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Image-guided and tumor-targeted drug delivery with radiolabeled unimolecular micelles

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ABSTRACT

Unimolecular micelles formed by dendritic amphiphilic block copolymers poly(amidoamine)–poly(L-lactide)-*b*-poly(ethylene glycol) conjugated with anti-CD105 monoclonal antibody (TRC105) and 1,4,7-triazacyclononane-N, N′, N-triacetic acid (NOTA, a macrocyclic chelator for ^{64}Cu) (abbreviated as PAMAM–PLA-*b*-PEG–TRC105) were synthesized and characterized. Doxorubicin (DOX), a model anti-cancer drug, was loaded into the hydrophobic core of the unimolecular micelles formed by PAMAM and PLA via physical encapsulation. The unimolecular micelles exhibited a uniform size distribution and pH-sensitive drug release behavior. TRC105-conjugated unimolecular micelles showed a CD105-associated cellular uptake in human umbilical vein endothelial cells (HUVEC) compared with non-targeted unimolecular micelles, which was further validated by cellular uptake in CD105-negative MCF-7 cells. In 4T1 murine breast tumor-bearing mice, ^{64}Cu-labeled targeted micelles exhibited a much higher level of tumor accumulation than ^{64}Cu-labeled non-targeted micelles, measured by serial non-invasive positron emission tomography (PET) imaging and confirmed by biodistribution studies. These unimolecular micelles formed by dendritic amphiphilic block copolymers that synergistically integrate passive and active tumor-targeting abilities with pH-controlled drug release and PET imaging capabilities provide the basis for future cancer theranostics.

1. Introduction

Polymeric micelles with a unique core–shell structure, formed by self-assembly of amphiphilic copolymers, have captured significant attention in diverse biomedical fields such as gene and drug delivery, and especially in tumor therapy [1–5]. The hydrophobic core of the polymeric micelle serves as a container to improve the solubility of water-insoluble agents. The outer hydrophilic shell, which is often composed of poly(ethylene glycol) (PEG), offers the micelles excellent dispersibility in an aqueous solution [6,7]. Polymeric micelles are desirable drug delivery vehicles for targeted cancer therapy due to their unique advantages, such as enhancing the aqueous solubility of hydrophobic drugs, prolonging the circulation time of the drug in the blood, improving the *in vivo* stability of the drug, providing both passive and active tumor-targeting abilities, and reducing nonspecific uptake by the reticuloendothelial system (RES) [8–11]. However, classical multi-molecular polymeric micelles suffer from insufficient stability *in vivo* due to the fact that their *in vivo* stability is sensitive to the surrounding environment, especially the concentration of the amphiphilic block copolymers. Upon dilution in the bloodstream, multi-molecular polymeric micelles disassemble, leading to a burst release of drug and loss of tumor-targeting abilities [12–14].

Unimolecular micelles formed by individual dendritic amphiphilic block copolymers are investigated to overcome this problem.

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Because of their covalent nature, properly engineered unimolecular micelles can possess excellent *in vitro* and *in vivo* stability [2,15–20]. The size of unimolecular micelles can also be tuned by controlling the number of amphiphilic block copolymer arms and the length of the hydrophobic and hydrophilic segments [21]. Despite these potential advantages, unimolecular micelles incorporating therapeutic agents, molecular targeting, and diagnostic imaging capabilities are rare.

CD105 (i.e., endoglin), which is almost exclusively expressed on proliferating tumor endothelial cells, serves as an ideal vascular target [22]. More importantly, the expression level of CD105 is correlated with poor prognosis in more than 10 solid tumor types [23], which makes it a generally applicable prognostic, diagnostic, and therapeutic vascular target in cancer [24,25]. TRC105, a human/murine chimeric IgG1 monoclonal antibody that binds to both human and murine CD105, was used as the targeting ligand in this work. Due to its high affinity for CD105 and the indispensable role of CD105 in cancer progression, multiple phase II clinical trials with TRC105 are currently ongoing [26]. In our previous studies, TRC105 showed a very high avidity for CD105 in different murine cancer models [27,28]. Positron emission tomography (PET) can provide excellent tissue penetration, higher detection efficiency, non-invasiveness, and superb quantitative accuracy [29], which can serve as an invaluable tool to study the *in vivo* behavior of different nanomaterials [18,30,31]. It was used here for unveiling the *in vivo* behavior of CD105-targeted drug/agent nanocarriers designed in the current study. Rationally designed CD105-targeted nanomedicine can be used for both cancer diagnosis and cancer therapy (i.e., theranostics), which will improve the management of cancer patients in the long run.

In this work, we report a type of unimolecular micelle (Fig. 1) formed by dendritic amphiphilic block copolymers poly(amidoamine)–poly(L-lactide)-*b*-poly(ethylene glycol) conjugated with an anti-CD105 monoclonal antibody (TRC105) and 1,4,7-triaza cyclononane-N, N′, N-triacetic acid (NOTA, a macrocyclic chelator for ^{64}Cu) (abbreviated as PAMAM–PLA-*b*-PEG–TRC105) for targeted cancer therapy and imaging. The effects of the TRC105 targeting ligands on the cellular uptake in cell lines with different CD105 expression levels (e.g., human umbilical vein endothelial cells [HUVEC] or MCF-7 human breast cancer cell line) were studied *in vitro*. The effects of TRC105 as the targeting ligand on the tumor accumulation of the unimolecular micelles in 4T1 murine breast tumor-bearing mice were studied *in vivo*.

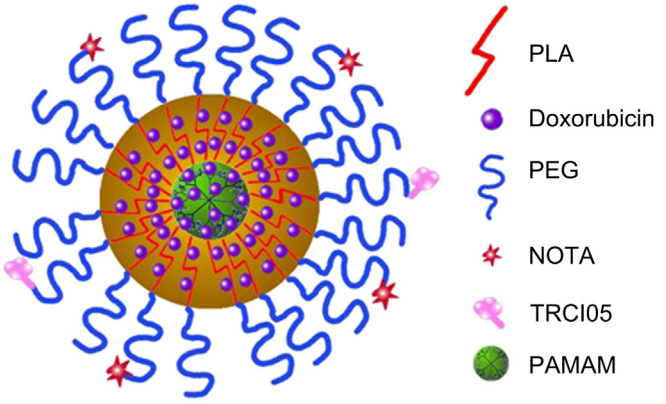

Fig. 1. A schematic illustration of the multifunctional PAMAM–PLA-*b*-PEG–OCH_3/TRC105/NOTA unimolecular micelles for tumor-targeted drug delivery and PET imaging.

2. Materials and methods

2.1. Materials

Poly(amidoamine) (Core:1,4-diaminobutane; {***dendri***-poly(amidoamine)-$(OH)_{64}$}; ($G = 4$)) dendrimer was purchased from NanoSynthons LLC (Mt. Pleasant, MI, USA). L-lactide (LA) (Sigma–Aldrich, Milwaukee, WI, USA) was recrystallized twice from ethyl acetate (Sigma–Aldrich) before use. The heterobifunctional poly(ethylene glycol) (PEG) derivatives, HOOC–PEG–maleimide (HOOC–PEG–Mal) (M_w = 5000) and HOOC–PEG–OCH_3 (M_w = 5000) were acquired from JenKem Technology (Allen, TX, USA). 4-Dimethylamino pyridine (DMAP), 1,3-dicyclohexylcarbodiimide (DCC), and aminoethanethiol hydrochloride (AET·HCl) were purchased from ACROS and used without further purification. Stannous (II) octoate ($Sn(Oct)_2$), (Sigma–Aldrich) was used as received. Tetrahydrofuran (THF), triethylamine (TEA), dimethyl sulfoxide (DMSO), anhydrous dimethylformamide (DMF), anhydrous dichloromethane (DCM), and tris(2-carboxyethyl)phosphine (TCEP) were purchased from Sigma–Aldrich. 5,5′-Dithio-bis-(2-nitrobenzoic acid) (Ellman's reagent) and 2-iminothiolane (Traut's reagent) were purchased from Thermo Scientific (Rockford, IL, USA). All other chemicals and reagents used were of analytical reagent grade. The anti-cancer drug doxorubicin hydrochloride (DOX·HCl) was purchased from Beijing Mesochem Technology Co., Ltd. (S)-2-(4-Isothio-cyanatobenzyl)-1,4,7-triazacyclononane-1,4,7-triacetic acid (p-SCN-Bn-NOTA) was purchased from Macrocyclics, Inc. (Dallas, TX, USA). TRC105 was kindly provided by TRACON Pharmaceuticals, Inc. (San Diego, CA, USA). All organic solvents were of analytic grade. Phosphate buffered solutions (PBS, pH 7.4) and acetate buffered solutions (ABS, pH 5.3) were prepared fresh in our laboratory. Ultrapure deionized water (DI water, Milli-QWater Systems) was used for all buffer solutions and experiments. The buffers were of Millipore grade and pretreated with Chelex 100 resin (50–100 mesh, Sigma–Aldrich, St. Louis, MO, USA) to ensure that the aqueous solution was free of heavy metals.

2.2. Synthesis of PAMAM–PLA

PAMAM–PLA was prepared by the ring-opening polymerization of LA monomer using PAMAM-OH as the micro-initiator (cf. Scheme 1). A 50 ml two-neck flask equipped with an argon gas inlet was charged with PAMAM-OH (40 mg, 2.80×10^{-6} mol) and placed in an oil bath. A predetermined amount of LA (645.6 mg, 4.48 mmol) was slowly introduced and a catalytic amount of $Sn(Oct)_2$ ($[Sn(Oct)_2]/[LA]$ = 1/1000 mol/mol) was added subsequently. The reaction was stirred at 120 °C for 24 h. THF was added to stop the reaction. The resulting reaction mixture was added drop-wise into the methanol (three times) to obtain the final polymer product via precipitation. The products were dried under vacuum.

2.3. Synthesis of PAMAM–PLA-b-PEG–OCH_3/Mal

The PAMAM–PLA (40 mg), HOOC–PEG–Mal (Maleimide content: 70% in molar, 72.9 mg, 14.6×10^{-6} mol), HOOC–PEG–OCH_3 (76.6 mg, 15.32×10^{-6} mol), DCC (5.27 mg, 25.54×10^{-6} mol), and DMAP (0.31 mg, 2.54×10^{-6} mol) were added in a 50 ml two-neck flask. The mixture was dried under vacuum for 1 h. Afterwards, 10 ml anhydrous DCM was added via a syringe. The mixture was stirred under argon gas at room temperature for 20 h and the by-product, dicyclohexylurea, was removed by filtration. Subsequently, the solution was added into cold diethyl ether to collect the crude product. The impurities and unreacted PEG were removed by dialysis against DI water using a cellulose dialysis membrane (molecular weight cut-off, 15 kDa). After 48 h dialysis, the product was dried by lyophilization.

2.4. Synthesis of the NOTA-SH

NOTA-SH was synthesized via the reaction between the SCN group of p-SCN-Bn-NOTA (2.8 mg, 0.005 mmol) and the amino group of AET·HCl (0.57 mg, 0.005 mmol) in the presence of TEA in PBS (pretreated with Chelex 100 resin to prevent oxidation of the thiol) for 2 h at room temperature under an N_2 atmosphere [18].

2.5. Synthesis of TRC105-SH

TRC105-SH was prepared by reacting TRC105 with Traut's reagent at a molar ratio of 1:20 in PBS (pretreated with Chelex 100 resin to prevent oxidation of the thiol) at a pH of 8.0. After 2 h of incubation at room temperature, TRC105-SH was purified by size-exclusive chromatography using PBS as the mobile phase. Based on our previous experience, there were about 5 thiol groups per TRC105-SH molecule based on Ellman's reagent titration [30].

2.6. Synthesis of the dendritic amphiphilic PAMAM–PLA-b-PEG–OCH_3/TRC105/NOTA block copolymers

A predetermined amount of NOTA-SH PBS solution and TRC105-SH PBS solution were added into the PAMAM–PLA-*b*-PEG–OCH_3/Mal PBS suspension with a feed molar ratio of TRC105:NOTA:PAMAM–PLA-*b*-PEG–OCH_3/Mal at 5:7:1. The reaction was carried out at a pH of 7.5 in the presence of TCEP (1.6 mmol/L) to prevent disulfide formation among TRC105-SH and NOTA-SH. The product was purified by