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RESEARCH PAPER

Anticancer Drug-Loaded Nanospheres Based on Biodegradable Amphiphilic ε-Caprolactone and Carbonate Copolymers

Guo-Ping Yan • Rong-Feng Zong • Liang Li • Ting Fu • Fan Liu • Xiang-Hua Yu

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ABSTRACT

Purpose The aim was to investigate anticancer drug-loaded poly(carbonate-ester) nanospheres as potential drug delivery systems for cancer therapy.

Methods Functional poly(carbonate-ester) copolymers (HPCP-SD) were synthesized by the incorporation of sulfadiazine as the tumor-targeting groups to hydroxyl groups of poly (carbonate-ester) copolymers. Two types of anticancer drug-loaded poly(carbonate-ester) nanospheres I and II were further prepared by dialysis method and high-voltage electrostatic field-assisted atomization, respectively, using HPCP-SD as polymeric carriers. These carriers and anticancer drug-loaded nanospheres were characterized, and their properties *in vitro* were evaluated.

Results These anticancer drug-loaded poly(carbonate-ester) nanospheres had steady drug release rates and good controlled release properties. Moreover, anticancer drug-loaded poly(carbonate-ester) nanospheres II had faster drug release rates than those of anticancer drug-loaded nanospheres I. These anticancer drug-loaded nanospheres possessed lower cytotoxicity to HEK 293 cells and exhibited obviously higher anticancer efficiencies to the HeLa tumor cells than that of 5-fluorouracil. Anticancer drug-loaded nanospheres I possessed lower cytotoxicity to HEK 293 cells and higher anticancer activity to HeLa cells than those of anticancer drug-loaded nanospheres II.

Conclusions These anticancer drug-loaded poly(carbonate-ester) nanospheres showed the potential as drug delivery systems for cancer therapy.

KEY WORDS biodegradable polycarbonate · copolymers · drug controlled release · nanospheres · sulfadiazine

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INTRODUCTION

Over the past few decades, the tumor-targeting polymeric drug-controlled release system has attracted extensive attention, since it can possibly achieve targeted delivery of anticancer drugs to tumors and then give drug continually, utilize drug more effectively, stabilize the drug in matrix, reduce side effects, prolong time periods and reduce frequency of doses. One important approach in this ideal drug delivery system, such as water-soluble polymeric prodrugs, nanopartcles and microspheres, was that the attached drugs were usually incorporated to biodegradable macromolecular carriers containing the tumor-targeting groups by chemical reaction or physical methods (1–13).

In recent years, biodegradable polycarbonates, poly(ε-caprolactone) (PCL), and their copolymers have been widely used as biodegradable materials in drug delivery and tissue engineering because of their good medicine permeability, low toxicity, low immunogenity, good biocompatibility and degradability (14–28).

In previous works, a series of poly(carbonate-ester) copolymers poly(ε-caprolactone-co-9-phenyl-2, 4, 8, 10-tetraoxaspiro-[5, 5]undcane-3-one) P(CL-co-PTC) were synthesized by the ring-opening bulk polymerization of ε-caprolactone (CL) and 9-phenyl-2, 4, 8, 10-

tetraoxaspiro-[5, 5]undcane-3-one (PTC). These poly (carbonate-ester) copolymers were further reduced by the palladium/carbonate (Pd/C) catalyst to obtain the partly deprotected poly(carbonate-ester) copolymers poly(ε-caprolactone-co-9-phenyl-2, 4, 8, 10-tetraoxaspiro-[5, 5]undcane- 3-one) containing hydroxyl groups (HPCP). The experimental results indicated that poly(carbonate-ester) copolymers HPCP possessed more hydrophilicity, faster degradation rates and faster drug release rates than those of the corresponding poly (carbonate-ester) copolymers P(CL-co-PTC), presumably because the hydrophilic hydroxyl groups enhanced the hydrophilicity and promoted water absorption and permeation into the copolymer matrix for improving their biodegradation rates (29,30). Moreover, the introduction of hydroxyl functional groups to the side chains of poly(carbonate-ester) copolymers HPCP improved amphiphilic property and potentially provided a platform for chemical modification to readily attach drugs, environmental sensitivity groups and tissue or organ-targeting groups in drug delivery systems.

Sulfadiazine (SD) derivatives were reported to be concentrated into Walker carcinoma or Yoshida sarcoma by a factor of about 2–3 compared with the uptake in the liver (31). Previously, sulfadiazine has been used as a tumor-selective group and then incorporated into gadolinium diethylenetriaminepentaacetic acid (Gd-DTPA) to obtain the potential tumor-specific contrast agents. The biodistribution and MRI studies indicated that the incorporation of SD into Gd-DTPA increased their uptake by Hepatoma and Ehrlich ascites carcinoma in mice, and these gadolinium complexes exhibited tumor-selective properties (32). Polyaspartamide gadolinium complexes containing sulfadiazine groups also possessed higher relaxation effectiveness than Gd-DTPA and the specific uptake by the Hepatoma. Moreover, MR imaging showed that these polyaspartamide gadolinium complexes containing sulfadiazine groups greatly enhanced the contrast of MR images of Hepatoma in the lower limb of mice and provided prolonged intravascular duration (33).

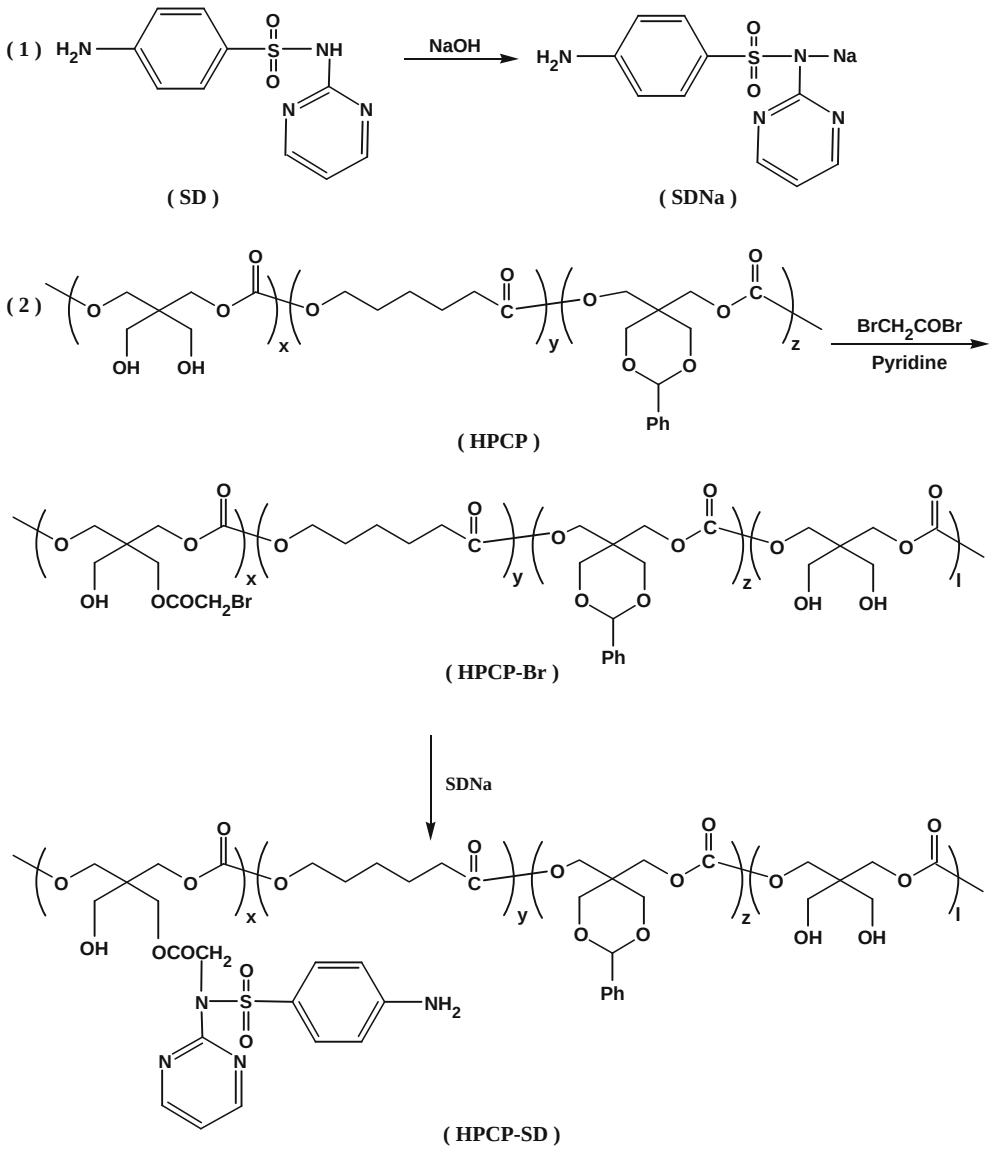
In this work, sulfadiazine as the tumor-targeting groups were incorporated to the hydroxyl groups of poly(carbonate-ester) copolymers HPCP to synthesize the functional poly(carbonate-ester) copolymers containing sulfadiazine and hydroxyl groups (HPCP-SD) (Scheme 1). Two types of anticancer drug-loaded poly(carbonate-ester) nanospheres I and II containing 5-fluorouracil (5-Fu) as a drug model were further prepared by dialysis method and high-voltage electrostatic field-assisted atomization, respectively, using the functional poly(carbonate-ester) copolymers HPCP-SD thus obtained as polymeric carriers. These anticancer drug-loaded poly(carbonate-ester) nanospheres were characterized, and their properties *in vitro* were evaluated.

MATERIALS AND METHODS

Instrumentation and Materials

The compounds prepared were characterized using a Nicolet is10 fourier transform-infrared (FT-IR) spectrophotometer (Thermo Fisher Scientific Inc., Madison, WI, United States of American), a UV-Vis spectrophotometer (UV-2800 series, Unico, Shanghai, China), a Varian Mercury-VX300 NMR spectrometer (Varian, Inc. Corporate, Palo Alto, CA, United States of American) and an automatic contact angle meter (SL200A/B/D Series, Solon Tech. Inc. Ltd., Shanghai, China). The molecular weight was measured by a gel permeation chromatography (GPC, Waters Corporation Milford, MA, United States of American) (Waters 2965D separations module, Waters 2414 Refractive Index Detector, Shodex K802.5 & K805 with Shodex K-G Guard Column, Polystyrene Standard, DMF solvent, 1.0 mL.min-1 flow rate, 323 K Column temperature and 318 K Detector temperature). The glass transition temperature (Tg) of copolymer was measured by a differential scanning calorimeter (DSC) (NETZSCH DSC 200 F3, Erich NETZSCH GmbH & Co. Holding KG, Gebrüder-Netzsch-Strasse, Selb, Germany). The micromorphology of the nanoparticles was studied using a transmission electron microscopy (TEM, Tecnai G2 20, FEI Company, Netherlands) and a zeta potential and laser diffraction particle size analyzer (Zetasizer Nano ZS, Malvern Instruments Ltd., United Kingdom). The optical densities (OD_{570}) were measured with a DG-3022A ELISA-Reader (Hercules, CA, United States of America). The 293T human embryonic kidney cells (HEK 293) and HeLa cells were provided by the China Center for Type Culture Collection of Wuhan University, China.

All the chemicals used were analytical reagents. Dichloromethane and pyridine were purified by the conventional methods before use. The growth medium was the RPMI-1640 media (10% fetal bovine serum (Gibco. Co., United States of America), 100 units/mL penicillium, 100 μg/mL streptomycin). The partly deprotected poly(carbonate-ester) copolymers poly(ε-caprolactone-co-9-phenyl-2, 4, 8, 10-tetraoxaspiro-[5, 5]undcane-3-one) P (CL-co-PTC) containing hydroxyl groups (HPCP) were synthesized according to the method described in literature (29,30). HPCP: Mn 0.96×10^4, Mw/Mn 1.21, contact angle 60.73°, glass transition temperature (Tg) 36.2°C, repeat structure unit CL/PTC in copolymer (mol/mol) 8.264:1, PTC repeat unit molar content ratio (mol%) in molecular structure 10.79%, reduction rate of PTC repeat unit



Scheme 1 Synthetic route to functional poly(carbonate-ester) copolymers containing sulfadiazine and hydroxyl groups (HPCP-SD).

61.86%, repeat unit molar content ratio (mol%) of hydroxyl groups in molecular structure 13.35%.

Synthesis of Functional Poly(Carbonate-Ester) Copolymers Containing Hydroxyl and Sulfadiazine Groups (HPCP-SD)

Sulfadiazine (SD, 1.5 g, 6 mmol) and sodium hydroxide (0.3 g, 7.5 mmol) were dissolved in 10 mL of distilled water. The mixture was stirred for 3 h at room temperature and then precipitated using ethanol (200 mL). The precipitated solid was centrifuged, filtered and dried under vacuum for 48 h to yield a white powder of sulfadiazine sodium (SDNa, 1.31 g, 80%).

The poly(carbonate-ester) copolymers HPCP (1.33 g, 9.8 mmol) were dissolved in 20 mL of dichloromethane, and then bromoacetyl bromide (0.389 g, 1.93 mmol) was added to the polymeric solution. Pyridine (0.778 g, 9.8 mmol) was slowly added dropwise to the mixture solution at 0°C and stirred for 12 h at room temperature. The mixture was filtered and then precipitated using anhydrous ether (200 mL). The precipitated solid was centrifuged, filtered, reprecipitated using anhydrous ether for three times, and dried under vacuum for 48 h to yield a white powder (HPCP-Br, 1.20 g, 77%). ^{1}H NMR ($CDCl_3$, δ, ppm): 7.25–7.45 (m, **C_6H_5**-), 5.44 (s, C_6H_5-**CH**-$(O)_2$-), 4.47 (s, -COO-CH_2C(**CH_2**O-)$_2$-CH_2O-), 4.11–4.13 (s, -COO-CH_2C(CH_2O-)$_2$-**CH_2**O-), 3.95–4.07 (s, -COO-**CH_2**C(CH_2O-)$_2$-CH_2O-), 3.84–3.91 (t, -OOC$CH_2CH_2CH_2CH_2$**CH_2**O-), 2.27–2.34 (t, -OOC**CH_2**$CH_2CH_2CH_2CH_2O$-), 1.61–1.64 (m, -OOCCH_2**CH_2**CH_2**CH_2**CH_2O-), 1.37–1.39 (m, -OOCCH_2CH_2**CH_2**CH_2CH_2O-); IR (KBr, cm^{-1}): 3450 (-OH), 2958, 2858 (-CH_2), 1752 (C=O), 1390 (C-C), 1104 (C-O); UV ($CHCl_3$, λ, nm): 256.

HPCP-Br (1.5 g, 9.4 mmol) was dissolved in 10 mL of dichloromethane, and then a solution of sulfadiazine sodium dissolved (2.56 g, 9.4 mmol) in 10 mL of distilled water was added. Subsequently, 10% Tetrabutylammonium hydroxide solution (0.03248 g, 0.8–1.2%) was added, and the mixture was stirred for 12 h at room temperature. The reaction solution was precipitated using ether, and the precipitated solid was washed by distilled water to remove unreacted sulfadiazine sodium, centrifuged, filtered, and dried under vacuum for 48 h to yield a white powder functional poly(carbonate-ester) copolymers poly(ε-caprolactone-co-9-phenyl-2, 4, 8, 10- tetraoxaspiro-[5, 5] undcane-3-one) containing sulfadiazine and hydroxyl groups (HPCP-SD, 1.332 g, 74%). ^{1}H NMR (DMSO-d_6, δ ppm): 8.83, 8.47, 8.3 (m, -CN-**CH**=**CH**-**CH**=N-), 8.10, 8.05, 7.45 (m, C_6H_4-, C_6H_5-), 6.44 (s, C_6H_5-**CH**-$(O)_2$-, C($\mathbf{CH_2}$O-)$_2$-CH), 5.40 (s, -OOC-CH_2C($\mathbf{CH_2}$O-)$_2$-CH_2O-), 4.9 (s, -OOC-CH_2C(CH_2O-)$_2$-$\mathbf{CH_2}$O-), 3.6–3.2 (m, -OOC$CH_2CH_2CH_2CH_2\mathbf{CH_2}$O-, -OOC-$\mathbf{CH_2}$C($CH_2$O-)$_2$-$CH_2$O-), 2.4 (t, -OOC$\mathbf{CH_2}CH_2CH_2CH_2CH_2$O-), 1.58 (m, -OOC$CH_2\mathbf{CH_2}CH_2\mathbf{CH_2}CH_2$O-), 1.3 (m, -OOC$CH_2CH_2\mathbf{CH_2}CH_2CH_2$O-); IR (KBr, cm^{-1}): 3355 (OH, NH_2), 2932, 2854 (C-H), 1737, 1642.4, 1620.6, 1595, 1581.3 (COO, CONH), 1397.2, 1172.5(S=O), 1154.6, 1094.2 (CN, C-C), 907.2, 840.3, 803.8(C_6H_4, $C_4N_2H_2$); UV ($CHCl_3$, λ, nm): 256, 274. The average grafted ratios of sulfadiazine groups (mol%) and hydroxyl groups of poly (carbonate-ester) copolymers HPCP-SD with the feed molar ratio of hydroxyl groups in HPCP/bromoacetyl bromide as 1:1 were determined from ^{1}H NMR as 5.67% and 7.68%, respectively.

The functional poly(carbonate-ester) copolymers HPCP-SD with different average grafted ratios of sulfadiazine groups (mol%) were synthesized by the same method at the different reaction conditions. Their average grafted ratios of sulfadiazine groups (mol%) and hydroxyl groups of poly (carbonate-ester) copolymers HPCP-SD with the feed molar ratio of hydroxyl groups in HPCP/bromoacetyl bromide as 2:1 were determined from ^{1}H NMR as 4.01% and 9.34%, respectively. Their average grafted ratios of sulfadiazine groups (mol%) and hydroxyl groups of poly (carbonate-ester) copolymers HPCP-SD with the feed molar ratio of hydroxyl groups in HPCP/bromoacetyl bromide as 1:2 were also determined from ^{1}H NMR as 8.01% and 5.34%, respectively.

Preparation of Anticancer Drug-Loaded Poly (Carbonate-Ester) Nanospheres

Anticancer drug-loaded poly(carbonate-ester) nanospheres I prepared by dialysis method (8): 10 mg of 5-fluorouracil (5-Fu, 10 mg) and 10 mg of HPCP-SD were dissolved in 2 mL of dimethyl sulphoxide (DMSO), and the solution was homogenized by sonication of 30 s. Subsequently, to remove free 5-Fu and form 5-Fu-incorporated nanoparticles, the solution was dialyzed against 2 L of distilled water using the dialysis membrane (EQ1040-4, 21 mm, MWCO: 8000–12000). During the first 3 h, the water was exchanged three times (every hour) and then two times during the following 6 h (every 3 h). After a total of 24 h of dialysis, the solution was lyophilized to form anticancer drug-loaded poly(carbonate-ester) nanospheres I.

Anticancer drug-loaded poly(carbonate-ester) nanospheres II prepared by high-voltage electrostatic field-assisted atomization: 10 mg of 5-Fu (10 mg) and 10 mg of HPCP-SD were dissolved in 3 mL of dimethyl sulphoxide (DMSO), and the solution was homogenized by sonication of 30 s. Subsequently, the solution was sprayed from a needle as the positive electrode into anhydrous ethanol in the negative electrode to precipitate to form anticancer drug-loaded poly(carbonate-ester) nanospheres using the Nano Anticancer Drug Manufacturer (with a high voltage electricity: 3694 V, needle diameter: 0.5 mm, high: 40 mm, volume of flow: 1.06 mL/h). The mixture solution was dialyzed for 24 h with distilled water and then was evaporated and lyophilized to make anticancer drug-loaded poly(carbonate-ester) nanospheres II.

In Vitro Drug Release Study

The anticancer drug-loaded poly(carbonate-ester) nanospheres (80–100 mg) were suspended in 10 mL of phosphate-buffered saline (PBS) in a dialysis bag. The dialysis bag was sealed and then slowly shaken in 90 mL of PBS at 37°C in a 250-mL Erlenmeyer flask. Aliqouts of the solution outside the dialysis membrane (25 mL) were replaced with 25 mL of PBS at various times intervals and tested at 256 nm by a HPLC spectrophotometer. The change of the concentrations of 5-Fu was obtained from curves of the absorption *A versus* concentration *C* of 5-Fu in PBS based on Lambert-Beer law.

In Vitro Cytotoxicity Assay

293T human embryonic kidney cells (HEK 293, 2×10^5/mL) were plated in 96-well plates in the growth medium (the RPMI-1640 media: 10% fetal bovine serum (Gibco. Co., USA), 100units/mL penicillium, 100 μg/mL streptomycin), and the number of cells in each well was 2×10^4. The cells were incubated for 24 h in incubator (37°C, 5% CO_2), and the growth medium was then removed and replaced with 100 μL of the growth medium containing 5-Fu or anticancer drug-loaded poly(carbonate-ester) nano-

Table I Effect of Molar Feed Ratio of Hydroxyl Groups in HPCP/Bromoacetyl Bromide on Functional Poly(Carbonate-Ester) Copolymers HPCP-SD

Feed molar ratio of hydroxyl groups/bromoacetyl bromide (mol/mol)	Mn(g/mol)	PDI (Mw/Mn)	Contact angle (degree)
1:0	9600	1.21	60.73
2:1	9264	1.01	75.20
1:1	9263	1.01	77.91
1:2	9241	1.01	78.77

spheres. After a 48-h incubation, 20 μL of a 5.0 mg/mL MTT (thiazolyl blue (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide) solution in PBS were added to each well. The cells were incubated for 3 h again, and 100 μL of DMSO was then added and shaken for 30 min at room temperature, after which the growth medium was removed. The optical densities (OD_{570}) were measured at 570 nm with a DG-3022A ELISA-Reader and expressed as a percentage relative to control (no anticancer drug-loaded nanospheres or 5-Fu) cells.

In Vitro Inhibition Assay to HeLa Tumor Cells

The HeLa tumor cells (1×10^5/ml) were plated in the growth medium and incubated for 24 h in incubator (37°C, 5% CO_2). The growth medium was removed and replaced with the growth medium containing 5-Fu or anticancer drug-loaded poly(carbonate-ester) nanospheres. After 48 h incubation, the cells were washed with PBS, fixed in the mixture solution of methanol and glacial acetic acid (v/v=3/1), rinsed again with PBS, stained with Hoechst 33258 (0.5 μg/ml) for 45 min and analyzed. Cell morphology was examined under a fluorescence microscope. The optical densities (OD_{570}) were measured at 570 nm with a DG-3022A ELISA-reader and expressed as a percentage relative to control (no polymeric nano drugs) cells.

Statistical Analysis

All results were expressed as mean differences and were tested for significance by *t*-test with $P<0.05$ being considered a significant difference.

RESULTS AND DISCUSSION

Preparation of Anticancer Drug-Loaded Poly (Carbonate-Ester) Nanospheres

A series of amphiphilic functional poly(carbonate-ester) copolymers containing sulfadiazine and hydroxyl groups (HPCP-SD) were synthesized by the attachment of sulfadiazine to the hydroxyl groups of partly deprotected poly(carbonate-ester) copolymers HPCP. The FT-IR spectra of functional poly (carbonate-ester) copolymers showed a characteristic peak in 1737 cm^{-1}, which represents the absorption peak of C=O groups and the characteristic peaks in 1397.2 cm^{-1} and 1172.5 cm^{-1}, which represent the absorption peaks of S=O. Meanwhile, the UV spectra of functional poly(carbonate-ester) copolymers showed a characteristic peak in 274 nm, which represents the absorption peak of SD groups.

In the ^{1}H NMR spectra of functional poly(carbonate-ester) copolymers HPCP-SD, the typical signals for CL and PTC repeat units in the backbone of copolymer structures can be observed at 8.83–8.3 ppm (SD groups: -CN-**CH**=**CH**-**CH**=N-) and 8.10–8.0 ppm (SD groups:: **-C_6H_4-**), 7.25–7.45 ppm (PTC repeat units: **-C_6H_5**) and 1.58 ppm (CL repeat units: -$OOCCH_2$**CH_2**CH_2**CH_2** CH_2O-), respectively. Thus, the average poly(carbonate-ester) copolymer compositions of SD groups, PTC and CL repeat units (mol%) can be calculated according to the integration values of the 8.83–8.3 ppm (-CN-**CH**=**CH**-**CH**=N-), the 7.25–7.45 ppm (**-C_6H_5**) peaks and the 1.58 ppm (-$OOCCH_2$**CH_2**CH_2**CH_2**CH_2O-) peak.

Compared to poly(carbonate-ester) copolymers HPCP containing hydroxyl groups, functional poly(carbonate-ester) copolymers HPCP-SD had the higher water contact angles than 60.73°, and water contact angles of HPCP-SD

Table II Effect of Molar Feed Ratio of Hydroxyl Groups in HPCP/Bromoacetyl Bromide on Anticancer Drug-Loaded Poly(Carbonate-Ester) Nanospheres I Prepared by Dialysis Method

Feed molar ratio of hydroxyl group/bromoacetyl bromide (mol/mol)	Drug content in nanospheres (w%)	Average particle size (Di) (nm)	PDI (D_v/D_n)	Average zeta potential (mV)
2:1	11.57	272.8	1.13	−7.73
1:1	13.48	288.9	1.21	−7.28
1:2	19.08	730.0	1.14	−6.00

D_i intensity-average diameter; D_v volume-average diameter; D_n number-average diameter; D_v/D_n size distribution; D_i, D_v and D_n values were measured for samples diluted with distilled water.

Table III Effect of Molar Feed Ratio of Hydroxyl Groups in HPCP/Bromoacetyl Bromide on Anticancer Drug-Loaded Poly(Carbonate-Ester) Nanospheres II Prepared by High-Voltage Electrostatic Field-Assisted Atomization

Feed molar ratio of hydroxyl groups/bromoacetyl bromide (mol/mol)	Drug content in nanospheres (w%)	Average particle size(Dn) (nm)	PDI (D_v/D_n)	Average zeta potential (mV)
2:1	15.89	31.0	1.05	−29.69
1:1	20.87	59.8	1.04	−21.84
1:2	25.14	80.9	1.03	−4.07

(Voltage: 3694 V, Liquid flow: 1.06 mL/h, Polymer concentration: 0.333 mg/mL)

increased appreciably from 75.20° to 78.77° when the feed molar ratio of hydroxyl groups in HPCP/bromoacetyl bromide decreased from 2:1 to 1:2. It could be considered that the hydrophilicities of HPCP-SD were reduced whilst the molar content of SD groups increased (Table I). Meanwhile, the glass transition temperatures of HPCP-SD increased from 48.3°C to 62.7°C when the molar feed ratio of hydroxyl groups in HPCP/bromoacetyl bromide decreased from 2:1 to 1:2, probably due to the steric location block effect enhancement and molecular rigidity improvement when the part hydroxyl groups were replaced by the rigid SD molecules after reaction.

Subsequently, the anticancer drug-loaded poly(carbonate-ester) nanospheres I were further prepared by dialysis method with the functional poly(carbonate-ester) copolymers HPCP-SD used as polymeric carriers. The average particle sizes of anticancer drug-loaded poly(carbonate-ester) nanospheres I increased from 272.8 nm to 730 nm, and the drug contents in nanospheres also increased from 11.57% to 19.08% when anticancer drug-loaded poly(carbonate-ester) nanospheres were made from the functional poly(carbonate-ester) copolymers HPCP-SD with molar feed ratio of hydroxyl groups in HPCP/bromoacetyl bromide varied from 2:1 to 1:2. (Table II)

The anticancer drug-loaded poly(carbonate-ester) nanospheres II were also prepared by the high-voltage electrostatic field-assisted atomization using the functional poly (carbonate-ester) copolymers HPCP-SD as polymeric carriers. The average particle sizes of anticancer drug-loaded nanospheres II increased from 31.0 nm to 80.9 nm, and the drug contents in nanospheres also increased from 15.89% to 25.14% when anticancer drug-loaded nanospheres II were made from the functional poly(carbonate-ester) copolymers HPCP-SD with molar feed ratio of hydroxyl groups in HPCP/bromoacetyl bromide varied from 2:1 to 1:2 (Table III).

The TEM morphologies of anticancer drug-loaded poly (carbonate-ester) nanospheres II prepared from the functional poly(carbonate-ester) copolymers HPCP-SD with molar feed ratio of hydroxyl groups in HPCP/bromoacetyl bromide as 2:1 are shown in Fig. 1. The AFM morphologies of anticancer drug-loaded poly(carbonate-ester) nanospheres II prepared from the functional poly(carbonate-ester) copolymers HPCP-SD with molar feed ratio of hydroxyl groups in

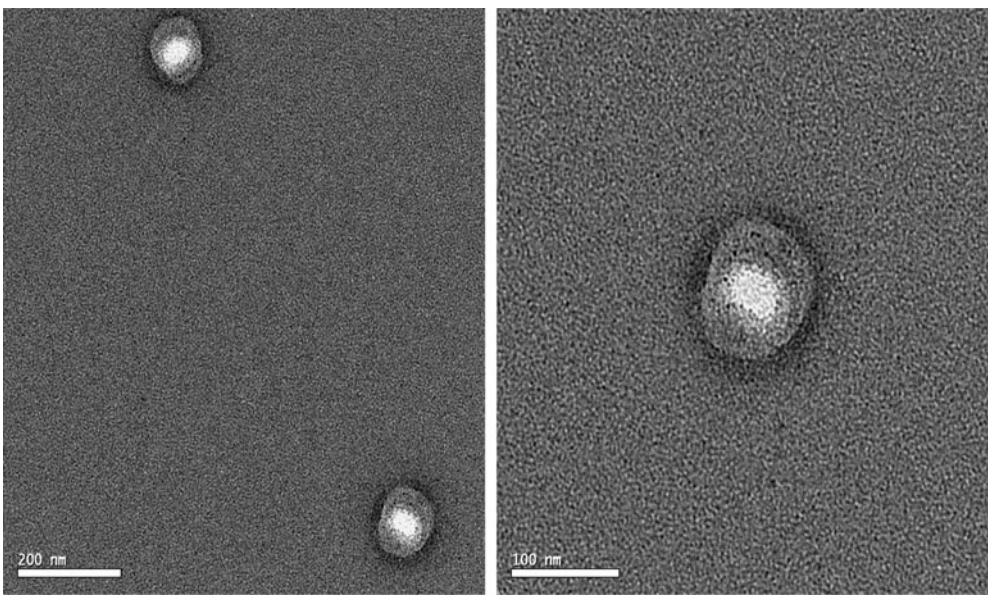


Fig. 1 TEM of anticancer drug-loaded poly(carbonate-ester) nanospheres II (Feed molar ratio of hydroxyl groups in HPCP/bromoacetyl bromide (mol/mol)(2:1), Voltage: 3694 V, Liquid flow: 1.06 mL/h, Polymer concentration: 0.333 mg/mL).

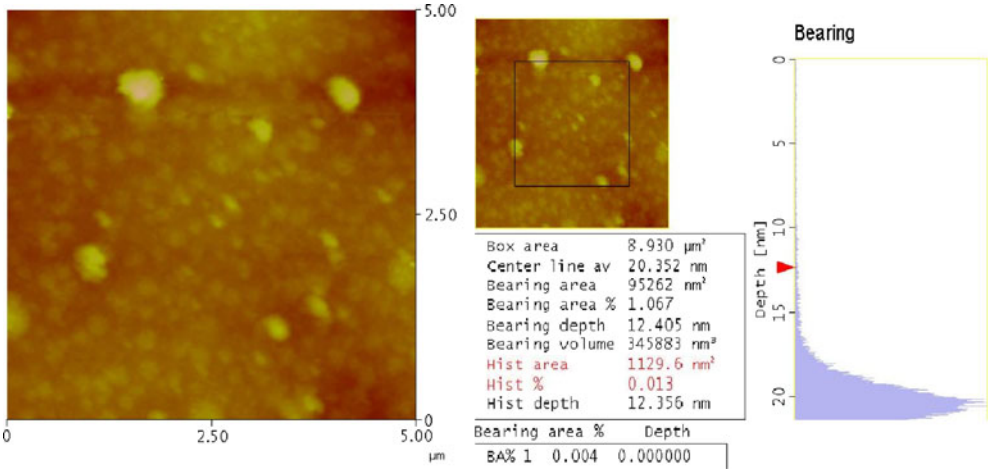


Fig. 2 AFM of anticancer drug-loaded poly(carbonate-ester) nanospheres II (Feed molar ratio of hydroxyl groups in HPCP/bromoacetyl bromide (mol/mol) (1:2), Voltage: 3694 V, Liquid flow: 1.06 mL/h, Polymer concentration: 0.333 mg/mL).

HPCP/bromoacetyl bromide as 1:2 are shown in Fig. 2. However, the results demonstrated that the particle sizes of anticancer drug-loaded nanospheres II prepared from the functional poly(carbonate-ester) copolymers HPCP-SD with molar feed ratio of hydroxyl groups in HPCP/bromoacetyl bromide as 1:2 and 2:1 showed about 20 nm and 100 nm, respectively, differing with the data detected by the zeta potential and laser diffraction particle size analyzer.

In Vitro Drug Release Property of Anticancer Drug-Loaded Poly(Carbonate-Ester) Nanospheres

The overall process of drug release from the anticancer drug-loaded poly(carbonate-ester) nanospheres was mostly controlled by drug diffusion, drug dissolution and polymeric degradation (18–22). The release profiles 5-Fu of anticancer drug-loaded poly(carbonate-ester) nanospheres are shown in Figs. 3 and 4. The substantial release rates of anticancer drug-loaded poly(carbonate-ester) nanospheres could be maintained for more than 28 days of measurement. The anticancer drug-loaded nanospheres had no obvious phenomenon of abrupt release. The release rate became faster, whilst anticancer drug-loaded nanospheres prepared from the functional poly(carbonate-ester) copolymers HPCP-SD with molar feed ratio of hydroxyl groups in HPCP/bromoacetyl bromide increased from 1:2 to 2:1, presumably due to the higher hydrophilicities of HPCP-SD and the increased drug diffusion coefficient.

The release rates of anticancer drug-loaded nanospheres II prepared by high-voltage electrostatic field-assisted atomization were higher than those of corresponding anticancer drug-loaded nanospheres I prepared by dialysis method with the same functional poly(carbonate-ester) copolymers HPCP-SD as the polymeric carriers. The

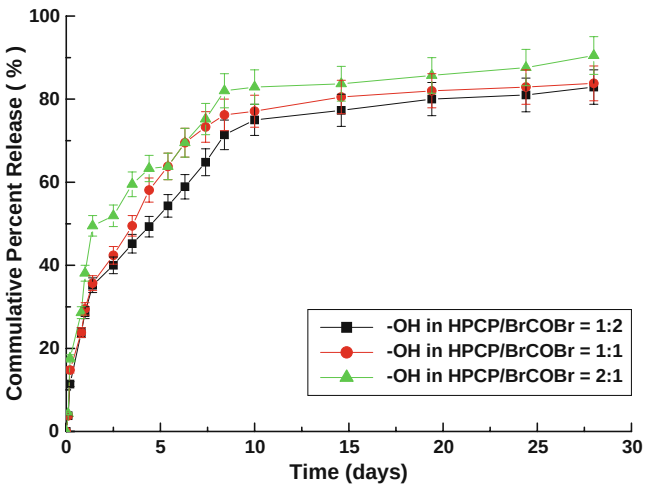


Fig. 3 Release profiles of 5-Fu from anticancer drug-loaded poly (carbonate-ester) nanospheres I in PBS at 37°C (Feed molar ratio of hydroxyl groups in HPCP/bromoacetyl bromide (mol/mol) were 1:2, 1:2, and 1:2, respectively).

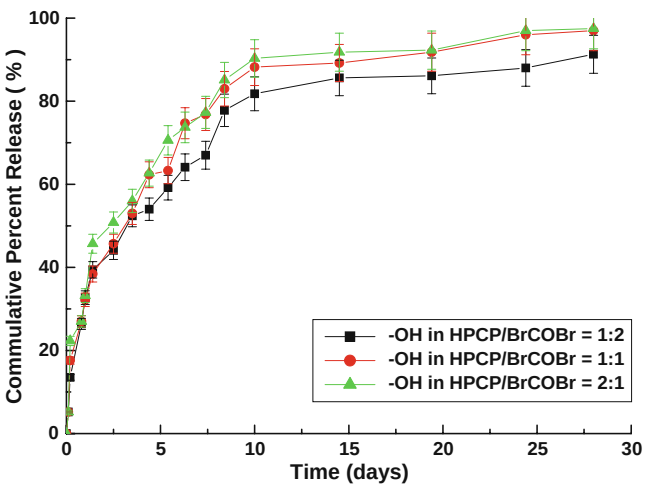


Fig. 4 Release profiles of 5-Fu from anticancer drug-loaded poly (carbonate-ester) nanospheres II in PBS at 37°C (Feed molar ratio of hydroxyl groups in HPCP/bromoacetyl bromide (mol/mol) were 1:2, 1:2, and 1:2, respectively).

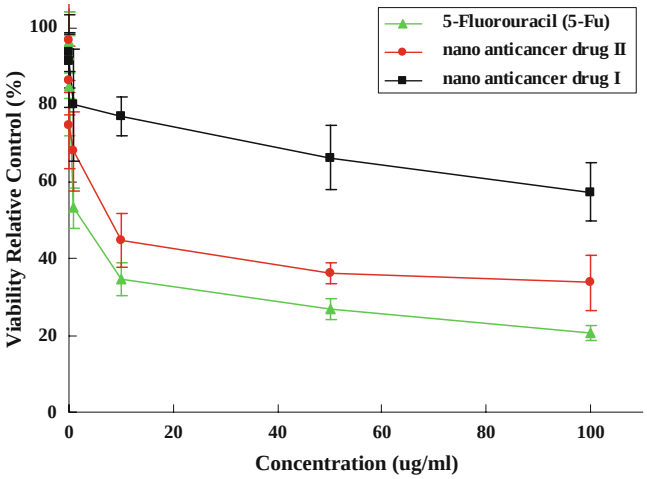


Fig. 5 *In vitro* cytotoxicity assay of anticancer drug-loaded poly(carbonate-ester) nanospheres to HEK 293 cells.

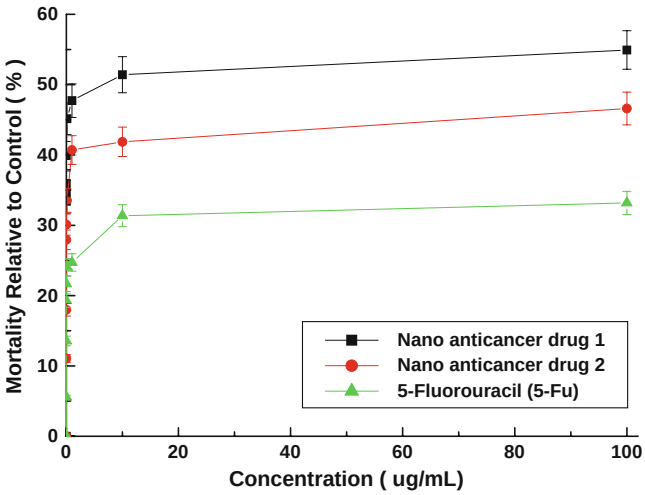


Fig. 7 Antitumor activity of anticancer drug-loaded poly(carbonate-ester) nanospheres to HeLa cells *in vitro*.

cumulative percent releases of anticancer drug-loaded poly (carbonate-ester) nanospheres I which were prepared by dialysis method with the functional poly(carbonate-ester) copolymers HPCP-SD with molar feed ratio of hydroxyl groups in HPCP/bromoacetyl bromide as 2:1, 1:1, 1:2, reached 89.91%, 85.47%, and 83.87%, respectively. Meanwhile, the cumulative percent releases of anticancer drug-loaded poly(carbonate-ester) nanospheres II which were prepared by high-voltage electrostatic field-assisted atomization with the functional poly(carbonate-ester) copolymers HPCP-SD with molar feed ratio of hydroxyl groups in HPCP/bromoacetyl bromide as 2:1, 1:1, 1:2, reached 96.53%, 94.44%, and 88.89%, respectively. This indicated that the larger surface of the smaller sized nanoparticles and higher hydrophilic hydroxyl groups enhanced the hydrophilicity and biodegradation rates and promoted water absorption and drug diffusion coefficient. Thus, the results indicated that these two type anticancer drug-loaded poly(carbonate-ester) nanospheres had the steady drug release rates and controlled release properties. Moreover, anticancer drug-loaded nanospheres II had faster drug release rates than those of anticancer drug-loaded nanospheres I.

In Vitro Cytotoxicity Assay

The effects of anticancer drug-loaded poly(carbonate-ester) nanospheres to HEK 293 cell growth and metabolism are shown in Fig. 5. At the concentration (100 μg/mL) of anticancer drugs in the growth medium, the viabilitiy of HEK 293 cells incubated with anticancer drug-loaded poly(carbonate-ester) nanospheres I or II and 5-Fu retained 57.3%, 33.7%, and 20.5%, respectively, relative to control. This illustrated that anticancer drug-loaded poly(carbonate-ester) nanospheres possessed lower cytotoxicity to HEK 293 cells than those of 5-Fu, and anticancer drug-loaded nanospheres I possessed lower cytotoxicity to HEK 293 cells than that of anticancer drug-loaded nanospheres II.

In Vitro Tumor Inhibition Assay

Sulfadiazine (SD) derivatives have been reported as good tumor-selective groups for MRI contrast agents and drug delivery systems (31–33). Sulfadiazine was incorporated to

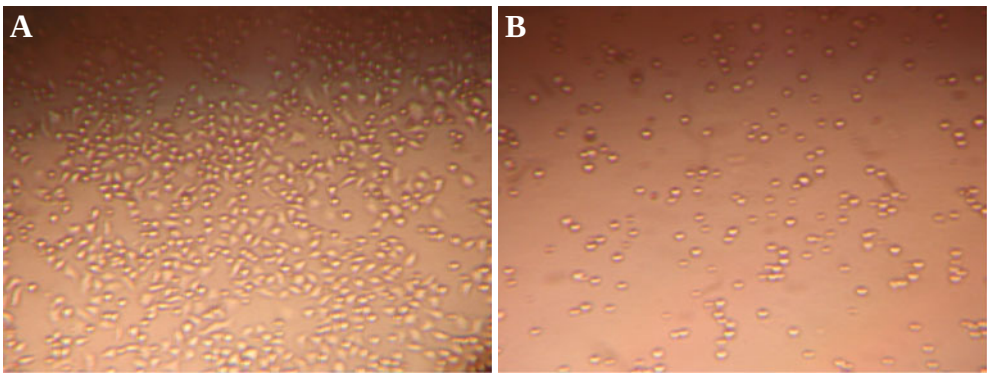


Fig. 6 Induced apoptosis photo of anticancer drug-loaded poly (carbonate-ester) nanospheres to HeLa tumor cells. **A** control cells; **B** anticancer drug-loaded poly (carbonate-ester) nanospheres.

the hydroxyl groups of poly(carbonate-ester) copolymers HPCP to synthesize the tumor-targeting poly(carbonate-ester) copolymers containing sulfadiazine and hydroxyl groups (HPCP-SD). Subsequently, anticancer drug-loaded poly(carbonate-ester) nanospheres I and II were made with HPCP-SD as the tumor-targeting polymer carriers and 5-Fu as an anticancer drug.

The HeLa cells with different concentrations of anticancer drugs in the growth medium in culture were incubated. Induction of apoptosis was confirmed by the induced apoptosis photo of anticancer drug-loaded poly(carbonate-ester) nanospheres to HeLa tumor cells (Fig. 6). 5-Fluorouracil, anticancer drug-loaded poly(carbonate-ester) nanospheres I and II at 100 μg/mL concentration induced apoptosis in about 33.2%, 54.9%, and 46.6%, respectively, of HeLa cells after 48 h incubation. When the concentration of anticancer drug-loaded poly(carbonate-ester) nanospheres I or II increased, the percentage of apoptosis to the HeLa cells became considerably larger (Fig. 7). The apoptosis experiments showed anticancer drug-loaded poly(carbonate-ester) nanospheres could exhibit obviously higher anticancer efficiencies to the HeLa cells than that of 5-Fu, and anticancer drug-loaded nanospheres I possessed higher anticancer activity to HeLa cells than that of anticancer drug-loaded nanospheres II. Anticancer drug-loaded poly(carbonate-ester) nanospheres could be taken up selectively by HeLa cells and easily accumulate into tumor cells due to the specific affinity of SD and tumor cells. Anticancer drug-loaded poly(carbonate-ester) nanospheres kept the steady drug release rates in tumor cells and induced the apoptosis of HeLa cells.

CONCLUSIONS

Sulfadiazine as the tumor-targeting groups were incorporated to hydroxyl groups of poly(carbonate-ester) copolymers HPCP to synthesize the functional poly(carbonate-ester) copolymers containing sulfadiazine and hydroxyl groups (HPCP-SD). Two types of anticancer drug-loaded poly(carbonate-ester) nanospheres I and II were further prepared by dialysis method and high-voltage electrostatic field-assisted atomization, respectively, with the functional poly(carbonate-ester) copolymers HPCP-SD thus obtained used as the polymeric carriers. These two type anticancer drug-loaded poly(carbonate-ester) nanospheres the steady drug release rates and controlled release properties. Moreover, anticancer drug-loaded poly(carbonate-ester) nanospheres I prepared by dialysis method had faster drug release rates than those of anticancer drug-loaded poly (carbonate-ester) nanospheres II prepared by high-voltage electrostatic field-assisted atomization. The experiments *in vitro* demonstrated that anticancer drug-loaded poly (carbonate-ester) nanospheres possessed lower cytotoxicity to HEK 293 cells and obviously higher anticancer efficiencies to the HeLa tumor cells than that of 5-fluorouracil. The anticancer drug-loaded poly(carbonate-ester) nanospheres I possessed higher cytotoxicity to HEK 293 cells and higher anticancer activity to HeLa cells than those of anticancer drug-loaded poly(carbonate-ester) nanospheres II.

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