Biodegradable Micelles Capable of Mannose-Mediated Targeted Drug Delivery to Cancer Cells

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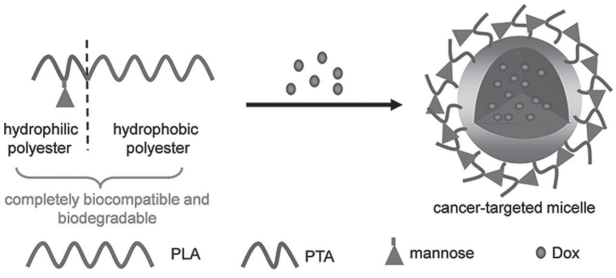
A targeted micellar drug delivery system is developed from a biocompatible and biodegradable amphiphilic polyester, poly(Lac-OCA)-*b*-(poly(Tyr(alkynyl)-OCA)-*g*-mannose) (PLA-*b*-(PTA-*g*-mannose), that is synthesized via controlled ring-opening polymerization of *O*-carboxyanhydride (OCA) and highly efficient "Click" chemistry. Doxorubicin (DOX), a model lipophilic anticancer drug, can be effectively encapsulated into the micelles, and the mannose moiety allows active targeting of the micelles to cancer cells that specifically express mannose receptors, which thereafter enhances the anticancer efficiency of the drug. Comprised entirely of biodegradable and biocompatible polyesters, this micellar system demonstrates promising potentials for targeted drug delivery and cancer therapy.



1. Introduction

Polymeric micelles, composed of amphiphilic block co-polymers to form a hydrophobic core and a surrounding hydrophilic shell, have attracted significant attention for the delivery of poorly water-soluble drugs as well as therapeutic genes and proteins, leading to substantially improved drug stability and bioavailability.[1–12] The nanoscale dimension of the micelles is well documented to contribute to the passive tumor targeting, facilitated drug permeation across biological barriers, and prolonged lifetime in the blood circulation.[1–12] To date, most amphiphilic block co-polymers are based on the hydrophobic polyesters and hydrophilic poly(ethylene glycol) (PEG). Polyesters, exemplified by poly(lactic acid) (PLA), poly(glycolic acid) (PGA), poly(ε-caprolactone) (PCL), and poly(lactic-*co*-glycolic acid) (PLGA), display desired biocompatibility and biodegradability, and thus serve as ideal materials to construct the hydrophobic core of micelles for the encapsulation of lipophilic drugs.[13–26] PEG demonstrates excellent biocompatibility and it forms a hydrophilic corona around the micelles, thereby reducing the nonspecific adhesion of blood components onto micelle surfaces that ultimately leads to phagocytosis.[27] However, PEG is nondegradable in vivo; it can interfere with the interaction between micelles and target cells and thus compromise the drug delivery efficiency.[28–31] Several other hydrophilic polymers, such as poly(*N*-isopropylacrylamide) (PNIPAAm), poly(acrylic acid) (PAA), and poly(*N*,*N*-dimethylaminoethylacrylate) (PDMAEA), have

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also been explored and utilized as the hydrophilic building block of micelles.[32–34] Although encouraging results have been obtained for these micelles when used as drug delivery systems, the in vivo safety profiles of these nondegradable polymers are yet to be established and concerns still remain regarding their potential latent immunogenicity and antigenicity, and difficulties with renal excretion.[32–34] To this end, micelles composed entirely of degradable polyesters would be attractive alternatives to these afore-mentioned polyester-based micelles, which can effectively encapsulate and deliver the lipophilic drugs and then allow degradation in vivo. Additionally, considering that hydrophilic polymers such as poly[*N*-(2-hydroxypropyl)methacrylamide] (HPMA), poly(amino acid), zwitterionic polymer, and polysaccharide could serve as alternatives to PEG to impart stealth functions,[35] it is expected that water-soluble polyester with strong hydrophilicity might also bring stealth functions to micelles to allow long circulation and improve pharmacokinetics of therapeutic cargos.

To realize such design strategy, it necessitates the synthesis of water-soluble polyester segment that can act as the hydrophilic shell. Ideally, the hydrophilic polyester needs to be noncharged to prevent nonspecific tissue interactions as well as to prolong the circulation half-life when applied in vivo. In traditional approaches, preparation of water-soluble polyesters is synthetically challenging due to the lack of side-chain functionalities. Recently, a novel strategy has been developed to synthesize side-chain-functionalized polyesters via ring-opening polymerization (ROP) of five-membered-ring *O*-carboxyanhydrides (OCAs) derived from amino acids.[36–42] By taking advantage of this strategy, we have developed a new water-soluble polyester with pendant hydroxyl groups via the living ROP of *O*-benzyl-L-serine carboxyanhydrides.[39] We have also synthesized side-chain-functionalized polyester with pendant alkyne groups that are liable to post-functionalization by Huisgen 1,3-dipolar cycloaddition or thiol-yne "Click" reactions.[43–45] In this regards, a variety of functional moieties such as targeting ligands, antibodies, and sugars can be readily incorporated onto the polymer side chains, rendering it not only hydrophilicity but also specific biological function.

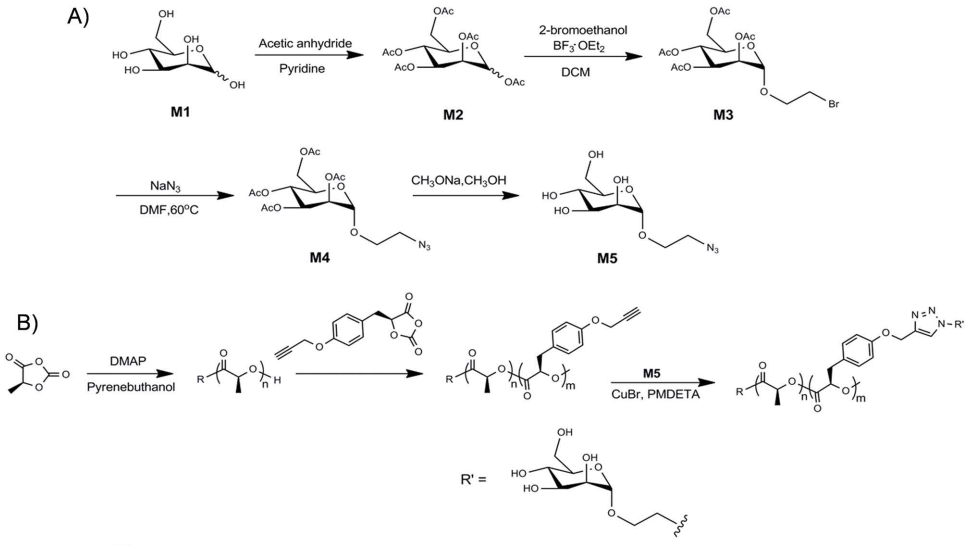
With the controlled ROP of OCAs, a novel amphiphilic diblock copolymer poly(Lac-OCA)-*b*-(poly(Tyr(alkynyl)-OCA)-*g*-mannose) (PLA-*b*-(PTA-*g*-mannose)) is herein developed to construct polymeric micelles that are composed entirely of biocompatible and biodegradable polyesters. Controlled ROP of L-LacOCA first yields the hydrophobic poly(Lac-OCA) block, and the terminal hydroxyl further initiated the controlled ROP of Tyr(alkynyl)-OCA to obtain the PTA block bearing alkyne side groups. The subsequent introduction of mannose residues via the highly efficient "Click" chemistry endows the PTA block with hydrophilicity, and thus the core–shell micelles are constructed from the amphiphilic PLA-*b*-(PTA-*g*-mannose) and used as delivery vehicles for doxorubicin (DOX), a lipophilic anticancer drug. The mannose ligand not only stabilizes the micelles under physiological conditions, but also allows active targeting to cancer cells expressing mannose receptors to potentiate the anticancer efficacy.

2. Results and Discussion

2.1. Characterization of PLA-*b*-(PTA-*g*-Mannose)

Well-defined PLA-*b*-PTA copolymer was prepared via a two-step sequential ROP of L-lacOCA and Tyr(alkynyl)-OCA that were reported previously.[39] PLA was first polymerized from L-lacOCA with pyrenebutanol as the initiator and 4-dimethylaminopyridine (DMAP) as the catalyst (Figure 1B). After a 16-h reaction, hydroxyl-terminated PLA with the degree of polymerization (DP) of 30 was obtained, which subsequently initiated the ROP of Tyr(alkynyl)-OCA to yield the diblock PLA-*b*-PTA. ^{1}H NMR was first used to confirm the structure of the polymers. As shown in Figure S1 (Supporting Information), the signals around 1.60 and 5.18 ppm corresponded to the resonance of methyl and methine protons of PLA, respectively, and representative resonance peaks (a–f) of PTA were also noted in the NMR spectrum of PLA-*b*-PTA. Gel permeation chromatography (GPC) analyses further revealed that M_n values of the resulting PLA and PLA-*b*-PTA at different M/I ratios (10, 30, and 50 for Tyr(alkynyl)-OCA) well agreed with the expected MWs, and they all showed narrow molecular weight distributions (MWDs, <1.1, Figure 1C and Figure S2, Supporting Information), which collectively demonstrated that DMAP mediated well-controlled ROP of OCA.

PLA-*b*-(PTA-*g*-mannose) was then prepared by grafting azido mannose (**M5**) onto the PTA block via the azide–alkyne Huisgen cycloaddition "Click" reaction.[46] Prior to this, **M5** was first synthesized as shown in Figure 1A. Briefly, D-mannose was globally protected with acetic anhydride to produce acylated compound **M2**. **M2** was then treated with 2-bromoethanol under Lewis acid-mediated conditions followed by treatment with NaN_3 in DMF at 60 °C for 6 h to yield the azide intermediate **M4**. **M5** was then prepared upon de-*O*-acetylation of **M4** with sodium methoxide in methanol. Catalyzed by CuBr and *N,N,N′,N″,N″*-pentamethyldiethylenetriamine (PMDETA), **M5** was conjugated to alkynyl-functionalized PLA-*b*-PTA to obtain the final amphipathic polyesters, PLA-*b*-(PTA-*g*-mannose) (Figure 1B). ^{1}H NMR spectrum showed the triazole peak at 8.13 ppm and the multiple peaks from 3.3 to 5.0 ppm that corresponded to the protons of the azide mannose (Figure S1, Supporting Information), which confirmed the success of the "Click" reaction and existence of the mannose moiety in the PLA-*b*-(PTA-*g*-mannose).



C)

Sample	M/I	$M_{n,cal}$(× 10^{-3} g/mol)	$M_{n,GPC}$(× 10^{-3} g/mol)	M_w/M_n
PLA_{30}	30	2.4	2.5	1.08
PLA_{30}-b-PTA_{10}	10	4.5	4.7	1.03
PLA_{30}-b- PTA_{30}	30	8.5	8.4	1.03
PLA_{30}-b- PTA_{50}	50	12.5	13.4	1.06

Figure 1. Synthesis of PLA-*b*-(PTA-*g*-mannose). A) Synthetic route of azido mannose (**M5**). B) Synthetic route of PLA-*b*-(PTA-*g*-mannose) via ROP of LacOCA and Tyr(alkynyl)-OCA with DMAP as the catalyst. C) MWs and PDI of PLA-*b*-(PTA-*g*-mannose).

2.2. Characterization of Micelles

Core–shell polymeric micelles were then prepared from the amphiphilic PLA-*b*-(PTA-*g*-mannose), which possessed a hydrophobic PLA core and a hydrophilic PTA-*g*-mannose shell. To prepare the micelles, the polymer (10 mg) was first dissolved in DMSO (2 mL), a good solvent for both the hydrophobic and the hydrophilic blocks, followed by addition of water (20 mL) under vigorous stirring. The freshly prepared micelles were further characterized by dynamic light scattering (DLS). As shown in Table S1 (Supporting Information), the micelles possessed hydrodynamic diameters of 80–130 nm, and an increase in the length of the hydrophilic PTA-*g*-mannose block led to increased particle size, presumably due to formation of a thicker hydrophilic out-layer. Transmission scanning microscopy (TEM) observation revealed similar particle size to DLS measurements, and spherical morphology was noted for assembled micelles in the aqueous solution (Figure 2A). Apart from the spherical shape obtained here, worm-like micelles might be achieved at a particular PLA/PTA ratio in the block copolymer, which could afford better performance than spherical micelles in terms of drug loading, systemic circulation, and cancer targeting.[47,48]

The critical micelle concentration (CMC) represents one of the most important parameters related to micellar stability, and thus was further determined by using Nile Red (NR) as a fluorescence probe.[49] As shown in Table S1 (Supporting Information) and Figure S3 (Supporting Information), all the PLA-*b*-(PTA-*g*-mannose) copolymers demonstrated low CMC values, and a decrease in the length of the hydrophilic PTA-*g*-mannose block led to a decrease in CMC values, indicating that shorter PTA-*g*-mannose block facilitated the formation of micelles due to higher hydrophobic interactions in the core. After incubation with PBS or cell culture media at 10-fold volume, unappreciable alteration in the diameter of the PLA_{30}-*b*-(PTA_{10}-*g*-mannose) micelles was noted (Figure 2C), indicating desired stability of these polyester-based micelles.

DOX is one of the most potent anticancer drugs in the treatment of different types of solid malignant tumors and is known to inhibit macromolecular biosynthesis by intercalating DNA.[50] Thus, DOX was selected as a model hydrophobic anticancer drug to evaluate the drug loading and release profiles of the polyester micelles. Drug encapsulation into micelles cores via physical entrapment is mainly driven by the hydrophobic interactions between the drug and the hydrophobic components of polymers.[51] Therefore, we selected PLA_{30}-*b*-PTA_{10} with the highest hydrophobic content to monitor the drug loading and release properties. At the theoretical drug- loading capacity (DLC) of 10%, DOX was well encapsulated into the PLA_{30}-*b*-PTA_{10} micelles at a loading efficiency of 51%, thus achieving the experimental DLC of 5.1%. Diameter of the micelles slightly increased to

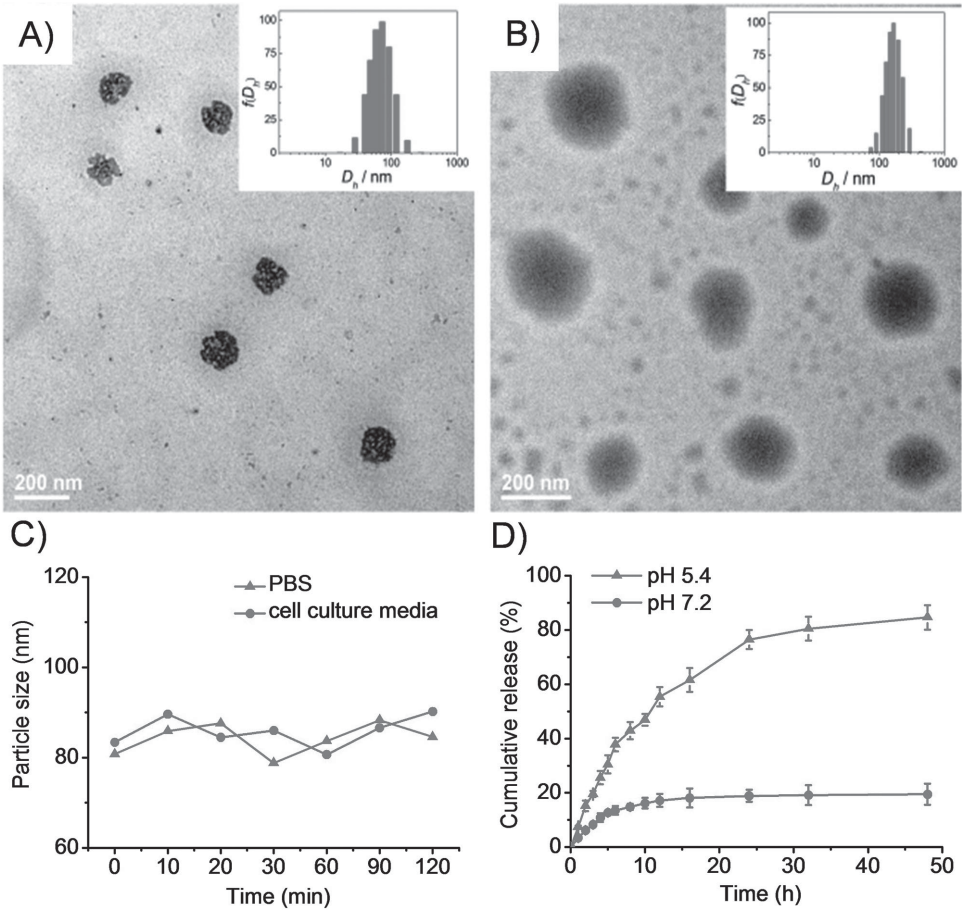


Figure 2. Characterization of PLA_{30}-*b*-(PTA_{10}-*g*-mannose) micelles. TEM image and size distribution (inset) of blank micelles (A) and DOX-loaded micelles (B). C) Stability of micelles following incubation in PBS or cell culture media. D) In vitro DOX release profiles from DOX-loaded micelles at different pH values (7.2 and 5.4) and 37 °C.

≈170 nm after drug loading, as measured by both DLS and TEM (Figure 2B).

The therapeutic index of anticancer drugs is closely related to their release behaviors from the delivery system. pH of the blood is neutral (≈7.2), while pH in the endosomal/lysosomal compartments of cancer cells is acidic (5.0–5.5). As such, the in vitro release studies on DOX-loaded micelles were carried out in phosphate buffer solution (pH 7.2) and acetate buffer solution (pH 5.4). As shown in Figure 2D, only 20% of the encapsulated DOX was released at pH 7.2 within 48 h. Comparatively, a notably faster DOX release was observed at pH 5.4, achieving a cumulative release amount of 80% within 48 h. Such disparities indicated that DOX release from PLA_{30}-*b*-PTA_{10} micelles was pH dependent, which may be attributed to the enhanced DOX solubility at lower pH.[52] The pH-dependent release profile thus allowed DOX to be encapsulated in the micelles during circulation in the blood while after reaching the acidic tumor sites and endosomal/lysosomal compartments inside the tumor cells, DOX could be extensively released to exert anticancer efficacy.

2.3. Cellular Internalization of Micelles

Human lung adenocarcinoma (A549) and hepatocellular carcinoma (HepG-2) cell lines that express mannose receptors were selected to probe the delivery efficiency and anticancer efficacy of DOX-loaded polyester micelles in vitro.[53,54] As shown in Figure 3A,B, DOX-loaded micelles showed comparable cell uptake levels to free DOX in HepG-2 cells while slightly higher uptake levels in A549 cells, and the uptake level increased with the incubation time and feeding amount. To verify the mannose receptor-mediated targeting effect of micelles, we performed the cell uptake study in the presence of free mannose. As shown in Figure 3C, an increase in the free mannose concentration significantly reduced the cell uptake level of DOX-micelles but not free DOX, which substantiated our design hypothesis that mannose-containing micelles targeted to cell membranes via recognition of mannose receptors that could be competitively occupied by free mannose. Because the intracellular kinetics is closely related to the internalization pathways, we then probed the mechanisms

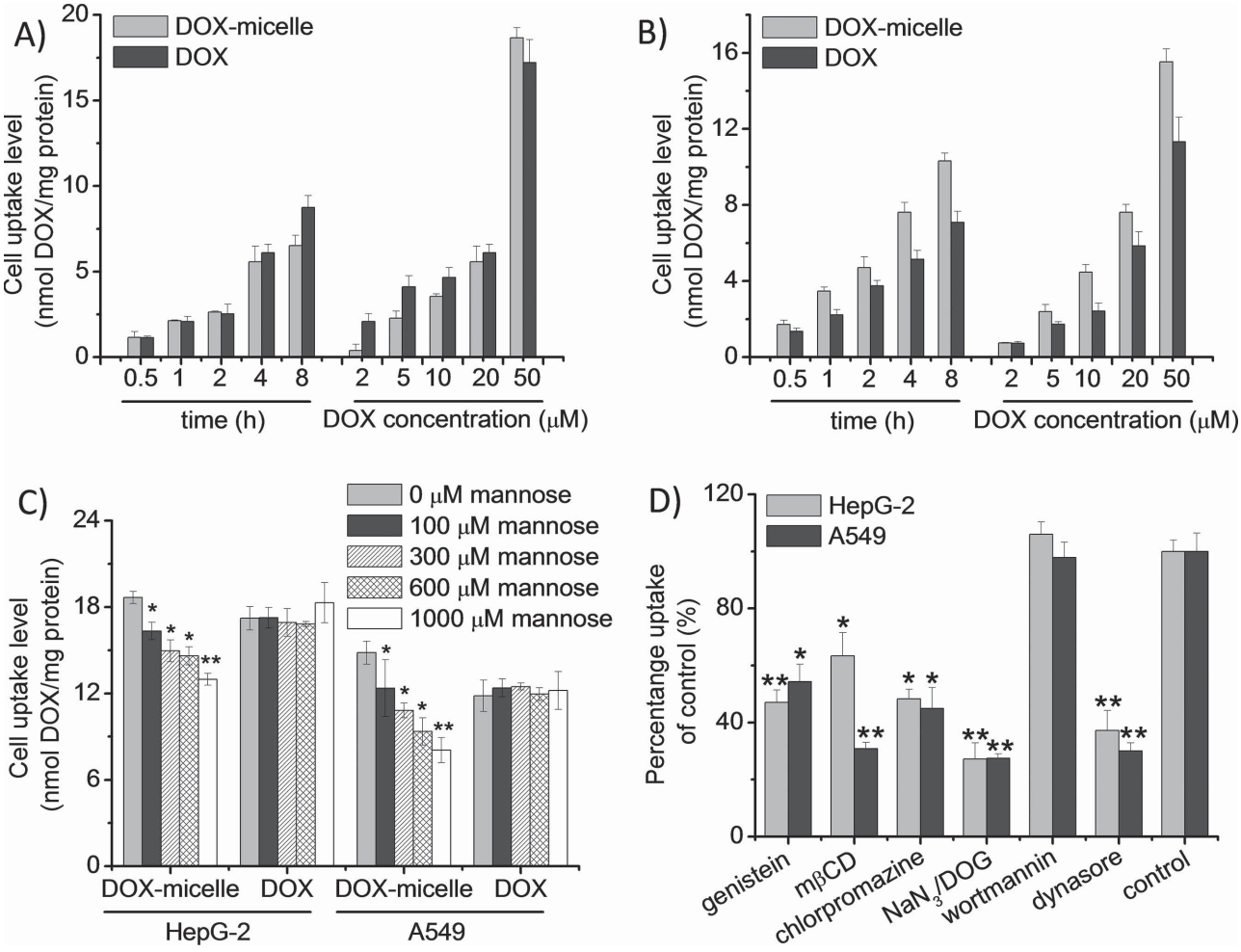


Figure 3. Cell uptake levels of DOX and DOX-micelles in HepG-2 (A) and A549 (B) cells at different DOX concentrations (incubation time kept constant at 4 h) or incubation time (DOX concentration kept constant at 20×10^{-6} M). C) Cell uptake levels of DOX and DOX-micelles in the presence of free mannose at different concentrations. Statistical difference to the cellular uptake level in the presence of 0×10^{-6} M mannose (*$p < 0.05$, **$p < 0.01$). D) Mechanistic probe of the internalization mechanism of DOX-micelles. Statistical difference to control (*$p < 0.05$, **$p < 0.01$).

underlying the cellular internalization of DOX-loaded micelles by performing the cell uptake study in the presence of various endocytic inhibitors. NaN_3/deoxyglucose (DOG) completely block endocytosis by depleting the energy; chlorpromazine inhibits clathrin-mediated endocytosis (CME) by triggering the dissociation of the clathrin lattice; genisteine and methyl-β-cyclodextrin (mβCD) inhibit caveolae by inhibiting tyrosine kinase and depleting cholesterol, respectively; dynasore inhibits both CME and caveolae by inhibiting dynamin; wortmannin inhibits macropinocytosis by inhibiting phosphatidyl inositol-3-phosphate.[55,56] As shown in Figure 3D, the cell uptake level of DOX-micelles was inhibited by 70%–80% by NaN_3/DOG, suggesting that majority of the micelles were endocytosed while the remaining were internalized via energy-independent physical binding and diffusion. The cell uptake was also inhibited by genistein, mβCD, chlorpromazine, and dynasore, suggesting that the endocytosis was related to both caveolae- and clathrin-mediated pathways. In comparison, wortmannin exerted unappreciable inhibitory effect, indicating that macropinocytosis was not involved during the internalization of DOX-micelles.

2.4. In Vitro Anticancer Efficacy

The in vitro anticancer efficacy of DOX-loaded micelles following 72-h treatment was further evaluated and compared to free DOX using the MTT assay. As shown in Figure 4A, DOX-loaded micelles exerted notable cytotoxicity towards both cell lines, affording IC_{50} values of 5.2×10^{-6} and 4.3×10^{-6} M DOX-equiv in A549 and HepG-2 cells, respectively. As a comparison, free DOX achieved IC_{50} values of 1.2×10^{-6} and 0.6×10^{-6} M in A549 and HepG-2

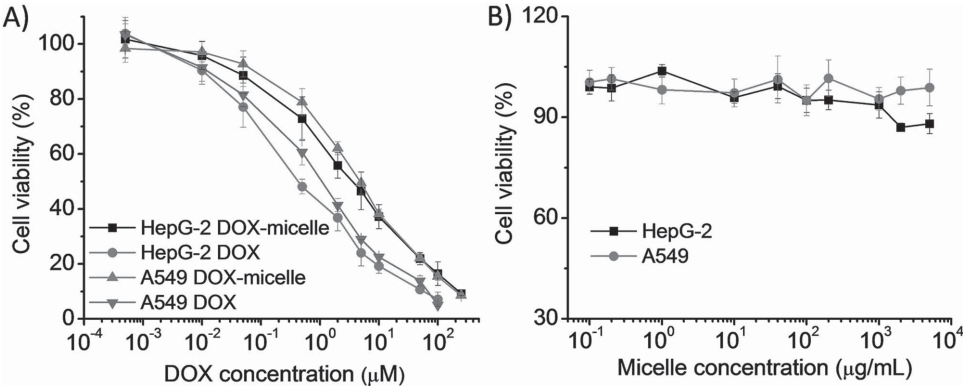


Figure 4. Cytotoxicity of DOX-loaded micelles (A) and blank micelles (B) towards HepG-2 and A549 cells following incubation for 72 h. Free DOX at various concentrations was incorporated as a control.

cells, respectively. More noteworthy is that blank micelles exhibited unappreciable cytotoxicity at high concentrations up to 5000 μg mL^{-1} (equals to DOX concentration of 500×10^{-6} M for DOX-loaded micelles, Figure 4B), indicating desired safety profiles of the micelles that are comprised entirely of biocompatible and biodegradable polyesters.

3. Conclusion

We developed a micellar delivery system for anticancer drugs that are comprised entirely of biocompatible and biodegradable polyesters. With the ROP strategy of OCA and "Click" chemistry, PLA-*b*-(PTA-*g*-mannose) diblock copolymers can be synthesized in a highly controlled manner such that the optimal structure toward micellation can be easily identified. Mannose residues on the polymer side chains allowed the micelles to target cancer cells expressing mannose receptors such that anticancer efficacy of the drug-loaded micelles can be improved. With their desired biocompatibility, biodegradability, and cancer cell targeting capabilities, these micelles demonstrate great potentials for targeted anticancer drug delivery towards cancer therapy. In a future study, the degradation kinetics of PLA-*b*-(PTA-*g*-mannose) will be clarified, and the potential of PLA-*b*-(PTA-*g*-mannose) to impart stealth functions to micelles will be explored.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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[1] H. Arimura, Y. Ohya, T. Ouchi, *Biomacromolecules* **2005**, *6*, 720.
[2] R. Gref, Y. Minamitake, M. T. Peracchia, V. Trubetskoy, V. Torchilin, R. Langer, *Science* **1994**, *263*, 1600.
[3] K. Kataoka, A. Harada, Y. Nagasaki, *Adv. Drug Delivery Rev.* **2001**, *47*, 113.
[4] J. Y. Liu, Y. Pang, W. Huang, X. Y. Zhu, Y. F. Zhou, D. Y. Yan, *Biomaterials* **2010**, *31*, 1334.
[5] S. Matsumoto, R. J. Christie, N. Nishiyama, K. Miyata, A. Ishii, M. Oba, H. Koyama, Y. Yamasaki, K. Kataoka, *Biomacromolecules* **2009**, *10*, 119.
[6] V. P. Torchilin, *Pharm. Res.* **2007**, *24*, 1.
[7] J. W. Bae, R. M. Pearson, N. Patra, S. Sunoqrot, L. Vukovic, P. Kral, S. Hong, *Chem. Commun.* **2011**, *47*, 10302.
[8] W. P. Wang, Z. M. Yang, S. Patanavanich, B. Xu, Y. Chau, *Soft Matter* **2008**, *4*, 1617.
[9] L. F. Zhang, A. F. Radovic-Moreno, F. Alexis, F. X. Gu, P. A. Basto, V. Bagalkot, S. Y. Jon, R. S. Langer, O. C. Farokhzad, *ChemMedChem* **2007**, *2*, 1268.
[10] J. Pyun, *Polym. Rev.* **2007**, *47*, 231.
[11] C. H. Luo, Y. Liu, Z. B. Li, *Soft Matter* **2012**, *8*, 2618.
[12] W. F. Ma, S. A. Xu, J. M. Li, J. Guo, Y. Lin, C. C. Wang, *J. Polym. Sci., Polym. Chem.* **2011**, *49*, 2725.
[13] A. C. Albertsson, I. K. Varma, *Biomacromolecules* **2003**, *4*, 1466.
[14] P. Bordes, E. Pollet, L. Averous, *Prog. Polym. Sci.* **2009**, *34*, 125.
[15] C. S. Ha, J. A. Gardella, *Chem. Rev.* **2005**, *105*, 4205.
[16] M. Okada, *Prog. Polym. Sci.* **2002**, *27*, 87.
[17] H. Seyednejad, D. Gawlitta, R. V. Kuiper, A. de Bruin, C. F. van Nostrum, T. Vermonden, W. J. A. Dhert, W. E. Hennink, *Biomaterials* **2012**, *33*, 4309.
[18] A. Sodergard, M. Stolt, *Prog. Polym. Sci.* **2002**, *27*, 1123.
[19] M. Sokolsky-Papkov, K. Agashi, A. Olaye, K. Shakesheff, A. J. Domb, *Adv. Drug Delivery Rev.* **2007**, *59*, 187.
[20] R. Tong, J. J. Cheng, *J. Am. Chem. Soc.* **2009**, *131*, 4744.
[21] R. Tong, L. D. Yala, T. M. Fan, J. J. Cheng, *Biomaterials* **2010**, *31*, 3043.

[22] K. E. Uhrich, S. M. Cannizzaro, R. S. Langer, K. M. Shakesheff, *Chem. Rev.* **1999**, *99*, 3181.
[23] O. C. Farokhzad, S. Y. Jon, A. Khademhosseini, T. N. T. Tran, D. A. LaVan, R. Langer, *Cancer Res.* **2004**, *64*, 7668.
[24] S. Sunoqrot, J. W. Bae, R. M. Pearson, K. Shyu, Y. Liu, D. H. Kim, S. Hong, *Biomacromolecules* **2012**, *13*, 1223.
[25] Y. Yu, J. Zou, L. Yu, W. Jo, Y. K. Li, W. C. Law, C. Cheng, *Macromolecules* **2011**, *44*, 4793.
[26] J. Zou, Y. Yu, L. Yu, Y. K. Li, C. K. Chen, C. Cheng, *J. Polym. Sci. Polym. Chem.* **2012**, *50*, 142.
[27] G. Pasut, F. M. Veronese, *J. Controlled Release* **2012**, *161*, 461.
[28] H. Hatakeyama, H. Akita, H. Harashima, *Adv. Drug Delivery Rev.* **2011**, *63*, 152.
[29] R. L. Hong, C. J. Huang, Y. L. Tseng, V. F. Pang, S. T. Chen, J. J. Liu, F. H. Chang, *Clin. Cancer Res.* **1999**, *5*, 3645.
[30] B. Romberg, W. E. Hennink, G. Storm, *Pharm. Res. Dord* **2008**, *25*, 55.
[31] S. Mishra, P. Webster, M. E. Davis, *Eur. J. Cell Biol.* **2004**, *83*, 97.
[32] S. Pioge, C. A. Fustin, J. F. Gohy, *Macromol. Rapid Commun.* **2012**, *33*, 534.
[33] C. C. Liu, G. Chen, H. Sun, J. Xu, Y. H. Feng, Z. Zhang, T. Wu, H. Y. Chen, *Small* **2011**, *7*, 2721.
[34] H. Y. Hong, Y. Y. Mai, Y. F. Zhou, D. Y. Yan, Y. Chen, *J. Polym. Sci., Polym. Chem.* **2008**, *46*, 668.
[35] C. M. J. Hu, R. H. Fang, B. T. Luk, L. F. Zhang, *Nanoscale* **2014**, *6*, 65.
[36] O. T. du Boullay, C. Bonduelle, B. Martin-Vaca, D. Bourissou, *Chem. Commun.* **2008**, *15,* 1786.
[37] O. T. du Boullay, E. Marchal, B. Martin-Vaca, F. P. Cossio, D. Bourissou, *J. Am. Chem. Soc.* **2006**, *128*, 16442.
[38] M. Kolitz, N. Cohen-Arazi, I. Hagag, J. Katzhendler, A. J. Domb, *Macromolecules* **2009**, *42*, 4520.
[39] Y. B. Lu, L. C. Yin, Y. F. Zhang, Z. H. Zhang, Y. X. Xu, R. Tong, J. J. Cheng, *ACS Macro Lett.* **2012**, *1*, 441.
[40] R. J. Pounder, D. J. Fox, I. A. Barker, M. J. Bennison, A. P. Dove, *Polym. Chem. UK* **2011**, *2*, 2204.
[41] Q. Yin, R. Tong, L. C. Yin, T. M. Fan, J. J. Cheng, *Polym. Chem. UK* **2014**, *5*, 1581.
[42] Q. Yin, R. Tong, Y. X. Xu, K. Baek, L. W. Dobrucki, T. M. Fan, J. J. Cheng, *Biomacromolecules* **2013**, *14*, 920.
[43] Z. H. Zhang, L. C. Yin, C. L. Tu, Z. Y. Song, Y. F. Zhang, Y. X. Xu, R. Tong, Q. Zhou, J. Ren, J. J. Cheng, *ACS Macro Lett.* **2013**, *2*, 40.
[44] Z. H. Zhang, L. C. Yin, Y. X. Xu, R. Tong, Y. B. Lu, J. Ren, J. J. Cheng, *Biomacromolecules* **2012**, *13*, 3456.
[45] H. Wang, L. Tang, C. Tu, Z. Song, Q. Yin, L. Yin, Z. Zhang, J. Cheng, *Biomacromolecules* **2013**, *14*, 3706.
[46] A. C. Engler, H. I. Lee, P. T. Hammond, *Angew. Chem. Int. Ed.* **2009**, *48*, 9334.
[47] Y. Geng, P. Dalhaimer, S. S. Cai, R. Tsai, M. Tewari, T. Minko, D. E. Discher, *Nat. Nanotechnol.* **2007**, *2*, 249.
[48] S. S. Cai, K. Vijayan, D. Cheng, E. M. Lima, D. E. Discher, *Pharm. Res. Dordr.* **2007**, *24*, 2099.
[49] C. J. Chen, G. Y. Liu, X. S. Liu, S. P. Pang, C. S. Zhu, L. P. Lv, J. Ji, *Polym. Chem. UK* **2011**, *2*, 1389.
[50] M. L. Adams, A. Lavasanifar, G. S. Kwon, *J. Pharm. Sci.* **2003**, *92*, 1343.
[51] X. T. Shuai, H. Ai, N. Nasongkla, S. Kim, J. M. Gao, *J. Controlled Release* **2004**, *98*, 415.
[52] C. Wei, K. Y. Wu, J. M. Li, W. F. Ma, J. Guo, J. Hu, C. C. Wang, *Macromol. Chem. Phys.* **2012**, *213*, 557.
[53] J. H. Ahire, I. Chambrier, A. Mueller, Y. P. Bao, Y. M. Chao, *ACS Appl. Mater. Int.* **2013**, *5*, 7384.
[54] K. Vonfigura, V. Gieselmann, A. Hasilik, *Biochem. J.* **1985**, *225*, 543.
[55] S. E. A. Gratton, P. A. Ropp, P. D. Pohlhaus, J. C. Luft, V. J. Madden, M. E. Napier, J. M. DeSimone, *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 11613.
[56] I. A. Khalil, K. Kogure, H. Akita, H. Harashima, *Pharmacol. Rev.* **2006**, *58*, 32.