from the reaction of the iron oxide powder and the mixture of oleic acid and octadecene at 300 °C and collected as the precipitate by adding chloroform and acetone into the reaction mixture. Amphiphilic riblock polymer with carboxylate groups were used to coat and stabilize the magIO nanoparticles. The core size of IO nanoparticles were controlled at the different sizes, i.e., 4, 10, 15, 20, 30, 40 nm, respectively, in this study. the thickness of triblock polymer coating is 5 nm typically.

Transmission Electron Microscopy (TEM) TEM images were taken on a JEOL-200 instrument with an acceleration voltage of 200 kV. All of the samples were purified by acetone precipitation from chloroform solution. Formvar film-coated copper grids were dipped in the chloroform solutions to deposit nanocrystals onto the film.

Magnetism and Surface Charges Magnetic measurements were performed at 10 kG in the temperature range 300 K with a SQUID MPMS-7 magnetometer. The surface charges were determined using Malvern Zetasizer (Nano ZS90).

Measurement of MRI Relaxivities T1 and T2 relaxivities of phantoms with different magIO suspensions were measured on 3T (Philips Intera) using sequences of inversion recovery for T1 and CPMG for T2, respectively.

Surface Functionalization and Conjugation The carboxylate groups on the triblock polymer coating can be used directly for cross-linking reaction to amine groups, or can be activated with coupling of Ni-NTA. The functionalization of this nanoparticle formulation was tested using Ni-NTA coupled particles conjugated to the amino terminal fragment (ATF) peptide (135 amino acids, **Fig. 1**) of urokinase plasminogen activator (uPA), a protease that regulates matrix degradation, cell motility and angiogenesis. Cellular receptor of uPA (uPAR) is highly expressed in various cancer cells. Gene construct for mouse ATF (1-135 aa) was cloned into pET bacteria-expressing plasmid and produced the His-tagged ATF peptides in E. coli. His-tagged ATF peptides were then purified using a Ni-protein purification column. Various experiments were performed to examine the target specificity of ATF-magIO to the different cell lines with different level of uPAR expression, using multi-echo T_2 measurement of phantoms of cells incubated with IO nanoparticles.

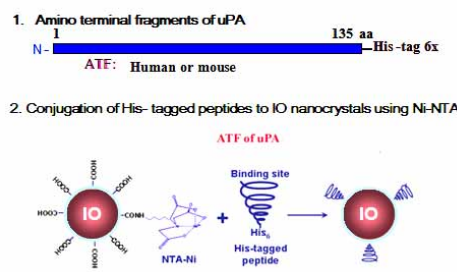

Fig. 1. ATF peptide from mouse or human breast cancer cells can be prepared with histidine-tag and conjugated to IO nanocrystals via –COOH of surface coating polymer.

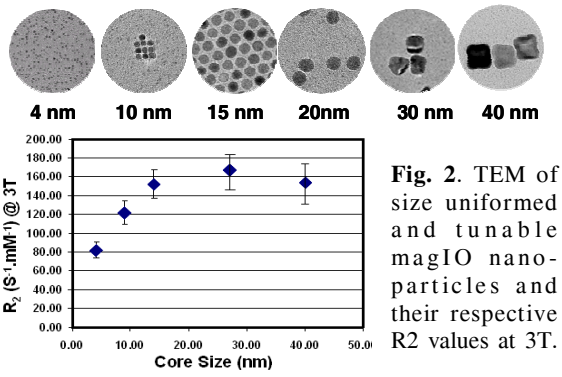

Fig. 2. TEM of size uniformed and tunable magIO nano-particles and their respective R2 values at 3T.

RESULTS

TEM showed highly uniform size of magIO nanoparticles (**Fig. 2**). Such size tunable IO nanoparticle exhibited size dependent chemical and physical properties between 4 - 30 nm, i.e., increased magnetism from 69 to 140 emuOe/g [Fe] and increased surface charge (ζ) from -11 to -45 mV, which indicates increased surface area and numbers of $–COO^-$ groups. The size related properties change also correlated to the change in the relaxivities, e.g., with R_2 increases from 67 to 156 ($S^{-1}.mM^{-1}$) at 3T (**Fig. 2**).

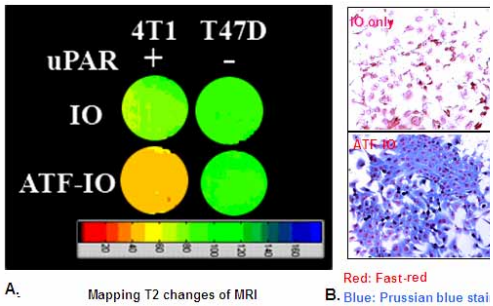

Fig 3. T_2 change (red) in MRI showed the specific binding of ATF-magIO to uPAR(+) 4T1 cells overexpressed with (**A**). IO-ATF binding to 4T1 cells was confirmed by Prussian blue staining (**B**).

When conjugated with receptor uPAR targeted ATF peptides, ATF-magIO nanoparticles exhibited highly specific binding to the mouse mammary cancer cells (4T1) with uPAR overexpression in vitro compared to those of uPAR- cells (T47D), which were demonstrated by T2 changes of cell phantoms (**Fig 3A**) in T2 relaxometry mapping. The receptor specific binding of ATF-magIO nanoparticles was further confirmed by positive Prussian blue staining of iron (Fig. 3B). In addition, confocal microscopy showed receptor mediated cellular internalization of megIO nanoparticles, suggesting this form of IO nanoparticles can be also used for magnetic cell labeling applications.