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A cancer-recognizable MRI contrast agents using pH-responsive polymeric micelle

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ABSTRACT

A cancer-recognizable MRI contrast agents (CR-CAs) has been developed using pH-responsive polymeric micelles. The CR-CAs with pH sensitivity were self-assembled based on well-defined amphiphilic block copolymers, consisting of methoxy poly(ethylene glycol)-*b*-poly(L-histidine) (PEG-p(L-His)) and methoxy poly(ethylene glycol)-*b*-poly(L-lactic acid)-diethylenetriaminopentaacetic acid dianhydride-gadolinium chelate (PEG-p(L-LA)-DTPA-Gd). The CR-CAs have a spherical shape with a uniform size of ~40 nm at physiological pH (pH 7.4). However, in acidic tumoral environment (pH 6.5), the CR-CAs were destabilized due to the protonation of the imidazole groups of p(L-His) blocks, causing them to break apart into positively charged water-soluble polymers. As a result, the CR-CAs exhibit highly effective T_1 MR contrast enhancement in the tumor region, which enabled the detection of small tumors of ~3 mm^3 *in vivo* at 1.5 T within a few minutes.

1. Introduction

Accurate diagnosis of cancer is essential for improving the survival rate of cancer patients [1,2]. Magnetic resonance (MR) imaging with a contrast agent has been widely used as a powerful tool for cancer diagnosis [3–7]. The most extensively and clinically used MRI contrast agents are paramagnetic gadolinium (Gd^{3+}) chelates, which enhance the signal intensity by reducing the longitudinal relaxation time (T_1) of water protons close to the Gd^{3+} ion [8,9]. Despite the biomedical potential of MRI, the use of clinically available Gd^{3+}-based contrast agents is compromised by their intrinsic low efficiency, which results in a need for high doses [10]. Furthermore, most MRI contrast agents are non-targeted for cancer and passively distributed throughout the body [11]. To overcome the limitations of small molecule MRI contrast agents, various nanoparticle systems, *e.g.*, micelles [12–14], gold [15] and inorganic nanoparticles [16,17], and lipid nanoparticles [18], have been explored as platforms for the conjugation of small molecule contrast agents. Although this approach has led to enhanced MR relaxivity, many of these systems do not achieve rapid diagnosis within a few minutes, which is attributed to their time-dependent accumulation and the heterogeneous nature of tumors [19–21].

In this respect, our goal herein is to develop a cancer-recognizable MRI contrast agents (CR-CAs) that are nano-sized and pH-responsive. In the structural design, a pH-sensitive block copolymer can be employed as a component of CR-CAs (Scheme 1a). The CR-CAs can be formed as stable micelles at a neutral pH (*e.g.,* in normal tissues, pH 7.4) and with decreased T_1-relaxivity. However, acidic environments (*e.g.,* in tumor tissues, pH 6.5) destabilize the CR-CAs, causing them to break apart into charged water-soluble polymers with increased T_1-relaxivity due to the interaction between water molecules around the acidic tumor tissue and exposed Gd^{3+} from the micelle core (Scheme 1b) [22]. In addition, the CR-CAs' core is positively charged by protonation of the pH-sensitive moiety (*i.e.*, imidazole groups) after extravasation, which rapidly facilitates accumulation of CR-CAs compared with pH-insensitive micelle-based CAs due to the strengthened interaction between the positively charged CR-CAs and the negatively charged cellular membrane. Therefore, CR-CAs enable accurate and speedy cancer diagnosis (Scheme 1c).

In this study, the CR-CAs was prepared and characterized *in vitro* and *in vivo*. *In vivo* optical and MR imaging techniques were used to exploit to the cancer specific targeting and diagnosis effect of the CR-CAs in a small sized CT26 tumor (~3 mm^3)-bearing mice model.

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2. Materials and methods

2.1. Materials

N_{α}-Boc-$N_{(im)}$-2,4-dinitrophenyl-L-histidine (Boc-His(DNP)-OH), isopropanol solvate and methoxy poly(ethylene glycol) amine hydrochloride salt (PEG-NH_2·HCl, 2 kDa) were purchased from Bachem Americas, Inc. (Torrance, CA) and Jenkem Technology USA, Inc. (Allen, TX), respectively. Methoxy poly(ethylene glycol) (PEG-OH, 2 kDa), L-Lactide (L-LA), Toluene, diethyl ether, 1,4-dioxane, acetone, dimethyl sulfoxide (DMSO), *N*,*N*-dimethylformamide (DMF), deuterium oxide (D_2O), tetrahydrofuran (THF), thionyl chloride, 2-mercaptoethanol, diethylaminoethyl (DEAE) Sephadex A-25, potassium tetraborate, ammonium bicarbonate, 4-di-methylaminopyridine (DMAP), *N*,*N*-de dicyclohexylcarbodiimide (DCC), *N*-hydroxysuccinimide (NHS), *N*-(3-Dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDC), stannous octoate, diethylene triamine pentaacetic (DTPA) dianhydride, and $GdCl_3·6H_2O$ were purchased from Sigma (St. Louis, MO). Cy5.5 was purchased from Lumiprobe corporation (Florida, USA). PEG-poly(L-histidine) (PEG-p(L-His)) was synthesized following our previously reported procedures (Scheme S1a) [23–25]. Dialysis membrane (molecular weight cut-off (MWCO, 1 and 3.5 kDa) was purchased from Spectrum Laboratories Inc. (Rancho Dominguez, CA). Fetal bovine serum (FBS), 0.25% (w/v) trypsin – 0.03% (w/v), EDTA solution, and RPMI1640 medium were purchased from Gibco (Uxbridge, UK). Chang Liver cells were acquired form from the American Type Culture Collection (ATCC CCL 13, USA). The ^{1}H NMR spectra were recorded using a Bruker NMR Spectrometer (300 MHz). The samples were prepared by adding aliquots of products (10 mg) into a deuterated solvent. The ^{1}H NMR chemical shifts are reported in ppm and calibrated against TMS (δ 0). The gadolinium (Gd^{3+}) contents were estimated using inductively coupled plasma-optical emission spectrometry (ICP-OES) (Perkin–Elmer, Optima 4300 DV, Norwalk, CT, USA).

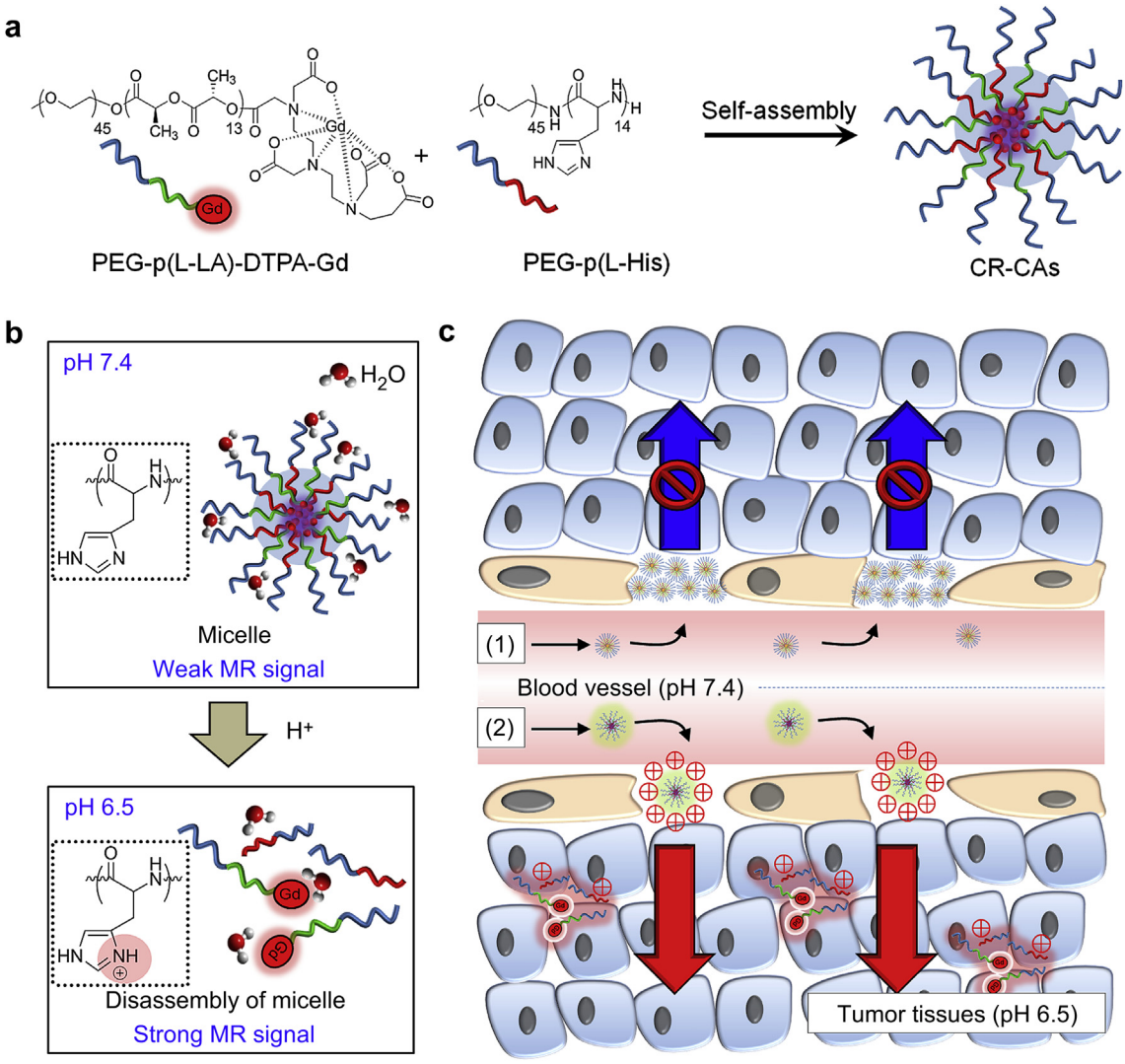
2.2. Synthesis of PEG-p(L-LA)-DTPA

PEG-p(L-LA) diblock copolymer was synthesized by ring-opening polymerization (ROP) of L-lactide initiated by the hydroxy group of PEG-OH in the presence of the catalyst stannous octoate (Scheme S1b) [26]. Briefly, L-lactide (10 g, 70 mmol) and stannous octoate (1 wt.% of L-lactide) were dissolved in 80 mL of toluene at 60 °C in a round-bottom flask equipped with a Dean–Stark trap. After L-lactide was completely dissolved, the reaction was carried out under a nitrogen environment for 24 h at 110 °C. The polymer was precipitated by pouring the cooled solution in an excess amount (~20-fold) of diethyl ether with vigorous stirring, and then it was filtrated. The collected white polymer was dried *in vacuo* for 24 h.

For PEG-p(L-LA)-DTPA, PEG-p(L-LA) (540 mg, 0.12 mmol) was added to DTPA dianhydride (54 mg, 0.15 mmol) in DMSO. The reaction mixture was stirred for 24 h at RT, and then the reactant mixture was dialyzed using a dialysis membrane (MWCO, 1 kDa) against deionized water (DIW) for 2 days. The final products (*i.e.*, PEG-p(L-LA)-DTPA) were lyophilized. The synthesized PEG-p(L-LA)-DTPA was characterized by ^{1}H NMR spectrophotometers (Fig. S1) and Fourier transform infrared spectrometry (FT-IR, Shimadzu FTIR-8700) (Fig. S2).

2.3. Gadolinium (III) chelation to PEG-p(L-LA)-DTPA

$GdCl_3·6H_2O$ (13 mg, 0.035 mmol) was added to PEG-p(L-LA)-DTPA (225 mg, 0.05 mmol) in 60 mL of water-dioxane (1:2 v/v) co-solvent. Subsequently, the pH of



Scheme 1. a) Schematic representation of the preparation of the cancer-recognizable MRI contrast agents (CR-CAs); Amphiphilic block copolymers (*i.e.*, PEG-p(L-LA)-DTPA-Gd and PEG-p(L-His) self-assemble into micelles in an aqueous solution at pH 7.4. b) Schematic representation of pH-dependent structural transformation and related MR signal change in CR-CAs. Inset: Chemical structural representation of the protonation of imidazole groups in PEG-p(L-His) at acidic pH. c) Schematic representation of the tumor-accumulation behavior of (1) conventional micelle-based CAs and (2) CR-CAs.