Bovine Serum Albumin (BSA) as Ultrasmall Nanoparticles Carrier: Application to MRI Contrast Agents

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Introduction

Biological molecules have attracted considerable interest for applications in nanomedicine because they are fully biocompatible and water-soluble. Bovine serum albumin (BSA) is an important carrier protein in blood plasma for several ions and molecules. Because it is large in size and heavy in mass (M_n = ~ 66.5 kD), it may carry ultrasmall nanoparticles (NPs) with a smaller size and mass than BSA. Biological molecules have several advantages over small molecules and polymers for biomedical applications in nanomedicine. First, the water-solubility of surface-modified NPs generally increases with increasing mass of the ligands and biological molecules will provide enhanced water-solubility for NPs. Second, the NPs conjugated to biological molecules can remain in the blood for a longer duration than free NPs and Gd-chelates, allowing longer imaging times (so called blood-pool contrast enhancing agents) and a higher likelihood of delivering NPs to the targeted areas in a body.

This study makes use of BSA to carry ultrasmall Gd_2O_3 NPs (GNPs) for magnetic resonance imaging (MRI). Ultrasmall GNPs have shown longitudinal (r_1) and transverse (r_2) water proton relaxivities larger than Gd-chelates because of the dense population of Gd(III) in NPs. Therefore, BSA, which can carry many ultrasmall GNPs may be useful for MRI. To conjugate GNPs to BSA, the GNPs were surface modified with polyethyleneglycol diacid (PEGD) and then conjugated to BSA via amide bonding. The particle sizes, surface modifications, water proton relaxivities, cellular toxicities, and in vivo MR images using mice were characterized. The BSA conjugated GNPs were applied to MRI and contrast enhancements in 3 tesla T_2 MR images were clearly observed.

Materials and Methods

PEGD coated ultrasmall gadolinium oxide nanoparticles were synthesized in one-pot. Two separate solutions were prepared: One is a precursor solution made from 5 mmol of $GdCl_3.xH_2O$ in 25 mL of triethylene glycol and the other is a NaOH solution made from 15 mmol of NaOH in 10 mL of triethylene glycol. The precursor solution was heated to 100 °C with magnetic stirring until the precursors were completely dissolved. The NaOH solution was then poured into the precursor solution. The mixed solution was magnetically stirred at 180 °C for 4 hours in air. The solution temperature was then lowered to 80 °C and 5 mmol of PEG was added to the solution, the solution temperature was again raised to 180 °C and stirred for additional 4 hours. The solution was then cooled to room temperature and transferred to a 1 L beaker containing 500 mL of triply distilled water. The supernatant was decanted and the remaining sample solution was washed with triply distilled water. This procedure was repeated three times. Conjugation of BSA with GNPs was completed by using the EDC/NHS coupling method. 5 mmol of EDC and 5 mmol of NHS were added to 20 mL of PBS (pH = 6) at room temperature and under atmospheric condition. Here, the pH = 6 of the PBS was obtained by slowly dropping 1mM HCl to the original PBS with pH = 7.2. After magnetic stirring for 15 minutes, PEG-diacid coated Gd_2O_3 nanoparticles were added to the solution and then, the solution was magnetically stirred for 2 hours. Then, 1.5 g of BSA was added to the above solution with magnetic stirring for additional 2 hours to obtain BSA coated Gd_2O_3 nanoparticles. The solution was then transferred to a 1 L beaker containing 500 mL of triply distilled water. The top transparent solution was decanted and the remaining sample solution was washed with triply distilled water. This procedure was repeated three times. The first half volume of the sample solution was used to prepare a MRI sample solution. The remaining half volume was subjected to a powder form by drying it in air for various characterizations. All chemicals purchased from Aldrich.

Result and Discussion

The surface coating of ultrasmall GNPs with PEGD followed by conjugation to BSA was investigated by FT-IR absorption spectroscopy (Fig. 1). FT-IR spectra of the BSA-PEGD-GNPs (blue line) are very different from that of the pure BSA (red line) and pure PEGD (black line). These stretching clearly confirmed the successful coupling of BSA with PEGD-GNPs. Figs. 2 present HVEM images of the BSA-PEGD-GNPs. The particle diameters of the ultrasmall GNPs ranged from 1 to 3 nm with d_{avg} of 2.0 nm. HVEM images of BSA-PEGD-GNPs indicated that many ultrasmall GNPs were conjugated to a BSA. The r_1 and r_2 values of BSA-PEGD-GNPs were estimated to be 6.0 s^{-1} mM^{-1} and 28.0 s^{-1} mM^{-1}, respectively (Fig. 3). The r_2 values are significantly larger than that of molecular Gd-DTPA, which is why only NPs are eligible as T_2 MRI contrast agents. Aqueous solutions of BSA-PEGD-GNPs showed clear dose-dependent contrast enhancements in their R_1 and R_2 map images (Fig. 4). The in vitro cytotoxicity of the aqueous sample solutions of BSA-PEGD-GNPs was measured using DU145 and NCTC1469 cells with Gd concentrations up to 500 mM (Figs. 5) The results showed that BSA-PEGD-GNPs were slightly toxic because GNPs in BSA-PEGD-GNPs are found mostly on the surface of BSA, as shown in the HVEM image (Fig. 2). In vivo MRI experiment was carried out using an aqueous sample solution of BSA-PEGD-GNPs. 3 tesla T_2 MR images of the mouse liver were taken before and after injecting the aqueous sample solution. As shown in Fig. 6, appreciable negative contrast enhancements were observed clearly in the liver 10 minutes after the injection, which returned to almost the original contrast after 24 hours due likely to the excretion of BSA-PEGD-GNPs.

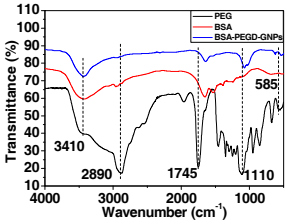

Figure 1. FTIR of PEG, BSA, and BSA-PEGD-GNPs.

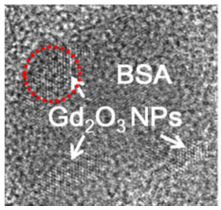

Figure 2. HRTEM images of BSA-PEGD-GNPs.

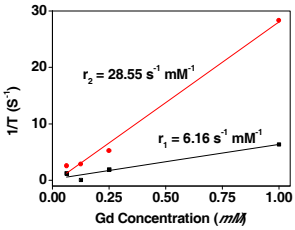

Figure. 3. Plot of $1/T_1$ and $1/T_2$ inverse relaxation times of sample solution of BSA-PEGD-GNPs.

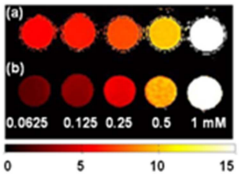

Figure 4. (a) In vitro T_1 and (b) T_2 map images showing contrast enhancements in both T_1 and T_2 map images with increasing dose of BSA-PEGD-GNPs.

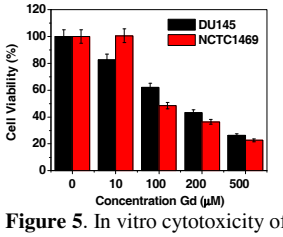

Figure 5. In vitro cytotoxicity of aqueous sample solutions of BSA-PEGD-GNPs using both DU145 and NCTC1469 cells.

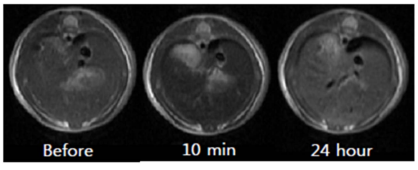

Figure 6. 3 tesla T_2 MR images of the liver of a mouse before and after injecting an aqueous sample solution of BSA-PEGD-GNPs into a mouse tail vein.