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Synthesis of star-branched PLA-*b*-PMPC copolymer micelles as long blood circulation vectors to enhance tumor-targeted delivery of hydrophobic drugs *in vivo*

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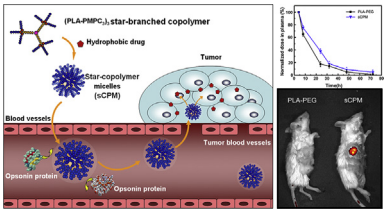
HIGHLIGHTS

- Star-branched amphiphilic copolymer micelles (sCPM) with zwitterionic shells were prepared.
- sCPM possess an ultra-hydrophilic surface and thus inhibited the protein absorption.
- sCPM can effectively prolong the cargo's plasma circulation time.
- sCPM can enhance the cargo's passive tumor-targeted delivery.

GRAPHICAL ABSTRACT



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ABSTRACT

Star-branched amphiphilic copolymer nanocarriers with high-density zwitterionic shell show great promise in drug delivery due to their controllable small size and excellent anti-biofouling properties. This gives the hydrophobic cargo with high stability and long blood circulation *in vivo*. In the present study, star-branched polylactic acid and poly(2-methacryloyloxyethyl phosphorylcholine) copolymers with $(AB_3)_3$–type architecture $(PLA\text{-}b\text{-}PMPC_3)_3$ were conceived as drug vectors, and the copolymers were synthesized by an "arm-first" approach via the combination of ring opening polymerization (ROP), atom transfer radical polymerization (ATRP) and the click reaction. The self-assembled star-branched copolymer micelles (sCPM) had an average diameter of about 64.5 nm and exhibited an ultra-hydrophilic surface with an ultralow water contact angle of about 12.7°, which efficiently suppressed the adhesion of serum proteins. *In vivo* experiments showed that the sCPM loading strongly enhanced the blood circulation time of DiI and the plasma half-life of DiI in sCPM was 19.3 h. The relative accumulation concentration in tumor of DiI delivered by sCPM was 2.37-fold higher than that of PLA-PEG, at 4 h after intravenous injection. These results demonstrated that the star-branched copolymer $(PLA\text{-}b\text{-}PMPC_3)_3$ is a promising alternative carrier material for intravenous delivery versus classic PEG-modified strategies.

1. Introduction

Nanoparticle formulations have achieved clinical success because of their pharmacological and toxicological parameters

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[1–5]. Of these attributes, the blood circulation time of nanoparticles is one of the most important determinants of their therapeutic potential. Regardless of active target or passive target, long circulation time is crucial to realize the enrichment of drugs in the target site. However, short circulation time might imply nonspecific uptake of the drug, which may trigger toxicity and side effects [6–8]. To realize long circulation in blood, nanoparticles must possess small size (generally <100 nm) to bypass filtration of the capillary bed [9–11]. At the same time, they must have an antibiofouling surface that resists nonspecific protein adsorption and escapes from opsonization of the reticuloendothelial system (RES). Although numerous approaches have employed polyethylene glycol (PEG) to improve the blood resistance of nanoparticles, the repeated administration of PEGylated liposomes or solid lipid nanoparticles results in accelerated blood clearance due to the anti-PEG antibody inducing complement activation [12]. Therefore, many researchers are looking for PEG substitutes.

In recent years, zwitterionic polymers such as polyphosphobetaine, polysulfobetaine, and polycarboxybetaine have been considered as improved long-circulating nano-carriers because of their anti-biofouling properties [13–17]. Zwitterionic surfaces can form a highly hydrated layer via strong charge interaction in addition to hydrogen bonds. Therefore, nanoparticles shielded with zwitterionic polymers have a strong resistance to nonspecific protein adsorption. This results in strong inhibition of opsonization and an increase in the blood circulation half-life. Zwitterionic gold [18] and magnetite nanoparticles [19] provide high systemic exposure and low clearance when administered intravenously. The softer zwitterionic nanogels reported by Zhang et al. [20] passed through splenic filtration more easily than their stiffer counterparts. This prolonged circulation time and reduced splenic accumulation. Recently, PCB-coated nanoparticles were reported to have a circulation half-life of 56 h. More importantly, no polymer-specific antibodies were found, and repeated administration did not induce the accelerated blood clearance [21].

In addition to the surface composition, the structure of the polymer has considerable effects on the surface properties of the material. It is universally known that the star polymer serves as a good support for drug delivery due to its unique structure and distinct physical properties [22,23]. The star polymer has a three-dimensional globular structure consisting of multiple arms or branches radiating from a central point or core. In contrast to linear amphiphilic copolymers, the star polymer does not rely on self-assembly and avoids disassembly when diluted, because of the covalently reinforced core-shell structure. The size of these unimolecular containers could be easily maintained below 100 nm by controlling the molecular structure. Particularly, the outer shells containing the hydration layer might protect the inner cores from protein adsorption when the zwitterions were employed. However, there has been no specific reporting on long-circulating characteristics or *in vivo* tumor targeting drug delivery of amphiphilic star zwitterionic copolymers.

Here, we synthesized amphiphilic star-branched zwiterionic copolymers to investigate the possible long blood circulation and tumor-targeting delivery properties of this type of molecule. PLA was chosen as the hydrophobic core due to its biocompatibility and biodegradability. PMPC was used to form the shell because of its excellent anti-fouling and blood compatibility. An $(AB_3)_3$-type architecture was designed, and we hypothesize that the shell with dense PC groups will maximize the inhibition of protein adsorption (Scheme 1). These star-branched PLA-*b*-PMPC copolymers were synthesized through combination of ROP, ATRP, and click chemistry. The ability of the star-branched copolymer micelles to prolong the circulation time of hydrophobic drugs in the bloodstream was investigated via DiI and Cy5.5 fluorescent pigment as model drugs.

2. Materials and methods

2.1. Materials

Block copolymer PLA-PEG (Mw = 12 kD) was purchased from Jinan Daigang Biomaterial Company (Jinan, China), and the molecular weight of the PEG segment was 2 kD. Copper (I) bromide (CuBr, 99.999%), sodium azide (NaN_3, 99%), 3-butyn-1-ol, 2,2′-bipyridine, and 2-bromo isobutyryl bromide were purchased from Alfa Aesar. 3-Bromo-2, 2-bis (bromomethyl) propanol, triethylamine (TEA), and stannous octoate ($Sn(Oct)_2$, 99%) were purchased from Sigma-Aldrich. 1,3,5-benzenetricarbonyl trichloride (BTC) was purchased from J&K Chemical. D, L-lactide was purchased from Glaco Ltd. (Beijing, China). MPC was purchased from Joy-Nature (Nanjing, China). Tetrahydrofuran (THF), dichloromethane, methanol, and *N*, *N*-dimethylformamide (DMF) were dried and distilled prior to use.

2.2. Synthesis and characterization of star-branched PLA-b-PMPC copolymers

2.2.1. Synthesis of azido-terminated PLA homopolymer (PLA-$(N_3)_3$) by ROP

Linear PLA capped with three azide groups was synthesized by a two-step reaction (Scheme 2, ①). First, 3-bromo-2, 2-bis(bromomethyl)propanol (4.00 g, 12.3 mmol) and sodium azide (molar ratio 1:4) were dissolved into 25 mL DMF. The mixture was flushed with stirring at 70 °C for 3 days under nitrogen. The remaining portion was filtered and flushed of DMF at reduced pressure. It was precipitated in 50 mL deionized water and extracted with 100 mL of dichloromethane twice to remove residual sodium salts. Pentaerythrioltriazide was finally obtained after drying over $CaCl_2$ and dichloromethane removal.

Second, PLA-$(N_3)_3$ was synthesized by ROP of D, L-lactide with pentaerythrioltriazide as the initiator. A typical procedure was as follows: D, L-lactide (5.0 g, 34.7 mmol), pentaerythrioltriazide (25 mg, 1.20 mmol), and Sn(Oct)2 (7.0 mg, 17.3 μm mol) were added into a dried tube with a Schlenk line. The exhaust-refill process was repeated three times, and then the tube was immersed into an oil bath at 130 °C for 12 h. The resulting product was purified by precipitation three times in methanol and dried in a vacuum at 40 °C for 24 h.

2.2.2. Synthesis of alkynyl-terminated PMPC homopolymer (alkynyl-PMPC) by ATRP

Synthesis of homopolymer PMPC capped with an alkynyl group is described as Scheme 2 ②. First, the initiator for ATRP (3-butyn-1-ol-2-bromoisobutyrate (BOBI)) was prepared. The 3-butyn-1-ol (0.5 mL, 6.6 mmol) and triethylamine (1 equivalent) were added into a flask with 5 mL of dichloromethane under nitrogen and in an ice bath. The 2-bromoiso-butyryl bromide (BiBB, 1.1 equivalent) diluted in 5 mL dichloromethane was added dropwise with stirring. The reaction was conducted at room temperature for 24 h. To remove impurities, the crude product was filtered and extracted with deionized water. After drying over $CaCl_2$ and condensing, the product was purified by silica gel chromatography with dichloromethane as the eluent. Second, MPC (5.0 g, 17 mmol), 2,2-bipyridine (0.15 g, 1.0 mmol) and CuBr (0.14 g, 1.0 mmol) were added into a flask in a vacuum line. The reaction mixture was degassed through exhausting and refilling three times. Then, the BOBI initiator (0.20 g, 1.0 mmol) in 10 mL of dried methanol was injected into the flask and further degassed by three freeze-vacuum-thaw cycles. The reaction was carried out with stirring at 60 °C for 48 h. The mixture was passed through a basic alumina column with methanol as the eluent. After vacuum concentration,