International Journal of Pharmaceutics 491 (2015) 152–161

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

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

Pharmaceutical nanotechnology

Thermo-responsive drug release from self-assembled micelles of brush-like PLA/PEG analogues block copolymers

Yanfei Hu[a], Vincent Darcos[a], Sophie Monge[b], Suming Li[a,*]

[a] Institut des Biomolécules Max Mousseron, UMR CNRS 5247—Equipe Biopolymères Artificiels, Université de Montpellier, 15 Avenue Charles Flahault, BP 14491, 34093 Montpellier, France

[b] Institut Charles Gerhardt, UMR CNRS 5253—Equipe Ingénierie et Architectures Macromoléculaires, Université de Montpellier, cc1702, Place Eugène Bataillon, 34095 Montpellier, France

ARTICLE INFO

Article history:
Received 26 February 2015
Received in revised form 8 June 2015
Accepted 13 June 2015
Available online 18 June 2015

Keywords:
Polylactide
PEG analogues
Brush-like copolymer
Micelle
Thermo-responsive
Drug release

ABSTRACT

Thermo-responsive brush-like amphiphilic poly[2-(2-methoxyethoxy) ethyl methacrylate-*co*-oligo (ethylene glycol) methacrylate]-*b*-poly(L-lactide)-*b*-poly[2-(2-methoxyethoxy) ethyl methacrylate-*co*-oligo(ethylene glycol) methacrylate] [P(MEO_2MA-*co*-OEGMA)-*b*-PLLA-*b*-P(MEO_2MA-*co*-OEGMA)] triblock copolymers were synthesized by atom transfer radical polymerization of MEO_2MA and OEGMA co-monomers using a α,ω-Bromopropionyl poly(L-lactide) (Br-PLLA-Br) macroinitiator. The resulting copolymers with MEO_2MA/OEGMA molar ratio ranging from 79/21 to 42/58 were characterized by ^{1}H nuclear magnetic resonance and size exclusion chromatography. Thermo-responsive micelles were obtained by self-assembly of copolymers in aqueous medium. The micelles are spherical in shape with sizes varying from 20.7 to 102.5 nm. A hydrophobic anticancer drug, curcumin, was encapsulated in micelles by using membrane hydration method. The properties of drug loaded micelles were determined by dynamic light scattering, transmission electron microscopy and lower critical solution temperature (LCST) measurements. The micelles size decreases from 102.5 nm for blank micelles to 37.6 nm with 10.8% drug loading, suggesting that the drug plays an important role in the micellization procedure. The LCST decreases from 45.1 °C for blank micelles to 40.6 and 38.3 °C with 5.9 and 10.8% drug loading, respectively. *In vitro* drug release was performed in pH 7.4 PBS at different temperatures. Data show that the release rate was significantly enhanced above the LCST comparing with that below the LCST. The amount of released drug at 41 °C was *ca.* 20% higher than that at 37 °C. Burst-like release was depressed due to enhanced interaction between drug with hydrophobic PLA and PMA chains.

1. Introduction

Cancer is nowadays a leading cause of death in the world. The World Health Organization reported approximately 14 million new cases and 8.2 million cancer related deaths in 2012. In the past decades, "magic bullet", which was first proposed by Ehrlich in 1960, has been widely investigated for anticancer drug delivery (Ehrlich, 1960). Generally, tumor targeting includes "passive targeting" and "active targeting" (Bertrand et al., 2014; Danhier et al., 2010; Iyer et al., 2006). The former is also called "enhanced permeability and retention (EPR)" due to the abnormal vascular architecture which plays a major role in tumor for selective drug targeting at tissue level (Maeda, 2001; Maeda et al., 2009; Maeda et al., 2000). The latter is achieved by using ligands such as folic acid, protein, peptide, DNA *etc.* (Danhier et al., 2010; Hrkach et al., 2012).

Recently, "activable" or "activated" nanocarriers were developed as a new targeting strategy due to the abnormal tumor microenvironment (~42 °C, pH 5.3) (Alarcon et al., 2005; Cammas et al., 1997; Chilkoti et al., 2002; Ganta et al., 2008; Kim et al., 2000; Rijcken et al., 2007; Sawant et al., 2006; Schmaljohann, 2006). The nanocarrier maintains the stealth function during circulation in the bloodstream. Upon arrival at the tumor site, transformation of the nanocarrier is triggered by the unique tumoral extracellular environment, which allows drug release or interaction with a specific target.

In this regard, thermo-responsive polymeric micelles have drawn great attention for targeted delivery of anticancer drugs. So far, micelles based on polylactide/poly(*N*-isopropylacylamide) (PLA/PNIPAAm) copolymers are most studied for biomedical applications (Cammas et al., 1997; Chilkoti et al., 2002; Hu et al.,

* Corresponding author. Current address: Institut Europeen des Membranes, UMR CNRS 5635, Université de Montpellier, cc 047, Place Eugène Bataillon, 34095 Montpellier, France.
E-mail address: suming.li@umontpellier.fr (S. Li).

http://dx.doi.org/10.1016/j.ijpharm.2015.06.020
0378-5173/

2014a; Li et al., 2011; Liu et al., 2005; Wei et al., 2009; Xu et al., 2013). These micelles generally have small sizes with low critical micelle concentration (CMC) allowing them to avoid the reticulo-endothelial system uptake thereby promoting prolonged blood circulation (Yokoyama et al., 1991). When the temperature is increased above the lower critical solution temperature (LCST), the outer shell chains dehydrate and collapse, allowing aggregation of micelles and favoring binding interactions with cell membrane surface (Cammas et al., 1997). *In vivo* studies indicated that enhanced accumulations at the tumor level and at the cell level are likely to be additive, leading to a 5 times enhancement over normothermic delivery of therapy using currently available thermally insensitive polymeric carriers based on polyethylene glycol (PEG) (Chilkoti et al., 2002). *In vitro* cell uptake suggested that the intracellular uptake of polypeptide or anticancer drug is obviously enhanced (4–5 times) above the LCST (Chilkoti et al., 2002; Li et al., 2011). In our previous work, a systemic study has been realized on the synthesis, self-assembly, cytotoxicity and drug release properties of amphiphilic triblock copolymers composed of a poly(L-Lactide) (PLLA) central block and two PNIPAAm or poly(NIPAAm-*co*-*N*,*N*-dimethylacrylamide) (P(NIPAAm-*co*-DMAAm)) lateral blocks (Hu et al., 2013; Hu et al., 2014a,b). Recently, Lutz et al. reported the synthesis and thermo-responsive properties of thermo-responsive copolymers from 2-(2-methoxyethoxy) ethyl methacrylate (MEO_2MA) and oligo (ethylene glycol) methacrylate (OEGMA) as alternative to PNIPAAm based copolymers (Lutz, 2008, 2011; Lutz and Hoth, 2006). These PEG analogues exhibited a LCST between 26 and 90 °C which can be precisely adjusted by varying the co-monomers ratio, which could be attractive for targeted drug delivery because of their thermo-responsive properties and outstanding biocompatibility similar to linear PEG. Later on, the synthesis and micellization of PLA/PEG analogues copolymers have been studied (Bakkour et al., 2013; Fenyves et al., 2014; Luzón et al., 2010; Saeed et al., 2009). Nevertheless, the LCST of these copolymers was above 75 °C, which greatly diminishes their potential as drug carrier. In fact, no drug release data have been reported.

This work aims to evaluate the potential of PLA/PEG analogues micelles as thermo-targeting drug carrier. A series of amphiphilic triblock copolymers composed of a biodegradable PLLA central block and two lateral thermo-responsive P(MEO_2MA-*co*-OEGMA) blocks were prepared by atom transfer radical polymerization (ATRP). An anticancer drug, curcumin, was encapsulated in the polymeric micelles by using membrane hydration method. The morphology and thermo-responsive properties of drug loaded micelles were determined by DLS, TEM and LCST measurements. For the first time, thermo-responsive drug release from such copolymer micelles was investigated below or above the LCST, and compared with that from P(NIPAAm-*co*-DMAAm)-*b*-PLLA-*b*-P(NIPAAm-*co*-DMAAm) micelles previously reported (Hu et al., 2014b)

2. Experimental

2.1. Materials

L-lactide was purchased from PuracBiochem (Goerinchem, The Netherlands). Stannous 2-ethylhexanoate ($Sn(Oct)_2$), 1,4-benzene dimethanol, 2-bromopropionyl bromide, tris(2-dimethyl aminoethyl) amine (Me_6TREN), copper (I) chloride (CuCl), *N*,*N*-dimethyl formamide (DMF) and curcumin were obtained from Sigma–Aldrich (St-Quentin Fallavier, France), and were used without further purification. Dichloromethane and toluene from Sigma–Aldrich were dried over calcium hydride for 24 h at room temperature and distilled under reduced pressure. MEO_2MA and OEGMA (M_n = 300 g mol^{-1}) were obtained from Sigma–Aldrich and purified through a basic aluminum oxide column. Triethylamine (Sigma–Aldrich) was dried over potassium hydroxide for 24 h at room temperature and distilled. Ultrapure water with a conductivity of 18 MΩ was produced using a Millipore Milli-Q water system.

α,ω-Bromopropionyl PLLA (Br-PLLA-Br) with degree of polymerization (DP) of 40 was prepared as previously reported (Hu et al., 2013).

2.2. Synthesis of P(MEO_2MA-co-OEGMA)-b-PLLA-b-P(MEO_2MA-co-OEGMA)

Triblock copolymers were prepared using standard Schlenk technique. Typically, 100 mg Br-PLLA-Br (30.4×10^{-3} mmol, $M_{n,NMR}$ = 3300 g mol^{-1}), 461 mg MEO_2MA (2.451 mmol), 1.1 g OEGMA (3.676 mmol) and 6 mg CuCl (60.6×10^{-3} mmol) were dissolved in 2 mL DMF. After five freeze-pump-thaw cycles, 16 μL Me_6TREN (60.8×10^{-3} mmol) was added under argon atmosphere. The mixture was stirred at 80 °C for 6 h. The reaction was stopped by exposition to air for 2 h at room temperature. Dialysis of the crude product was then carried out for 3 days in a dialysis tube (cut-off: 3500 D) to remove residual monomers and catalyst against Mill-Q water containing ethylenediaminetetraacetic acid tetrasodium salt hydrate. The product was finally collected by lyophilization.

2.3. Preparation of curcumin loaded micelles

The micelles were prepared by solvent evaporation/membrane rehydration method. Typically, 1.2 mg curcumin and 18.8 mg copolymer T5 (Sample 1, Table 2) were dissolved in 10 mL acetone, and then the solvent was evaporated in rotary evaporator at room temperature to yield a membrane at the wall of the round flask. After vacuum drying for 24 h, 20 mL Mill-Q water was added to the flask, yielding self-assembled micelles at room temperature. The resulting micellar solution was then centrifuged at 5000 rpm for 10 min to remove unloaded curcumin. The supernatant was

Table 1
Molecular characteristics of P(MEO_2MA-*co*-OEGMA)-*b*-PLLA-*b*-P(MEO_2MA-*co*-OEGMA) triblock copolymers.

Copolymer[a]	$[MEO_2MA]_0/[OEGMA]_0$	$[MEO_2MA]/[OEGMA]$[b]	x/y/z[b]	$M_{n,NMR}$[c]	$M_{n,SEC}$[d]	$Đ$[d]
T1	80/20	79/21	40/85/22	25900	25700	1.42
T2	70/30	68/32	40/86/33	28900	27100	1.37
T3	60/40	57/43	40/67/49	30600	18000	1.39
T4	50/50	49/51	40/65/53	31400	11700	1.37
T5	40/60	42/58	40/48/66	32100	9800	1.38

[a] ATRP conditions: [PLLA]/[comonomers]/[CuCl]/[Me_6TREN] = 1/200/2/2, *[M]* = 1.74 M, *T* = 80 °C, reaction time = 6 h.
[b] x, y and z are the degree of polymerization of [LA], [MEO_2MA] and [OEGMA] in copolymer, respectively.
[c] Molecular weight ($M_{n,NMR}$) calculated from NMR.
[d] Molecular weight ($M_{n,SEC}$) and dispersity ($Đ$) Obtained from SEC.