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Hierarchical self-assembly of magnetic nanoclusters for theranostics: Tunable size, enhanced magnetic resonance imagability, and controlled and targeted drug delivery

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ABSTRACT

Nanoparticle-based imaging and therapy are of interest for theranostic nanomedicine. In particular, superparamagnetic iron oxide (SPIO) nanoparticles (NPs) have attracted much attention in cancer imaging, diagnostics, and treatment because of their superior imagability and biocompatibility (approved by the Food and Drug Administration). Here, we developed SPIO nanoparticles (NPs) that self-assembled into magnetic nanoclusters (SAMNs) in aqueous environments as a theranostic nano-system. To generate multi-functional SPIO NPs, we covalently conjugated β-cyclodextrin (β-CD) to SPIO NPs using metal-adhesive dopamine groups. Polyethylene glycol (PEG) and paclitaxel (PTX) were hosted in the β-CD cavity through high affinity complexation. The core-shell structure of the magnetic nanoclusters was elucidated based on the condensed SPIO core and a PEG shell using electron microscopy and the composition was analyzed by thermogravimetric analysis (TGA). Our results indicate that nanocluster size could be readily controlled by changing the SPIO/PEG ratio in the assemblies. Interestingly, we observed a significant enhancement in magnetic resonance contrast due to the large cluster size and dense iron oxide core. In addition, tethering a tumor-targeting peptide to the SAMNs enhanced their uptake into tumor cells. PTX was efficiently loaded into β-CDs and released in a controlled manner when exposed to competitive guest molecules. These results strongly indicate that the SAMNs developed in this study possess great potential for application in image-guided cancer chemotherapy.

Statement of Significance

In this study, we developed multi-functional SPIO NPs that self-assembled into magnetic nanoclusters (SAMNs) in aqueous conditions as a theranostic nano-system. The beta-cyclodextrin (β-CD) was immobilized on the surfaces of SPIO NPs and RGD-conjugated polyethylene glycol (PEG) and paclitaxel (PTX) were hosted in the β-CD cavity through high affinity complexation. We found that nanocluster size could be readily controlled by varying the SPIO/PEG ratio in the assemblies, and also demonstrated significant improvement of the functional nanoparticles for theranostic systems; enhanced magnetic resonance, improved cellular uptake, and efficient PTX loading and sustained release at the desired time point. These results strongly indicate that the SAMNs developed in this study possess great potential for application in image-guided cancer chemotherapy.

1. Introduction

Over the past decades, advances in nanotechnology have led to the development of disease diagnostic and therapeutic agents [1–15]. Nanoparticle-based imaging and therapy using superparamagnetic iron oxide (SPIO), quantum dot, silica, and gold are of interest to the field of theranostic nanomedicine. In particular, SPIO

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nanoparticles (NPs) have attracted increasing attention in the fields of cancer imaging, diagnostics, and treatment because of their excellent imagability and their Food and Drug Administration-approved biocompatibility [6]. SPIO NPs possess various desirable features that have been widely applied in magnetic resonance imaging (MRI) [1–6,9,16], drug delivery [1–6,9], and hyperthermia cancer treatments [7]. However, a number of limitations hinder clinical use, including relatively rapid body clearance, low drug loading, and lack of target specificity that ultimately result in insufficient imaging signal intensity or drug concentration at target sites. Many scientists have attempted to address these shortcomings by modifying surface properties, encapsulating SPIOs in micro/nanocarriers, and using internal or external navigation systems [17].

Surface modification, such as the introduction of amphiphilic molecules, bifunctional polymeric ligands, or biomolecules can stabilize SPIO NPs and prevent particle agglomeration. Surface modification of SPIO NPs with polyethylene glycol (PEG), known as PEGylation, has become a common method for inhibiting phagocytosis by the reticuloendothelial system (RES), extending the half-life in blood circulation and promoting the enhanced permeability and retention (EPR) effect *in vivo*. In addition, SPIO NP size can be increased by clustering them in larger nanocarriers, including nanogels, liposomes, micelles, or capsules, since NP size plays a major role in pharmacokinetics and tissue distribution *in vivo*. For example, particles <10 nm are rapidly removed by the kidney, whereas particles >200 nm become concentrated in the spleen or are taken up by phagocytic cells, leading to low plasma concentrations. Interestingly, the encapsulation of SPIO NPs in other carrier systems can also increase magnetic resonance signals. SPIO NP clustering has been found to increase T_2 relaxivity and this increase is more significant in larger clusters.

To date, many PEGylation methods have been applied for SPIO NPs. For example, PEGs were covalently or ionically linked to iron oxide nanoparticles during particle synthesis using silane coupling agents or a ligand exchange method. However, this process is usually time-consuming and often changes the magnetic properties of SPIO NPs [18]. Amstad et al. identified catechol-derivative anchor groups possessing an irreversible binding affinity to iron oxide; using these anchor groups, ultrastable iron oxide nanoparticles could be obtained [19]. However, such SPIO NPs exhibit low efficacy for drug loading and rapid drug release since the drugs are weakly entrapped by physical adsorption.

It is well established that cyclodextrins (CDs) can form inclusion complexes with a wide variety of lipophilic molecules in their hydrophobic cavity, thus improving water solubility, stability, and biological activity in the body [20,21]. Recent studies have attempted to introduce CDs on the surface of SPIO NPs [1,22–26]; SPIO NPs were prepared by precipitation of iron salts in the presence of ammonia and the particles were mixed with β-CD and a pluronic polymer (F127). However, this method yielded large aggregates or irregular clusters.

In this study, a new method for preparing multifunctional self-assembled magnetic nanoclusters (SAMNs) was developed using β-CD coated SPIO (SPIO@CD) NPs (Fig. 1). The β-CD was modified with dopamine to enable stable conjugation on SPIO. Surface immobilized CD was utilized to introduce PEG and load paclitaxel (PTX). The terminal PEG was functionalized with a tumor-targeting ligand, cyclo-Arg–Gly–Asp-D-Phe–Cys c(RGDfC), since targeted drug delivery to a specific site through the receptor-mediated endocytosis could improve therapeutic efficiency and minimize side effects. The conjugation of specific ligands (e.g., Arg–Gly–Asp; RGD) is an accepted strategy used to facilitate efficient targeting of drug carriers to tumors since the RGD sequence can recognize molecular signatures on the surface of cancer cells (bio-conjunction) [6,27]. SAMNs were characterized by powder X-ray diffraction (XRD), Fourier transform infrared spectra (FT-IR), transmission electron microscopy (TEM), dynamic light scattering (DLS), thermogravimetric analysis (TGA), vibration sample magnetometer (VSM), and relaxivity measurements. The enhanced cellular uptake of c(RGDfC)-SAMNs was evaluated by flow cytometry and confocal laser scanning microscopy (CLSM). Finally, *in vitro* MRI visibility was tested.

2. Material and methods

2.1. Materials

Superparamagnetic iron oxide nanoparticles (SPIO), β-cyclodextrin (CD), 1-adamantylamine (ADA), dopamine hydrochloride (DOPA), poly(ethylene glycol) (PEG, Mw 4000), potassium iodide, silver (I) oxide, p-toluenesulfonyl chloride (TsCl), potassium thioacetate (KSAc), 1,10-phenanthroline, rhodamine B (Rho), N,N-dimethylformamide (DMF), tetrahydrofuran (THF), and stannous 2-ethylhexanoate were obtained from Sigma Aldrich (St Louis, MO, USA). Triethylamine (TEA) and aluminum oxide were purchased from Acros Organics (Morris Plains, NJ, USA). Divinyl sulfone (DVS) and cyclo(Arg–Gly–Asp-D-Phe–Cys) (c(RGDfC)) were supplied by TCI (Tokyo, Japan) and Peptide International (Louisville, KY, USA), respectively. Paclitaxel (PTX) was supplied from Samyang Corporation (Seoul, Korea). All reagents and solvents were used as received without further purification.

2.2. Synthesis of monotosyl-PEG, ADA-PEG, and VS-PEG-ADA

Monotosyl-PEG was synthesized using a stoichiometric amount of TsCl in the presence of silver (I) oxide and a catalytic amount of potassium iodide, as previously reported [28]. Briefly, PEG dissolved in MC was added to silver (I) oxide, potassium iodide, and TsCl. Following 2 h of stirring, the solution was filtered and evaporated. The product was obtained by recrystalyzation using MC-ether co-solvent and the yield was approximately 90%. The degree of substitution was determined to be 93%, calculated based on the integral value of PEG (δ 3.67) and the methylene protons adjacent to the tosyl group (δ 4.15). ^{1}H NMR (400 MHz, $CDCl_3$, δ in ppm): 7.79 (Ar), 7.34 (Ar), 4.15 (CH_2OTs), 3.67 (O–$(CH_2)_2$–O), 2.75 (CH_2–OH), and 2.45 (Ar–CH_3) (Fig. S1i).

The obtained monotosyl-PEG (8 g, 2.25 mmol) and ADA (0.45 g, 3 mmol) were dissolved in 200 mL of acetonitrile and the mixture was heated at 75 °C for 3 days under nitrogen atmosphere. The solution was allowed to cool to room temperature, dialyzed against water in a dialysis membrane (molecular weight cutoff, MWCO = 3.5 kDa) for 3 days, and subsequently lyophilized. Product yield was approximately 95%. The degree of substitution was determined to be 57%, calculated based on the integral value of PEG (δ 3.67) and the methylene protons of the ADA group (δ 1.75). ^{1}H NMR analysis results indicated a nearly quantitative transformation of TsCl into ADA moieties. ^{1}H NMR (400 MHz, $CDCl_3$, δ in ppm): 3.67 (O–$(CH_2)_2$–O), 1.91 (CH–$(CH_2)_3$), 1.75 (CH–CH_2–CH), 1.50 (CH–CH_2–CH) (Fig. S1ii).

For the final step of PEG modification, the conjugation of DVS to ADA-PEG was carried out using the following procedure: a mixture of ADA-PEG (4 g, 1 mmol) and potassium tert-butoxide (t-BuOK; 0.56 g, 5 mmol) in 150 mL of MC was slowly dropped into an excess amount of DVS (20-fold) in 30 mL of MC and stirred at 30 °C for 2 days under a nitrogen atmosphere. The solution was then evaporated and precipitated into cold diethyl ether and dried *in vacuo*. Product yield was approximately 90%. The degree of substitution was determined to be 45%, calculated based on the integral value of PEG (δ 3.67) and the proton on the vinyl group (δ 6.72). ^{1}H NMR (400 MHz, $CDCl_3$, δ in ppm): 6.72 (SO_2CH),