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Surface charge switching nanoparticles for magnetic resonance imaging

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ABSTRACT

In this study, polypeptide-based nanoparticles [constituted using poly(L-lysine) coupled with deoxycholic acid (DOCA) and conjugated with 2,3-dimethylmaleic acid (DMA)] have high tumor selectivity once electrostatically switched by the acidic milieu of solid tumors. These nanoparticles exhibited a significantly increased *in vitro* cellular uptake and high accumulation in the acidic tumor site *in vivo*. Consequently, Fe_3O_4-loaded nanoparticles enabled high contrast magnetic resonance (MR) imaging of the tumor *in vivo*.

1. Introduction

The precise control of nanoparticle systems for highly efficient magnetic resonance (MR) imaging is challenging (Mornet et al., 2004; Gupta and Gupta, 2005; Sun et al., 2008; Na et al., 2009; Veiseh et al., 2010; Yoo et al., 2011; Rümenapp et al., 2012; Lee et al., 2011a,b, 2012a,b). Only a few nanoparticles have been applied clinically because of the complicated clinical condition (Veiseh et al., 2010; Yoo et al., 2011; Rümenapp et al., 2012; Lee et al., 2012a,b). For example, the permeability and targeting of these nanoparticles to tumor sites may be not the same for different tumor tissues (Peer et al., 2007). Possible tumor microenvironmental intra- and intercellular variables and the heterogeneity of tumor cells affect the efficiency of MR imaging by nanoparticles (Peer et al., 2007; Veiseh et al., 2010). These factors may eventually complicate the development of MR imaging nanoparticles on a case-by-case basis. Fortunately, characteristic features (*e.g.*, acidic tumor pH) in tumor biology can serve as an excellent platform to develop novel MR imaging nanoparticles. The neutral or acidic pH of the tumor extracellular environment resulting from the high rate of anaerobic glycolysis (the Warburg effect) of tumor cells can be targeted using sophisticatedly designed nanoparticles (Tannock and Rotin, 1989; Gerweck and Seetharaman, 1996; Kim and Dang, 2006). Indeed, pH-sensitive positron emission tomography (PET) radiotracers, MR spectroscopy, MR imaging, and other optical imaging have shown that the pH of the tumor extracellular environment does not exceed pH 7.4 and is rather acidic (pH 5.8–7.2) when compared with normal tissue or blood (pH 7.4) (Tannock and Rotin, 1989; Gerweck and Seetharaman, 1996; Kim and Dang, 2006; Oh et al., 2012). Remarkably, the low pH of the tumor site consequently inspires the development of pH-responsive nanoparticles that are able to improve tumor recognition efficiency in MR imaging (Oh et al., 2009a,b, 2010, 2012, 2013; Lee et al., 2010, 2011b; Park et al., 2011; Baik et al., 2011). These nanoparticles should prove valuable for tumor diagnosis with greater efficacy in the future.

In this study, we developed a polypeptide-based nanoparticle with molecular sensitivity for tumor extracellular pH (tumor pH_e). First, we modified a backbone polymer, poly(L-lysine) [poly(Lys)] via a coupling process of deoxycholic acid (DOCA) to terminal amino groups of poly(Lys) and the conjugation of 2,3-dimethylmaleic acid (DMA) to the pendant ε-amino groups of poly(Lys) using *N*,*N*′-dicyclohexylcarbodiimide (DCC) and *N*-hydroxysuccinimide (NHS) (Supporting Fig. S1), finally producing deoxycholic acid-*b*-poly(Lys-2,3-dimethylmaleic acid) [DOCA-*b*-poly(Lys-DMA)]. We prepared DOCA-*b*-poly(Lys-DMA) nanoparticles via a self-assembly pathway of DOCA-*b*-poly(Lys-DMA) in aqueous solution and investigated the effectiveness of DOCA-*b*-poly(Lys-DMA) nanoparticles for tumor-recognizable MR imaging.

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2. Materials and methods

2.1. Materials

N^{ε}-Benzyloxycarbonyl-L-lysine, triphosgene, anhydrous 1,4-dioxane, n-hexane, anhydrous diethyl ether, hexylamine, anhydrous dimethylformamide (DMF), deoxycholic acid (DOCA), *N*, *N*′-dicyclohexylcarbodiimide (DCC), *N*-hydroxysuccinimide (NHS), trifluoroacetic acid (TFA), 33% HBr in acetic acid, 2,3-dimethylmaleic anhydride, succinic anhydride, triethylamine (TEA), pyridine, dimethyl sulfoxide (DMSO), iron (III) acetylacetonate, 1,2-hexadecanediol, dodecanoic acid, dodecylamine, benzyl ether, and sodium tetraborate ($Na_2B_4O_7$) were purchased from Sigma–Aldrich (USA). Chlorin e6 (Ce6) was obtained from Frontier Scientific Inc. (USA). RPMI-1640, fetal bovine serum (FBS), penicillin, and streptomycin were purchased from Welgene Inc. (Korea). Wheat germ agglutinin, Alexa Fluor® 488 Conjugate (WGA-Alexa Fluor® 488) was supplied from Lifetechnologies (USA). Feridex® was obtained from Advanced Magnetics, Inc. (USA). Fe_3O_4 nanoparticles (size 12 nm) were synthesized as described in detail in our previous report (Lee et al., 2011b). Briefly, iron (III) acetylacetonate (10 mmol), 1,2-hexadecanediol (50 mmol), dodecanoic acid (30 mmol), and dodecylamine (30 mmol) were dissolved in benzyl ether (50 mL) and heated to 150 °C for 30 min and to 300 °C for 30 min. After the reaction, the product was purified with an excess quantity of ethanol (400 mL).

2.2. Synthesis of *DOCA*-b-*poly(Lys-DMA)*

Poly(N^{ε}-benzyloxycarbonyl-L-lysine) [poly(Lys-cbz)], a backbone polypeptide, was prepared via the ring opening polymerization of *N*-carboxy-(N^{ε}-benzyloxycarbonyl)-L-lysine anhydride (9 mmol) (recrystallized in excess n-hexane after the chemical reaction of N^{ε}-benzyloxycarbonyl-L-lysine and triphosgene in anhydrous 1,4-dioxane at 50 °C for 2 h) in the presence of hexylamine (initiator) (0.3 mmol) in anhydrous DMF (30 mL) at room temperature for 3 days, as described by van Dijk-Wolthuis et al. (1997). The resulting solution was recrystallized in excess diethyl ether, resulting in yielding poly(Lys-cbz). The terminal amine group of poly(Lys-cbz) (1 mmol) was reacted with the succinylated DOCA (DOCA-NHS, 3 mmol) (prepared after the pre-activation process of the carboxyl group of DOCA using DCC and NHS) in DMF (30 mL) containing TEA (1 mL) at room temperature for 1 day. The solution was filtered and was lyophilized after adding excess anhydrous diethyl ether, resulting in the production of DOCA-*b*-poly(Lys-cbz). Next, the benzyloxycarbonyl groups of the resulting polymer (Lys repeating unit 18.9) were removed with TFA (5 mL)/33% HBr in acetic acid (5 mL) at room temperature for 30 min. The resulting solution was recrystallized in excess ethanol/diethyl ether (50:50 vol.%), filtered and lyophilized. The obtained DOCA-*b*-poly(Lys) (1 mmol) was again reacted with 2,3-dimethylmaleic anhydride (50 mmol) in DMSO (30 mL) containing TEA (1 mL) and pyridine (1 mL) at room temperature for 5 days, yielding DOCA-*b*-poly(Lys-2,3-dimethylmaleic acid) [DOCA-*b*-poly(Lys-DMA)] (Supporting Fig. S1). After the reaction was completed, the final solution was filtered and transferred to a pre-swollen dialysis membrane tube (Spectra/Por® MWCO 5 K) and was dialyzed against fresh DMSO (for 2 days) and $Na_2B_4O_7$ solution (pH 8.0, 5 mM, for 2 days) to remove non-reacted chemicals. The solution was withdrawn from a dialysis membrane tube and was freeze-dried for 2 days. In addition, DOCA-*b*-poly(Lys-succinic acid) [DOCA-*b*-poly(Lys-SA)] was synthesized using succinic anhydride instead of 2,3-dimethylmaleic anhydride as a control group. DOCA-*b*-poly(Lys) [DOCA-*b*-poly(Lys)] was synthesized after the deprotection of the benzyloxycarbonyl groups of DOCA-*b*-poly(Lys-cbz) using TFA (5 mL)/33% HBr in acetic acid at room temperature for 30 min.

2.3. Fe_3O_4-loaded nanoparticles preparation

Polymer (10 mg) and Fe_3O_4 nanoparticles (2 mg) dissolved in DMSO (2 mL) were transferred to a pre-swollen dialysis membrane tube (Spectra/Por® MWCO 15 K) and dialyzed against a phosphate buffer saline (PBS, 150 mM, pH 7.4) solution for 24 h. The outer phase was replaced three times with fresh PBS solution. The solution was subsequently lyophilized by freeze-drying for 2 days (Park et al., 2011). Here, Fe_3O_4 concentrations in nanoparticles were measured using a Jobin-Yvon Ultima-C inductively coupled plasma-atomic emission spectrometer (JY-Ultima-2, France). Before the test, nanoparticles (10 mg) dissolved in 2 mL of concentrated nitric acid were heated to 110 °C for 45 min and then diluted with deionized water.

In addition, nanoparticles containing fluorescent Ce6 dye were prepared in the same way using Ce6 instead of Fe_3O_4 for the *in vitro* cellular uptake test and *in vivo* photoluminescence imaging.

2.4. Particle size distribution

Particle size analysis was conducted using a Zetasizer 3000 (Malvern Instruments, USA) equipped with a He–Ne laser beam at a wavelength of 633 nm and a fixed scattering angle of 90° (Park et al., 2011).

2.5. Hyperspectral imaging analysis

The Hyperspectral imaging (HSI) system with a Nikon microscope equipped with a VNIR hyperspectral camera system, CytoViva dual mode fluorescence (DMF) module, and CytoViva high resolution adaptor (CytoViva, Auburn, AL, USA), was used to visualize the core and shell of the nanoparticles. Sample was prepared after drying 1–2 drops of nanoparticle solution (PBS pH 7.4) on a glass slide. Light illumination (for sample analysis) was manipulated using a DC-stabilized 150 W halogen light source. Lamp voltage was a constant 11 V. The internal neutral density filter was used to decrease light intensity. The core and shell of nanoparticles was visualized after collecting all visible-near infrared (VNIR) spectral data within each pixel of the scanned area and mapping spectral signatures from the newly scanned samples against the library database [stored in HyperVisual software (from the Institute for Technology Development (ITD), Stennis, Miss, USA] (Lee et al., 2012b).

2.6. Zeta-potential analysis

The zeta-potential change of each nanoparticle sample (0.1 mg/mL, PBS 150 mM) at different pH values (pH 7.4–5.0) was measured with a Zetasizer 3000 (Malvern Instruments, USA) (Oh et al., 2012). Before the test, each sample was stabilized at room temperature for 4 h.

2.7. In vitro *cellular uptake test*

Human nasopharyngeal epidermal carcinoma KB cells (from the Korean Cell Line Bank) were maintained in RPMI-1640 medium with 1% penicillin-streptomycin and 10% FBS in a humidified standard incubator at 37 °C with a 5% CO_2 atmosphere. Prior to testing, cells (1×10^5 cells/mL) that were grown as a monolayer were harvested via trypsinization using a 0.25% (wt./vol.) trypsin/0.03% (wt./vol.) EDTA solution. KB cells suspended in RPMI-1640 medium were seeded onto each well plate and cultured for 24 h prior to the *in vitro* cell testing. The tumor cellular uptake of each