Nanoprobe for Optical Molecular Imaging

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ABSTRACT

Near-infrared fluorescent (NIRF) dyes are intensively being developed for optical molecular imaging applications. This work focuses on the design, synthesis and application of a nanoparticle-based nanoprobe with a NIRF Cy-7-like dye chemically bonded in polymeric micelles composed of a crosslinked hydrophobic core and a hydrophilic shell with polyethylene glycol (PEG). Pharmacokinetic analysis, *ex vivo* biodistribution and imaging approaches revealed that nanoparticles with an average diameter of 24 nm exhibited prolonged blood half-life ($t_{1/2,\alpha}$ = 1.25 hr. $t_{1/2,\beta}$ = 46.18 hr) and moderate uptakes in organs of the mononuclear phagocytic system. Dual modality NIRF optical imaging and gamma scintigraphy of mice bearing subcutaneously inoculated human MDA-MB468 breast tumor clearly revealed accumulation of nanoparticles in the tumors over a period of 5 days after intravenous injection.

Keywords: nanoprobe, near-infrared fluorescence, optical molecular imaging, block copolymer, polymeric micelles

1 INTRODUCTION

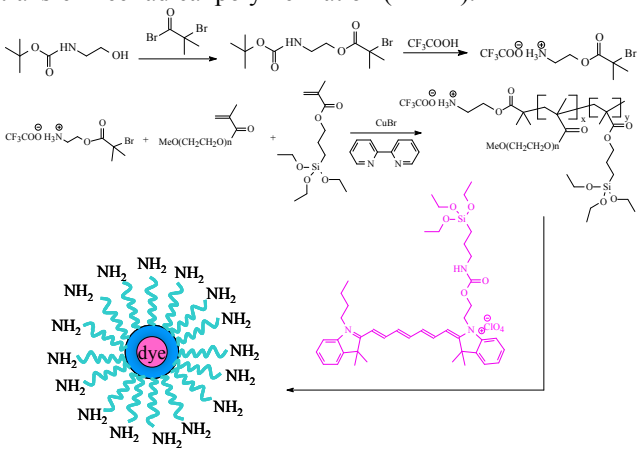
Optical molecular imaging (OMI) is a very powerful tool for studying the temporal and spatial dynamics of specific biomolecules and their interactions in real time in vivo and has been increasingly used to probe protein function and gene expression in live animals. It exhibits the great advantages of high temporal (picosecond) and spatial (submicron) resolutions, high sensitivity (single molecule level) and minimal invasion. Of the various optical imaging techniques investigated to date, near-infrared (NIR, 700- to 900-nm wavelength) fluorescence (NIRF) imaging is of particular interest for noninvasive *in vivo* imaging because of the relatively low tissue absorbance and minimal autofluorescence of NIR light.[1]

Nanoparticles have been increasingly used in a wide range of biomedical applications such as drug carriers and imaging agents. They are engineered materials with dimensions typically smaller than 100 nm, small enough to reach almost anywhere in the body and can be easily derivatized with a variety of targeting ligands, or loaded with multiple molecules of a contrast agent, providing a huge boost in signal intensity for diverse imaging modalities.[2] NIRF imaging based on nanoparticulate imaging probes is rapidly emerging as an advanced technology for noninvasive cancer detection, diagnostic and therapeutical applications.[3] The design and synthesis of smart nanoprobe is an enabler for NIRF imaging to be successful.

In this paper, we report the design, synthesis and application of PEG-shelled core-crosslinked polymeric micelle system derived from an amine-terminated amphiphilic block copolymer. The block copolymer self-assembled to form micellar nanoparticles, and NIRF dye was chemically bonded in the core bearing reactive ethoxysilane functional groups through subsequent sol-gel process. The covalent crosslinking provides physical stability to nanoparticle and to Cy7-like dyes, enhanced fluorescence efficiency and improved optical stability. The terminal amines allows for labeling with a gamma emitter indium-111 (^{111}In) through a metal chelator DTPA (diethylenetriaminepentaacetic acid) to facilitate pharmacokinetic and biodistribution studies.

2. EXPERIMENTAL

The synthesis of the block copolymer was shown in scheme 1. The block copolymer was synthesized by atom transfer free radical polymerization (ATRP).



Scheme 1

Block Copolymer PPEGMA-b-PESPMA (x = 31, y = 46): A 50 mL 3-neck flask was equipped with an additional funnel, a stopper, and a septum. Initiator trifluoroacetic acid salt of 2-aminoethyl 2-bromoisobutyrate (0.106 g, 0.33 mmol) and bipyridine (174 mg, 1.11 mmol) were dissolved in 2.4 mL of dry methanol in the flask, and methoxy-PEG methacrylate (PEG-MA, MW 475) (4.8 g, 0.01 mol) was added. Triethoxysilylpropyl methacrylate

(ESP-MA) (vacuum distilled from CaH_2) (4.5 mL, 4.41 g, 0.015 mol) was placed in the additional funnel with 2.0 mL of dry methanol. Both mixtures in the flask and additional funnel were degassed by bubbling nitrogen for 15 minutes. Then CuBr (80 mg, 0.56 mmol) was added quickly to the flask and the solution turned dark brown. The flask was heated in an oil bath at 50 °C for 30 minutes and ESP-MA solution was added quickly to the flask. The polymerization was continued overnight at 50 °C. To work up, the polymerization mixture was diluted with dry THF, passed through a pad of celite and basic aluminum twice, and concentrated to give clear viscous semi solid. 1H NMR indicated the ratio of x/y to be close to 1/1.5. GPC measurement yielded a number-average molecular weight (Mn) of 28,600 and polydispersity value (Mw/Mn) of 1.3.

NIRF Nanoparticles. Block copolymer PPEGMA-*b*-PESPMA (100 mg) and 3-(triethoxysilyl)propyl-Cy7 (0.4 mg) were dissolved in 10 mL of THF. Ten mL of distilled water was then added slowly to the THF solution. The mixture was stirred at room temperature in the dark for 8 hs, followed by addition of acetic acid (0.05 mL), and stirred at room temperature overnight. The nanoparticle-containing solution was dialyzed against distilled water for 24 hr (MW cutoff, 3000). The solution was then filtered sequentially through 0.7, 0.45, 0.2, and 0.1 μm filters. The volume average size of DTPA-nanoparticle was 24 ± 8.9 nm determined by dynamic light scattering measurements

DTPA-Conjugated nanoparticle. An aqueous solution of nanoparticle (20 mL, 9.3 mg dry weight/mL water) was placed in an amber vial. The pH of the solution was adjusted to 7.5 using 2% sodium bicarbonate solution. DTPA-Bz-NCS (0.6 mg, 1 mg/mL water) was added to the vial. The reaction was stirred under nitrogen overnight. Unreacted DTPA-Bz-NCS was removed by centrifugal filtration using membrane with molecular weight cutoff of 30,000. Approximately 70% of DTPA used was attached to the particles.

Radiolabeling. Aliquots of DTPA-nanoparticle in 0.1 M sodium acetate solution (pH 5.2) was mixed with an aqueous solution of $^{111}InCl_3$ for 30 min. Radiolabeled nanoparticles were analyzed using an instant thin layer chromatography (ITLC) system. The ITLC strips were developed with phosphate buffered solution (pH 7.4) containing 4 mM EDTA and quantified with Bioscan IAR-2000 TLC Imaging Scanner (Washington, DC). The labeling efficiency for CCPM was >98%. The specific activity was 1.18 mCi/nmol.

3. RESULTS AND DISCUSSION

The physicochemical properties of nanoparticles were characterized with regard to composition, size, surface charge, and optical properties. The nanoparticles had weak positive charge with ζ potential of +1.2 in PBS buffer at pH 7.5 and each nanoparticle contained approximately 21 Cy7-like dye and 19 DTPA molecules. The excitation and emission light intensities for NIRF nanoparticles peaked at 755 nm and 781 nm, respectively. The nanoparticle showed an increase quantum yield for Cy7-like dye of 0.081, whereas for free Cy7, the quantum yield was 0.061.

Following intravenous administration, ^{111}In-conjugated nanoparticles exhibited blood half-lives of 1.25±0.66 hr in the distribution phase ($t_{1/2,\alpha}$) and 46.18±6.86 hr in the clearance phase ($t_{1/2,\beta}$), respectively. Model parameters were obtained by fitting the %ID/g blood–time data to the following equation:

$$C_t\,(\%\text{ID/g blood}) = Ae^{-\alpha t} + Be^{-\beta t}$$

Biodistribution of nanoparticles at 48 hr and 120 hr after injection determined by *ex vivo* measurements of radioactivity and *ex vivo* analysis of the fluorescence signal intensities of resected tissues At 48 and 120 hr postinjection, nanoparticles showed considerably high blood retention, significant amount of the nanoparticles had been cleared from most organs studied with the exception of the liver, the spleen, and the tumor. In fact, uptakes in the liver and the tumor increased moderately from 48 hr to 120 hr, indicating continuous distribution into these tissues.

Accumulation of NIRF nanoparticles in the tumor could be readily visualized at the injected dose (4.5 nmol eq. Cy7/mouse) with optical imaging. In fact, tumors could be detected at doses up to 100-fold lower than used in the current study (i.e. 0.045 nmol eq. Cy7/mouse), indicating that NIRF nanoparticles displayed strong and persistent fluorescence signal *in vivo*. Clearly, γ-sciantigraphy and NIRF optical imaging techniques provided complementary information. While optical imaging technique offers inherently high sensitivity for superficially localized lesions, γ-scintigraphy reveals global distribution of nanoparticles particularly their uptakes in internal organs.

In conclusion, both *ex vivo* biodistribution and *in vivo* dual optical/gamma imaging were utilized to evaluate the ^{111}In-labeled NIRF nanoparticles *in vivo*. It was found that NIRF nanoparticles showed a prolonged circulation time, resulting in effective accumulated in solid tumors. This class of nanoparticles is a promising candidate for dual optical and nuclear imaging applications in tumor detection and molecular imaging. NIRF nanoparticles offer opportunity for further surface nano-engineering for targeted molecular imaging applications. Other imaging agents including MRI and therapeutic agents may also be incorporated in the core or shell of the nanoparticles.

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