Fluorescein - PEI surface Functionalized Gd_2O_3 Nanoparticles for Dual Imaging

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Introduction

MRI is a very useful technique in diagnosing diseases because of its high spatial resolution and good sensitivity. Detection of diseases can be further improved by using MRI contrast agents through contrast enhancement. Fluorescein coated gadolinium oxide nanoparticles are promising candidates as either *T1* MRI-CL or MRI-FI dual agents because gadolinium oxide nanoparticles show a longitudinal relaxivity (*r1*) which is much larger than those of Gd (III) -chelates while dyes generally provide a very strong fluorescent intensity. The enhanced *r1* of gadolinium oxide nanoparticles with respect to those of Gd (III) - chelates is due to a high density of probing Gd (III) ions in nanoparticles.

Gadolinium oxide nanoparticles must be biocompatible and completely excreted from the body through the renal system to avoid any danger such as nephrogenic systemic fibrosis. Therefore, nanoparticles should be well-coated with water-soluble and biocompatible ligands. In this work, fluorescein is conjugated to hydrophilic and biocompatible polyethyleneimine (PEI) (Mn = y1200 amu) to improve both water-solubility and biocompatibility of fluorescein through the EDC/NHS coupling method. Finally, fluorescein-PEI was conjugated to gadolinium oxide nanoparticles. We demonstrated MRI-CL dual functionality of fluorescein-PEI coated gadolinium oxide nanoparticles both in vivo and in vitro.

Materials and methods

2 mmol of $GdCl_3.xH_2O$ was added to 20 mL of triethylene glycol in a three-necked flask and magnetically stirred at 80 °C. Then, 6 mmol of NaOH was added and stirred for 2 h. Then, 3.5 mL of H_2O_2 solution was added. After 2 h the product solution was cooled to room temperature and then washed three times with triply distilled water. Fluorescein-PEI was synthesized by using the EDC/NHS coupling method. 2 mmol of EDC and 2 mmol of NHS were added to 20 mL of PBS (pH = 6) at room temperature and under atmospheric condition. Here, a pH = 6 of PBS was obtained by slowly adding 1 mM HCl dropwise to the original PBS with pH = 7.2. After magnetic stirring for 15 min, 0.1 mmol of fluorescein was added to the solution and then the solution was magnetically stirred for 2 h. Then, 1.2 mL of PEI solution was added to the above solution with magnetic stirring for an additional 2 h to obtain fluorescein-PEI. Gadolinium oxide nanoparticles were added to the above fluorescein-PEI solution. The reaction mixture was magnetically stirred for 16 h; the product solution was transferred into a 1 L beaker containing 500 mL of triply distilled water, and then washed with triply distilled water three times.

Result and discussion

HRTEM images of fluorescein-PEI coated gadolinium oxide nanoparticles range from 2 to 6 nm. The core davg and αavg of fluorescein-PEI coated gadolinium oxide nanoparticles were estimated to be 3.92 and 7.5 nm, respectively. The surface coating of the nanoparticles by fluorescein-PEI was confirmed by recording FT-IR absorption spectra of a powder samples.

The magnetic properties of fluorescein-PEI coated gadolinium oxide nanoparticles were characterized by recording both *M–H* curves (-5 ≤ H ≤ 5 tesla) at *T* = 5 and 300 K and ZFC *M–T* Curves (5 ≤T ≤ 330 K) at *H* = 100 Oe. The net magnetization of gadolinium oxide nanoparticles was estimated by using the net mass of gadolinium oxide nanoparticles estimated from a TGA analysis. These nanoparticles showed *r1* and *r2* of 6.76 and 20.27 s^{-1} mM^{-1}, respectively, and fluorescence at ~527 nm (green region). The MRI functionality was demonstrated through a clear positive contrast enhancement in 3 tesla *T1* MR Images of a rat with a N1S1 liver tumor after intravenous injection of a sample solution into a rat tail vein while the CL functionality, through fluorescent confocal images of DU145 cells after incubation with a sample solution. These two results together clearly show the excellent *T1* MRI-CL dual functionality of fluorescein-PEI coated gadolinium oxide nanoparticles.

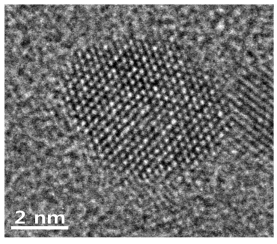
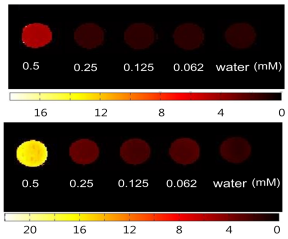


Fig. 1 HRTEM images



R1 map

R2 map

Fig. 2 Map images

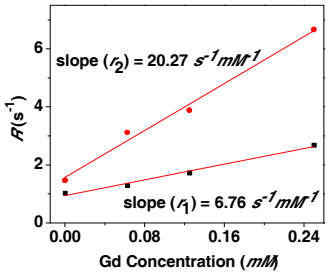


Fig. 3 Measurement of relaxivity

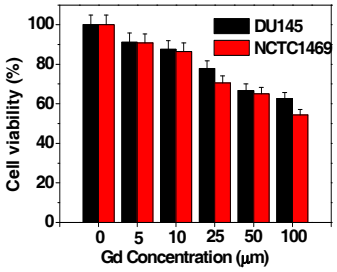


Fig. 4 In vivo cytotoxicity tests

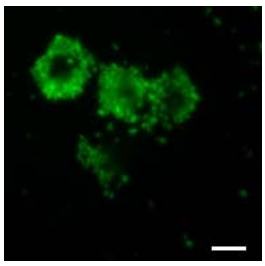


Fig. 5 Confocal images

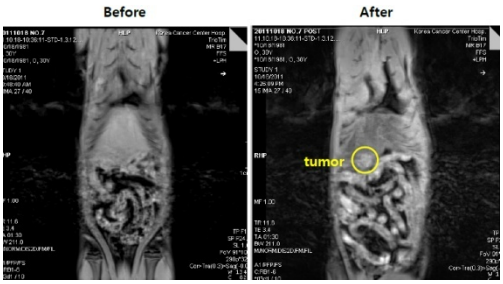


Fig. 6 3 tesla T_1 spin echo MR images of a rat with a N1S1 liver tumor before and 4.5 h after injection of fluorescein-PEI coated gadolinium oxide nanoparticles.