Materials Science and Engineering C 43 (2014) 207–213

Contents lists available at ScienceDirect

Materials Science and Engineering C

journal homepage: www.elsevier.com/locate/msec

Facile synthesis and characterization of novel biodegradable amphiphilic block copolymers bearing pendant hydroxyl groups

Gaicen Hu, Xiaoshan Fan, Bingcan Xu, Delong Zhang, Zhiguo Hu *

Collaborative Innovation Center of Henan Province for Green Manufacturing of Fine Chemicals, Key Laboratory of Green Chemical Media and Reactions, Ministry of Education, School of Chemistry and Chemical Engineering, Henan Normal University, Xinxiang 453007, PR China

ARTICLE INFO

Article history:
Received 9 February 2014
Received in revised form 4 June 2014
Accepted 3 July 2014
Available online 10 July 2014

Keywords:
Amphiphilic block copolymer
"Click" reaction
Self-assembly
Micelle

ABSTRACT

Novel amphiphilic block copolymers bearing pendant hydroxyl groups polylactide-b–poly(3,3-bis(Hydroxymethyl-triazolylmethyl) oxetane)-b–polylactide (PLA-b–PHMTYO-b–PLA) were synthesized via a facile and efficient method. First, the block copolymer intermediates polylactide-b–poly(3,3-Diazidomethyloxetane)-b–polylactide (PLA-b–PBAMO-b–PLA) were synthesized through ring-opening polymerization of lactide using PBAMO as a macroinitiator. Following "Click" reaction of PLA-b–PBAMO-b–PLA with propargyl alcohol provided the targeted amphiphilic block copolymers PLA-b–PHMTYO-b–PLA with pendant hydroxyl groups. The composition and structure of prepared copolymers were characterized by ^{1}H nuclear magnetic resonance (^{1}H NMR) spectroscopy, Fourier transform infrared (FT-IR) and gel permeation chromatography (GPC). The self-assembly behavior of the copolymers in water was investigated by transmission electron microscope (TEM), dynamic light scattering (DLS) and static light scattering (SLS). The results showed that the novel copolymers PLA-b–PHMTYO-b–PLA self-assembled into spherical micelles with diameters ranging from 100 nm to 200 nm in aqueous solution. These copolymers also exhibited low critical micellar concentrations (CMC: 6.9×10^{-4} mg/mL and 3.9×10^{-5} mg/mL, respectively). In addition, the in vitro cytotoxicity of these copolymers was determined in the presence of L929 cells. The results showed that the block copolymers PLA-b–PHMTYO-b–PLA exhibited better biocompatibility. Therefore, these well-defined copolymers are expected to find some applications in drug delivery or tissue engineering.

1. Introduction

It is well-known that amphiphilic block copolymers can spontaneously self-assemble into nano-sized aggregates in aqueous solution, such as micelle, vesicles, cylinders and other complex forms [1–6]. These nanostructures have great potential applications in drug delivery, gene therapy, diagnostic biosensors and tissue engineering [7–13], owing to their unique characteristics such as nanoscale size, core–shell structure, relatively high stability and prolonged circulation in the blood [14]. Therefore, the assemblies of amphiphilic block copolymers have received increasing attention. In particular, polymeric nanoparticles (NPs) formed from PLA-based amphiphilic block copolymers (ABPs) have drawn considerable interests.

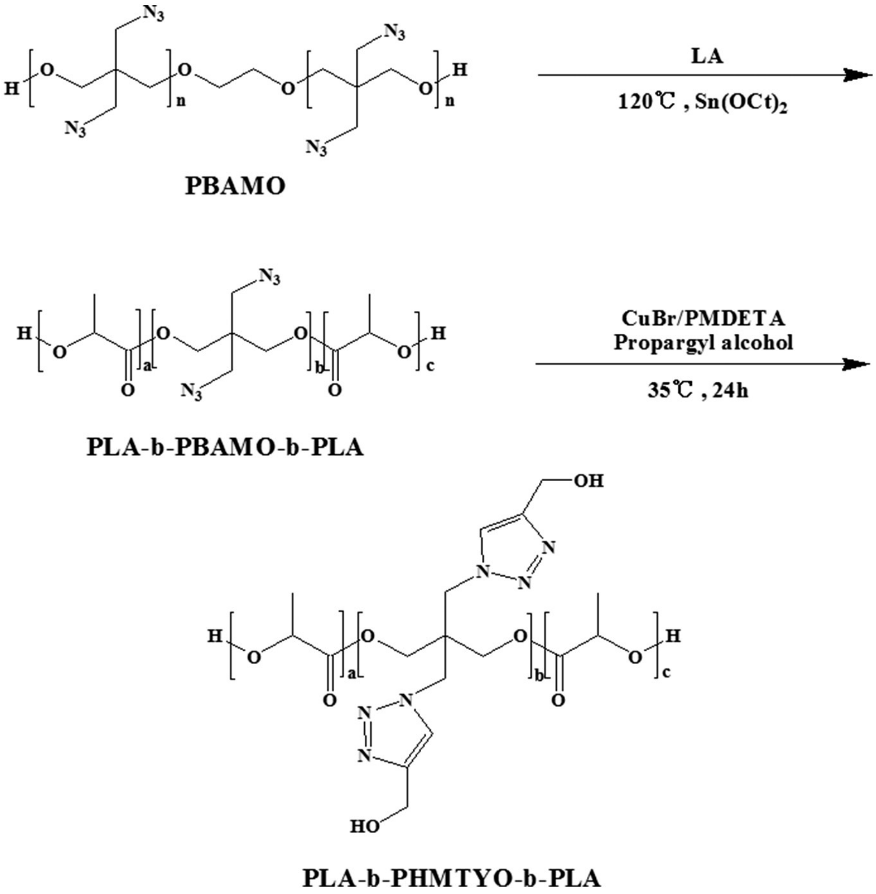
Polylactide (PLA), a plant-derived biodegradable polymer, has been widely used in various biological fields [15–18] due to its excellent shaping, good mechanical property and necessary biodegradability and biocompatibility. Because of its good characteristics, PLA represents excellent building blocks for the formation of hydrophobic domains in amphiphilic copolymers. And a number of these PLA-based ABPs have been investigated for biomedical applications [1,19–24]. However, these conventional copolymers show a lack of functional groups for further modification (except at the end), which may restrict their applications to some extent. To overcome this problem, some functional groups including hydroxyl, carboxyl and amine have been introduced into the side chain of PLA-based ABPs [20,25–28]. In this context, PLA-based amphiphilic copolymers possessing functional moieties of hydroxyl groups receive increasing attention from the polymeric chemists. As biologically relevant functional groups, the hydroxyl groups are ubiquitous in biological systems [29,30]. And the free hydroxyl groups in PLA-based ABPs are capable of further modification, which opens new possibilities of construction of "intelligent" nanoparticles for the controlled drug delivery [14,31–33]. Yet, due to the high reactivity of hydroxyl groups, synthesis of the PLA-base ABPs bearing hydroxyl groups in the previous studies [14,31,32] requires tedious protection and deprotection procedure performed in acidic or alkaline conditions, which may lead to degradation of PLA blocks. Thus, a straightforward and efficient strategy to PLA-based functional amphiphilic copolymers is highly desirable.

In this article, PLA-based biodegradable amphiphilic copolymers bearing multiple pendant hydroxyl groups PLA-b–PHMTYO-b–PLA were synthesized via a facile and efficient method as shown in Scheme 1.

---

* Corresponding author. Tel.: +86 373 3326335.
E-mail address: zghu@htu.cn (Z. Hu).

http://dx.doi.org/10.1016/j.msec.2014.07.020
0928-4931/© 2014 Elsevier B.V. All rights reserved.



Scheme 1. Synthetic approach to copolymers PLA-b–PHMTYO-b–PLA.

First, the functional block copolymer intermediates PLA-b–PBAMO-b–PLA were prepared by ROP of LA with PBAMO as a macroinitiator. Then, the transformation of the hydrophobic middle chain poly(3,3-Diazidomethyloxetane) (PBAMO) into hydrophilic poly(3,3-bis (Hydroxymethyl–triazolylmethyl) oxetane) (PHMTYO) via "Click" reaction provided the targeted copolymers. The composition and structure of the intermediates and targeted copolymers were well characterized. And the self-assembly behavior of the amphiphilic copolymers in aqueous solution was investigated. The results of the in vitro cytotoxicity showed that the copolymers PLA-b–PHMTYO-b–PLA exhibited better biocompatibility. The features of good biocompatibility and functionality render this new type of amphiphilic block copolymers promising for drug delivery application.

2. Experimental section

2.1. Materials

Stannous octanoate (Tin(II) ethyl hexanoate) was purchased from Sigma and was used as received. L-Lactide was obtained from Glaco Ltd and was recrystallized three times from ethyl acetate. Then it was dried 24 h under vacuum at 40 °C before use. PBAMO was synthesized according to the literature [34] procedures. Xylene was dried by refluxing over Na metal according to standard procedures prior to use. Dialysis tubing (molecular weight cutoff 2000 Da) was provided by Shanghai Green Bird Technology Development Ltd. Phalloidin (fluorescein isothiocyanate labeled) was obtained from Enzo Life Sciences. Penicillin–streptomycin solution and 4,6-diamidino-2-phenylindole dihydrochloride (DAPI) were purchased from Invitrogen. All other chemicals were of AR grade and were dried according to the routine methods.

2.2. Measurements

The ^{1}H NMR spectra were recorded on a Bruker-400 NMR instrument in $CDCl_3$ and DMSO-d_6 solvents. FT-IR spectra were conducted with a FTS NEXUS FT-IR spectrometer using the KBr pellet method. Molecular weights and molecular weight distribution of copolymers were estimated by a gel permeation chromatography (GPC) system using a LC-10AVP apparatus equipped with three columns (SHIMADZU Shim-pack GPC-803) in series. DMF was used as eluent at a flow rate of 1.0 mL/min at 40 °C. Polystyrene standards with narrow molecular weight distribution were used for GPC calibration. Laser light scattering, including both dynamic laser light scattering (DLS) and static laser light scattering (SLS), was employed to characterize the results of the self-assembly aggregates of copolymers in water. And the DLS and SLS experiments were performed on a laser scattering spectrometer (BI-200SM) equipped with a digital correlator (BI-9000AT) at 25 °C. Transmission electron microscope (TEM) images were obtained using a high resolution transmission electron microscopy (HRTEM, JEOL-100CX) operating at an acceleration voltage of 200 kV. The dialyzed solutions (0.1 mg/mL for PLA_{4K}-b–$PBAMO_{4K}$-b–PLA_{4K} and 0.01 mg/mL for PLA_{8K}-b–$PHMTYO_{4K}$-b–PLA_{8K}, respectively) were used for TEM detection. A drop of the solution was placed onto TEM copper/carbon grid and the excess solution was blotted up using a strip of filter paper, then the sample was allowed to dry at room temperature before the observation.

2.3. Synthesis of PLA-b–PBAMO-b–PLA copolymers

A typical procedure for synthesis of PLA_{8K}-b–$PBAMO_{4K}$-b–PLA_{8K} copolymer is described as follows: 11.99 g of LA (83.26 mmol), 3.00 g of PBAMO (Mn = 4000; 0.75 mmol) and 0.10 mL of $Sn(Oct)_2$ in