Multifunctional GdNPs; from probe design to imaging targeting of cancer

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Introduction

Magnetic resonance imaging (MRI) has proved to be a powerful non-invasive technique. The contrast of the resulting image can be enhanced by injection of paramagnetic or superparamagnetic agents. In recent years, interest in gadolinium nanoparticles (GdNPs) has risen in the expectation that these systems would serve not only as MRI probes but also as therapeutic agents through Gd-based neutron capture therapy (Gd-NCT) and chemotherapy in conjunction with those possessing anti-tumor activity. Benzothiazoles are known to possess tumor-targeting and antitumor properties in select breast, ovarian and renal cancer cell lines. More recently, conjugates of benzothiazole with DO3A (DO3A-BTA) has been provided as bimodal imaging probes such as optical/MRI and SPECT/MRI. Motivated by this fact to develop a new gadolinium based nano-system of multifunctional MRI contrast agents (CAs), herein we reported the synthesis, tumor-targeting properties, and *in vivo* application of Gd@SiO_2–NH_2 coated with DO3A-BTA, Gd@SiO_2-NHCO-DO3A-BTA, as a new nano-system of a T_1 weighted potential theranostic agents.

Material and Methods

All reactions were carried out using the standard techniques. Solvents were purified and dried using standard procedures. Characterization of new systems have been performed by analytical and various spectroscopic techniques (MRI, TEM, FT-IR). T_1 measurements were carried out using an inversion recovery method with variable inversion time (TI) at 1.5 T (64 MHz). T_1 relaxation times were obtained from the non-linear least square fit of the signal intensity measured at each TI value. T_2 relaxation times were obtained from the non-linear least squares fit of the mean pixel values for the multiple spin-echo measurements at each echo time (TE). Seventeen-week-old female balb/c nude mice bearing MDA-MB-231 tumor were used for the MRI. The mice were anesthetized with 1.5% isoflurane in oxygen. Measurements were made before and after injection of Gd@SiO_2-NHCO-DO3A-BTA via intraperitoneal. The amount of CA per each injection is 0.05 mmol [Gd]/kg for MR images. Whole body MR images were obtained with a 1.5 T MR unit (GE Healthcare, Milwaukee, WI, U.S.) equipped with a homemade small animal rf coil. The coil was of the receiver type with its inner diameter being 50 mm. The imaging parameters for SE (Spin echo) are as follows: repetition time (TR) = 500.0 ms; echo time (TE) = 13.0 ms; 7.0 mm field of view (FOV); 192×128 matrix size; 1.2 mm slice thickness; number of acquisition (NEX) = 6. Images were obtained during 330 min after injection. MCF-7, MDA-MB-231, SK-HEP-1, Caki, HeLa cells were plated on 35 mm corning dishes and cultured for 24 h. The medium was removed. Gd@SiO_2-NHCO-DO3A-BTA in DMEM serum-depleted media (containing 0.1% DMSO as a co-solvent) added, and incubation continued for 24 h. The stained cells were washed once with PBS buffer (pH 7.4). The cells were harvested with a solution of trypsin-EDTA (GIBCO, 0.25% trypsin, 1 mM EDTA•Na) after which DMEM was added. The cells were transferred to a 15 mL centrifuge tube to be centrifuged at 1000 rpm for 3 min. After removing the supernatant, DMEM was added and the cells transferred to a micro tube to be centrifuged at 6200 rpm for 3 min. The tube was filled with DMEM for MR measurement using 8-channel knee coil. T_1-weighted MRI parameters are as follows: FSE (Fast spin echo) sequence, TR = 500 ms; TE = 11 ms; 12 mm FOV; 192×128 matrix size; 1.5 mm slice thickness; NEX = 15.

Results and Discussion

Scheme 1 shows the route leading to the formation of amino functionalized gadolinium based nano-system conjugated with DO3A-BTA (Gd@SiO_2-NHCO-DO3A-BTA) for use as a new family of potential theranostic CAs. This system is completely soluble in water with the gadolinium concentration [Gd] up to 31.45 mg/mL (0.2 M). The R_1 relaxivities of Gd@SiO_2-NHCO-DO3A-BTA in water is 8.72 $mM^{-1}s^{-1}$ (Table 1). Figure 1 shows T_1-weighted MR images of MCF-7, MDA-MB-231, SK-HEP-1, Caki, HeLa cells incubated with Gd@SiO_2-NHCO-DO3A-BTA (100 μM) for 24 h, present the tumor-specific nature of the new nano-system. Figure 2 shows the coronal T_1-weighted images of mice obtained by intraperitoneal injection. The pattern of *in vivo* MR images compares well with conventional low molecular-weight ECF MRI CAs based on Gd-chelates such as Dotarem® with signal enhancement in liver, kidney, and bladder. In addition, this system is to be noted that excretion is made via bile duct, confirming hepatobiliary uptake. Further studies were carried out to investigate the antitumor activity of Gd@SiO_2-NHCO-BTA. Indeed, anti-tumor activity toward the various cancer cells such as toward MCF-7, MDA-MB-231, SK-HEP-1, Caki, HeLa was clearly observed to make this system a new family of theranostic agents. These results will also be presented in detail in the poster.

Conclusions

The study represnted successfully synthesized Gd@SiO_2-NHCO-DO3A-BTA as a new nano-system of potential multifunctional imaging probes with tumor-specificity. The same systems, when modified with BTA, exhibit anti-tumor activity in the other various cancer cells as well as Gd-NCT imaging properties.

Scheme 1. Synthesis of Gd@SiO_2-NHCO-DO3A-BTA.

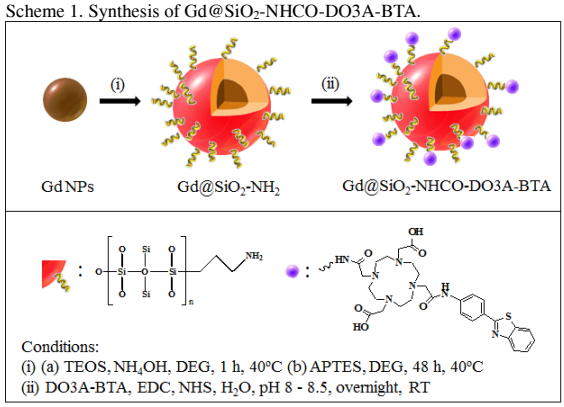

Table 1. Relaxivities of Gd@SiO_2-NHCO-DO3A-BTA at the 1 mM concentrations and 293 K.

	Gd@SiO_2-NHCO-DO3A-BTA
R_1 [mM^{-1} s^{-1}]	8.72 ± 0.74
R_2 [mM^{-1} s^{-1}]	7.67 ± 0.08

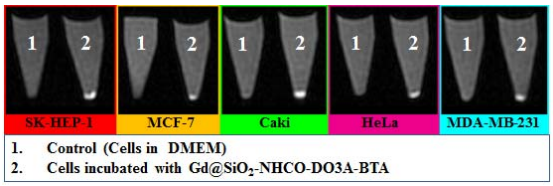

Figure 1. *In vitro* T_1-weighted MR images of SK-HEP-1, MCF-7, Caki, HeLa, MDA-MB-231 cells incubated with Gd@SiO_2-NHCO-DO3A-BTA (100 μM) for 24 h.

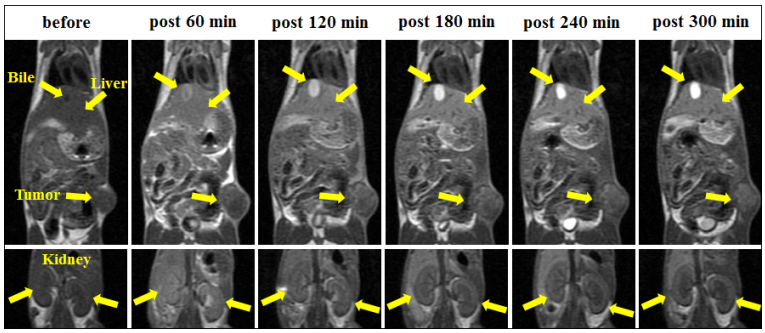

Figure 2. *In vivo* MR coronal images of mice obtained with Gd@SiO_2-NHCO-DO3A-BTA at 1.5 T and 293 K.