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In vivo monitoring of neural stem cells after transplantation in acute cerebral infarction with dual-modal MR imaging and optical imaging

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ABSTRACT

Stem cell therapies are promising strategies for the treatment of stroke. However, their clinical translation has not been fully realized due, in part, to insufficient ability to track stem cell migration and survival longitudinally over long periods of time in vivo. In this work, we synthesized a new class of manometer-sized cationic polymersomes loaded with superparamagnetic iron oxide nanoparticles and quantum dots for in vivo dual-modal imaging of stem cells. The results demonstrated that the synthesized cationic polymersomes can act as an effective and safety carrier to transfer image labels into neural stem cells, upon which the distribution and migration of grafted stem cells could be monitored by MR imaging up to 6 weeks and by fluorescence imaging within 4 weeks in the context of ischaemic brain injury. Cationic polymersomes hold great promise in the longitudinal monitoring of transplanted stem cells by using dual-modal MRI and optical imaging.

1. Introduction

Acute ischaemic stroke causes a disturbance of neuronal circuitry and disruption of the blood-brain-barrier that can lead to functional disabilities. The commonly used therapeutic strategy is thrombolytic therapy inducing recanalization of the occluded vessels in the cerebral infarcted area [1]. Nowadays, stem cell therapies, such as adult stem cell transplantation, are considered as promising strategies for the treatment of stroke. Stem cell therapies could act in a trophic, neuroprotective capacity, reducing the damage site and aiding in endogenous neurogenesis. Cells could also be administered at a later stage to replace nonviable tissues and restore function [2]. However, clinical translation of stem cell therapies has not been fully realized due, in part, to insufficient ability to track stem cell migration and survival longitudinally over long periods of time in vivo [3].

Current molecular imaging allow monitoring of stem cell implant or transplant in the same live recipient over time, thereby provide critically important information of their biodistribution, migrational dynamics, differentiation processes and regeneration potential [4,5]. Several approaches such as magnetic resonance imaging (MRI), optical imaging and radionuclide imaging had been extensively used to in vivo track the grafted cells in the host organism. Among them, cellular MRI is entirely clinically translatable with the advantages of non-invasiveness, non-radiation, superior resolution and simultaneous supplemental information about the surrounding tissue relevant to graft status [6], and so far its clinical studies have been initiated [7]. Whereas, the application of molecular imaging to interrogate transplanted cells may require one or even two imaging modalities to provide a reasonable assessment of transplanted cells in specific organs [8].

Previously, we synthesised a new class of nanometer-sized polymeric vesicles with a unique hollow structure where superparamagnetic iron oxide nanoparticles (SPIONs) were loaded, and with adjustable positive charges on the membrane to label bone marrow mesenchymal stem cells (MSCs). Using these polymersomes, highly efficient labelling of stem cells was achieved in a simple and controllable way, with varying outcome of in vivo tracking grafted stem cells in the cerebral ischaemia injury by using MR imaging [9]. In this study, we synthesised a new type of cationic polymersomes in which SPIONs and quantum dots (QDs) were incorporated to label neural stem cells (NSCs) for simultaneous

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cellular MRI and fluorescence imaging, and to determine the feasibility of dual-modal imaging to track grafted stem cells in the acute ischaemic stroke based on these cationic polymersomes.

2. Materials and methods

2.1. Animals

Sprague–Dawley (SD) rats were obtained from the Animal Experiment Centre of our university. All animals were housed in a standard animal facility and allowed standard food and water ad libitum. All procedures adhered to the guidelines for the care and use of laboratory animals and were approved by the Institutional Animal Care Committee. Twelve newly born rats (postnatal 1 day) were used as the donors of NSCs. Twenty-four adult male rats weighing 280–300 g were used to establish focal ischaemic cerebral injury.

2.2. Synthesis and characterization of PEI-PDDLA copolymers

Cationic polymersomes were prepared from diblock copolymers of poly(-etherimide) and poly(D, L-lactide) (PEI-PDLLA). PEI-PDLLA copolymer was synthesized as shown in Fig. 1. At first, lPEI was synthesized as reported before [10]. Briefly, 0.372 g of methyl tosylate (2 mmol, Sigma–Aldrich, St. Louis, MO, USA) was dissolved in 10 mL of acetonitrile (Guangzhou Chemical Industry, China). 4.8 g of 2-ethyl-2-oxazoline (48 mmol, Sigma–Aldrich) was added, and the mixture was stirred for 72 h at 70 °C and then reacted with ammonia for 1 h to terminate the reaction. After acetonitrile was removed via rotary evaporation, the mixture was dissolved in chloroform, precipitated in diethyl ether (Guangzhou Chemical Industry) and dried to get poly(2-ethyl-2-oxazoline) (PEtOx-NH_2). 4.8 g of PEtOx-NH_2 (1 mmol) was added to 15 mL of aqueous HCl (10 wt%, Guangzhou Chemical Industry). The mixture was stirred for 24 h under Ar protection at 100 °C, and then was centrifuged and lyophilized to yield linear polyetherimide (lPEI) (M_n = 900 Da, calculated by ^{1}H NMR spectrum).

0.15 mL of vacuum-dried dodecanol (0.66 mmol, Sigma–Aldrich) and 6.6 g of D, L-lactide (Shenzhen Esun Industry, China) were dissolved in 20 mL of anhydrous toluene (Guangzhou Chemical Industry). After appropriate stannous octoate (0.5 wt %, Sigma–Aldrich) was added as catalyst [11], the mixture was heated in 120 °C oil bath and refluxed for 12 h under a nitrogen atmosphere. After the completion of polymerization, the solution was precipitated into hexane and dried to obtain PDLLA-OH (M_n = 9900 Da, calculated based on ^{1}H NMR spectrum). 3.4 g of PDLLA-OH (0.34 mmol) and 0.55 g carbonyldiimidazole (CDI, 10 eq., Sigma–Aldrich) was dissolved in 20 mL and 15 mL of tetrahydrofuran, respectively. The solution of PDLLA-OH was dropwise added into the solution of CDI. After reacting under Ar protection for 24 h at room temperature, the mixture was precipitated into cool diethyl ether, centrifuged and dried to get PDLLA-CDI.

Finally, 0.45 g of lPEI (0.5 mmol) and 2 g of PDLLA-CDI (0.2 mmol) was dissolved in chloroform and stirred at room temperature for 24 h. After chloroform was removed, the mixture was precipitated into cool diethyl ether. The obtained precipitate was then dissolved in DMSO (Guangzhou Chemical Industry) and dialysed (MW = 3.5 kDa) for 24 h to remove excessive PEI and lyophilized to get PEI-PDLLA. Structure of the synthesized copolymers was confirmed by ^{1}H nuclear magnetic resonance (NMR) which was carried out by using a 300-MHz NMR spectrometer (Mercury-Plus 300; Varian, California, USA) in deuterated chloroform at room temperature and a Fourier transform infrared spectrometer (FT-IR, Nicolet/Nexus 670; Thermo Nicolet, Madison, WI, USA) by using KBr as the reference in the range of 500–4000 cm^{-1}. Gel permeation chromatograph (Waters Breeze; Milford, MA, USA) equipped with 1525 Binary HPLC Pump and Waters 2414 Refractive Index Detector was used to quantify the obtained copolymers.

2.3. Synthesis and characterization of PEG-PAsp copolymers

Poly(ethylene glycol) and Poly(aspartic acid) (PEG-PAsp) were synthesized as previously reported [12]. Methoxy poly(ethylene glycol) and poly(β-benzyl L-aspartate) (mPEG-PBLA) was synthesized by ring-opening polymerization of BLA-NCA (1.25 g, 5 mmol) using mPEG-NH_2 (1 g, 0.5 mmol) as an initiator, in the mixture of DMF (5.0 mL, Sigma–Aldrich) and CH_2Cl_2 (50 mL) at 35 °C for 72 h. Subsequently, the mixture was precipitated into a mass of cool diethyl ether, filtered, washed with diethyl ether, and dried in vacuum to get mPEG-PBLA (M_n = 4000 Da, calculated by ^{1}H NMR spectrum). After that, the benzyl group in PEG-PBLA was removed to get mPEG-PAsp by an acid deprotection method. Briefly, 1.5 g of PEG-PBLA was dissolved in 4 mL of trifluoroacetic acid, followed by the addition of 3 mL of HBr/HAc mixture. After stirring at room temperature for 1 h, the mixture was precipitated into a mass of cool diethyl ether, filtered and dried in vacuum. Then the dried product was dissolved in ultrapure water and the pH value was adjusted to neutral using NaOH solution (1 mol/L). Finally, the mixture was dialysed against ultrapure water and freeze-dried. Chemical structures of the products were analysed by ^{1}H NMR spectroscopy in deuterated dimethyl sulfoxide (DMSO-d_6).

2.4. Synthesis and characterization of SPION and QD-loaded polymersomes

The hydrophobic SPIONs of 6 nm were synthesized according to a reported method [13]. Next, the hydrophobic Fe_3O_4 nanoparticles (20 mg) was added to a solution of tetramethylammonium 11-aminoundecanoate in dichloromethane (10 mg/mL). The mixture was shaken for 20 min, during which the hydrophilic particles precipitated out of the solution. The precipitate was washed with dichloromethane, dried under argon and then dispersed in deionized water to get water soluble SPIONs. The SPION and QD-loaded polymersomes were synthesized via double-emulsion solvent evaporation method [14]. Since it was difficult to avoid particle aggregation when co-encapsulating SPIONs and QDs inside the polymersome

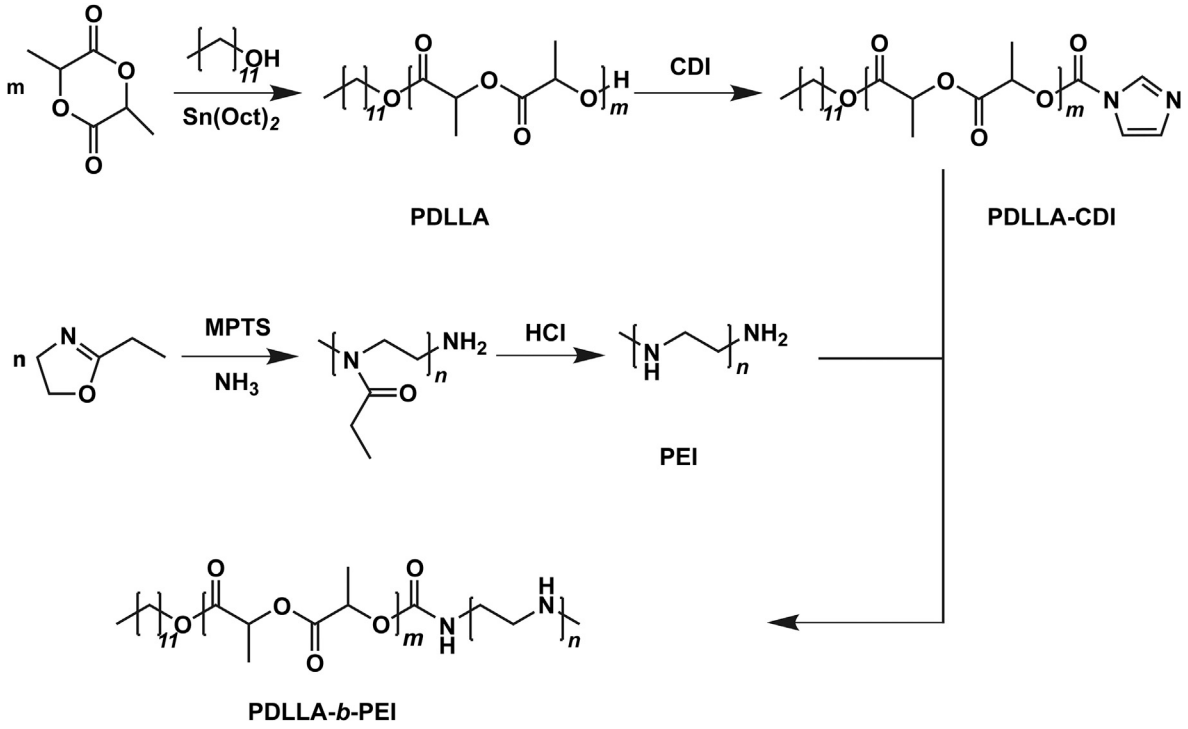

Fig. 1. Schematic diagram of synthesis of PEI-PDLLA copolymer.