Microwave-assisted synthesis of thermo- and pH-responsive antitumor drug carrier through reversible addition–fragmentation chain transfer polymerization

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Abstract. This paper reports the rapid synthesis of a dual-responsive copolymer through reversible addition–fragmentation chain transfer (RAFT) polymerization under microwave irradiation. Through use of 2-ethoxycarbonothioylthio acetic acid (ECTA) as a RAFT agent, the microwave-assisted polymerization rate of *N*-isopropylacrylamide (NIPAM) was approximately 150 times faster than that observed under conventional heating conditions, and the resulting homopolymer can be reactivated as a macroinitiator to produce poly(*N*-isopropylacrylamide-*block*-methacrylic acid) (PNIPAM-*b*-PMAA) block copolymers through a similar method. Research into the detailed polymerization kinetics of the PNIPAM and PNIPAM-*b*-PMAA revealed living characteristics that included a linear relationship between M_n and conversion, controlled molecular weights, and a relatively narrow molecular weight distribution. The solution of the block copolymers in phosphate-buffered saline buffer displayed a phase transition at a lower critical solution temperature transition of 42 °C, and altering the pH from 7 to 3.5 resulted in various degrees of polymer aggregation in the solution. Cisplatin was loaded to the polymeric carrier through a ligand exchange to form a macromolecular prodrug. The observed critical micelle concentration was 0.25 mg/mL. Overall, these polymers offer considerable potential for developing a new multifunctional drug delivery system.

Keywords: smart polymers, microwave-assisted synthesis, reversible addition fragmentation chain transfer polymerization, polymer drug conjugate, anticancer drug

1. Introduction

Reversible addition–fragmentation chain transfer (RAFT) polymerization has been extensively used to prepare polymers with desired molecular weights, a narrow molecular weight distributions, and well-defined nanostructures. A remarkable feature of RAFT is its general versatility with respect to monomer choice, which can be used to build various amphiphilic nanostructures such as micelles, vesicles, and nanoparticles with potential (bio)pharmaceutical applications [1, 2]. In the design of ‘smart’ polymers based on RAFT techniques, the well-controlled structures of these systems offer numerous advantages

compared with free radical systems such as more uniform response behavior, controlled size, and molecular weight. A wide range of RAFT-derived polymers has now been explored for applications in optoelectronics, drug delivery vehicles, and star polymer rheology control agents [3]. Nevertheless, the relatively low polymerization rates as well as heterogeneity of the by-products render its biomedical application difficult, and the often high reaction temperatures and extended polymerization times can be problematic for achieving a consistent polymer composition. Therefore, it is of great interest to develop novel methods that can prepare stimuli-responsive

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polymeric nanoparticles with high polymerization rates, while simultaneously maintaining reasonable molecular weight control and livingness of nature for biomedical applications.

Microwave irradiation appears to be an attractive synthetic tool for solving these drawbacks because of several advantages such as reduced reaction times, increased product yields, limited generation of by-products, and environmentally benign preparation. Microwave-assisted organic synthesis has been widely applied in the medicinal chemistry over the last decade [4]. For example, Imatinib (Gleevec), a marketed anticancer drug, can be produced through microwave heating in five steps with shorter reaction times and higher yields than what can be obtained through traditional heating methods [5]. Various inhibitors and pharmaceutical molecules have been synthesized through microwave-assisted synthetic protocols to treat infectious diseases such as tuberculosis, HIV/AIDS, malaria, and hepatitis C [6–9]. Other notable examples, including biologically active heterocycles for Sildenafil analogs [10, 11], non-toxic biofilm inhibitors (antimicrobial derivatives) [12, 13], sirtuin 2-selective inhibitors [14, 15], cross-coupling reactions [16, 17], and positron emission tomography [18, 19], have also been reported, and some reactions have been evaluated in a scale-up fashion [20, 21]. In contrast to these small molecules, reports of polymer drugs or biomaterials synthesized under microwave conditions are comparatively limited [22–24]. In the field of polymer synthesis, microwave irradiation has been widely used in step-growth polymerizations [25], free radical and ring-opening polymerizations [26–27]. Studies concerning controlled-radical polymerizations such as atom transfer radical polymerization (ATRP) or nitroxide-mediated radical polymerization (NMP) under microwave conditions, however, are relatively sparse [28–31]. Recently, microwave-assisted RAFT polymerization has appeared in the literature [32], although only a few types of hydrophilic monomers, such as *N,N*-dimethylacrylamide, *N*-isopropylacrylamide [33, 34], *N*-(2-hydroxypropyl)methacrylamide [35], and diallyldimethylammonium chloride [36], have been examined for amphiphilic copolymer applications. In this paper, we report the first and fast microwave-assisted polymer–drug conjugate synthesis featuring both pH and temperature responsiveness through RAFT polymerization. The responsiveness and self-assembling behavior of these polymers

were investigated, and the kinetic data for both PNIPAM and the subsequent chain extension to a diblock copolymer under microwave irradiation were determined.

2. Experimental

2.1. Materials

NIPAM (Tokyo Chemical Industry Co., Ltd., Japan) was recrystallized from mixtures of ethyl acetate and hexane (1/5 by volume) and dried in a vacuum prior to use. Methacrylic acid (MAA, Showa, Tokyo, Japan) was purified through distillation and stored in N₂ prior to use. 2,2-Azobis-isobutyronitrile (AIBN, Showa, Tokyo, Japan) was recrystallized in ethanol before use. Diethyl ether (>99.0%), toluene (>99.0%) and methyl alcohol (99.9%) were purchased from ECHO, Miaoli, Taiwan. Trimethylsilyldiazomethane (TMSD, 2M solution in hexanes) and cisplatin (99.99%) were purchased from ACROS Organics, Belgium. Tris(hydroxymethyl)aminomethane (99.9%) was purchased from MP Biomedicals, Ohio, USA. These reagents were used without further purification. 2-Ethoxycarbonothioylthio acetic acid (ECTA) was obtained through a reaction of 2-bromoacetic acid with potassium-*o*-ethyl dithiocarbonate, according to procedures outlined previously [37]. Mouse osteoblast-like cells (7F2), an osteoblastic cell line isolated from p53^{-/-} mice was obtained from the Bioresource Collection and Research Center (Hsinchu, Taiwan).

2.2. Instrumentation and measurements

¹H spectra were recorded using a 300 MHz (Varian–Mercury⁺300, Oxford, USA) spectrometer by employing a deuterated solvent. Fourier transform infrared (FTIR) spectra were measured using a Perkin Elmer spectrophotometer (Spectrum one, Oxford, UK). Gel permeation chromatography (GPC, Waters 1515, USA) were equipped with WATERS STYRAGEL HR4E DMF, HR4 DMF, HR1 DMF and a refractive index detector (Waters 2410). The molecular weights and dispersity (*D*) of the products were calibrated using poly(methyl methacrylate) molecular weight standards in the molecular weight range of 2,500–520 000 g/mol (Polymer Standards Service, USA). GPC was performed using a dimethylformamide (DMF) eluent containing 0.05 mol/L lithium bromide at 80 °C with a flow rate of 0.8 mL/min. Prior to the GPC analysis, the polymers were modified by methylating the carboxylic acid

groups with trimethylsilyldiazomethane in the DMF. Microwave-assisted polymerization was conducted inside a single-mode stainless steel cavity (CEM Discover S-Class NP-1008, USA) equipped with an infrared temperature sensor and simultaneous cooling system. The pH- and temperature-sensitive behaviors of the polymers were characterized by applying the UV2100+ spectrophotometer (Unico, New Jersey, USA) at 1–2 mg/mL at 600 nm. The pH of the test solutions was controlled by adding tris(hydroxymethyl)aminomethane or phosphoric acid. Dynamic light scattering (Zetasizer Nano ZS90, Malvern, England) was used to confirm the particle size distribution of polymer drugs at 4 mW He-Ne laser. Transmission electron microscopy (TEM) images were made with a JEM-2100 (JEOL, USA) electron microscope operated at an acceleration voltage of 100 kV. Platinum contents were determined using an inductively coupled plasma mass spectrometry (ICP-MS) instrument (Agilent 7500ce, Japan), and fluorescence measurements were performed on a HITACHI F-4500 FL spectrophotometer (Hitachi, Tokyo, Japan).

2.3. Synthesis

2.3.1. Microwave RAFT polymerizations of PNIPAM

Poly(*N*-isopropylacrylamide) (PNIPAM) was prepared through RAFT polymerization by using 2,2'-azobis-isobutyronitrile (AIBN) as the primary radical source and ECTA as the chain transfer agent (CTA). A 20 mL glass tube equipped with a magnetic stirrer was loaded with NIPAM (1 g, 8.83 mmol), AIBN (0.7 mg, $4.26 \cdot 10^{-3}$ mmol), and ECTA (159 mg, 0.883 mmol). The tube was deoxygenated by evacuation and back-flushed with nitrogen three times. Next, 4 mL of toluene was added with a syringe, and the resulting solution was purged with nitrogen for 20 min and placed in the cavity of the microwave instrument. The temperature was programmed to 75 °C at a maximum 100 W irradiation dose with cooling control. After 10 min, the polymer was dissolved in acetone (2 mL) and re-precipitated in diethyl ether (200 mL). Subsequently, the polymer was filtered and dried in a vacuum (0.71 g, yield: 71%). ^1H NMR (CDCl_3) δ : 1.13–1.33 ($\text{CH}(\text{CH}_3)_2$ of NIPAM), 1.51–1.97 (the methylene protons of polymer backbone), 2.03–2.50 (the methine protons of the polymer backbone), 3.99 ($-\text{NHCH}(\text{CH}_3)_2$ of NIPAM), and 4.62 ($-\text{OCH}_2\text{CH}_3$ of ECTA); ^{13}C NMR δ : 13.9, 22.6,

32.2–38.1, 32.2, 41.2, 42.4, 70.6, and 174.3; and IR (KBr) ν [cm^{-1}]: 1172 (C–O), 1574 (N–H), 1651 (C=O), 2951, 2936 (C–H), and 3300 (N–H). This polymer was used as the macro-CTA for subsequent copolymerization.

Kinetic studies were performed similarly, except that the samples were removed periodically by using a syringe during polymerization to determine the monomer conversion and molecular weight. To compare the results with those obtained using a conventional heating method, the reaction mixture with the same weight ratios and reaction conditions was performed in an oil bath.

2.3.2. Microwave block copolymerization of PNIPAM with MAA

The block copolymer PNIPAM-*b*-MAA was prepared in methanol using PNIPAM as the macro-CTA (M_n = approximately 6000 g/mol, $\bar{D}$ = 1.3) and AIBN as the primary radical source. First, a 20 mL glass tube equipped with a magnetic stirrer was loaded with PNIPAM (120 mg, 0.02 mmol), methacrylic acid (172 mg, 2 mmol), AIBN (0.2 mg, $1.22 \cdot 10^{-3}$ mmol), and DMF (60 μL). The resulting solution was purged with nitrogen for 20 min, and 5 mL of toluene was then added with a syringe. The tube was placed in the cavity of the microwave instrument, and the temperature was programmed to 75 °C at a maximum 100 W irradiation dose with cooling control. After 10 min, the polymer was dissolved in methanol (2 mL), precipitated in diethyl ether (200 mL) and collected as a white powder by filtration. The polymer was purified by dissolving it in a minimal amount of methanol and re-precipitated in a 10-fold excess of an Et_2O solution. Thereafter, the polymer was collected using filtration, washed with Et_2O , and dried in a vacuum overnight to provide 0.15 g of the polymer. The carboxylic acid content was subsequently determined by titration with 0.1 N NaOH. ^1H NMR (CDCl_3) δ : 0.78–1.28 (the methyl protons of the PMAA and $\text{CH}(\text{CH}_3)_2$ of PNIPAM), 1.28–2.20 (the methylene and methine protons of the polymer backbone), 3.83 ($-\text{NHCH}(\text{CH}_3)_2$ of PNIPAM), 4.62 ($-\text{OCH}_2\text{CH}_3$ of ECTA), 7.01–7.05 ($-\text{NH}$), and 12.30–12.50 ($-\text{COOH}$); ^{13}C NMR δ : 16.7–19.8, 24.2, 32.8–34.0, 40.6, 46.5, 173.7, and 179.3; and IR (KBr) ν [cm^{-1}]: 1172 (C–O), 1547 (N–H), 1651 (C=O), 1717 (C=O), 2951, 2936 (C–H), 3300 (N–H), and 3472 (O–H).

2.3.3. Cisplatin loading

PNIPAM-*b*-MAA (80 mg, 13.3 mmol) and *cis*-(Pt[NH₃]₂Cl₂) (19.2 mg, 64 mmol) were placed into a three-neck flask, and the system was evacuated and back-flushed with nitrogen three times. Next, 2 mL of DMF was injected into the reaction mixture and stirred in an oil bath at 40 °C for 24 h. Finally, the polymer was precipitated into a 120 mL of Et₂O solution and, following filtration, was washed with the Et₂O and dried in a vacuum (yield: 58.2 mg).

2.4. Determination of critical micelle concentrations of the Pt@PNIPAM-*b*-MAA

Four microliters of pyrene stock solution in acetone (1.26 mM) were added to each of a series of 10 mL volumetric flasks and the acetone was evaporated. Subsequently, 10 mL of polymer solution with various concentrations (0.001 to 2.0 mg/mL) was added to each flask, and the pH of the solutions was adjusted to 7.5 by adding tris(hydroxymethyl)aminomethane. Fluorescence spectra were measured from 360 to 460 nm following excitation at 339 nm. The intensities of the excitation peaks at 373 and 393 nm were recorded, and the ratios were plotted as a function of the polymer concentrations.

2.5. Cell viability assay

The cytotoxicity of the diblock copolymer was evaluated by determining the cell viability using 3-(4,5-

dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assays. The cells (7F2 osteoblast-like cells) were seeded in 96-well plates at a density of 10⁴ cells/well and incubated in a growth medium with different polymer concentrations (1, 35, 70 and 140 µg/mL (w/v)) for 24 h at 37 °C. At the end of incubation, 200 µL of MTT (0.1 mg/mL) was added and incubated for 4 h in an incubator. Subsequently, 200 µL of DMSO was added to dissolve formazan and the UV absorbance intensity was measured at 570 nm using the ELISA reader (Tecan, Infinite M200, Switzerland). All experiments were performed in quadruplicate, and the relative cell viability [%] was expressed as a percentage relative to the untreated control cells.

3. Results and discussion

3.1. Preparation and kinetic study of microwave-assisted RAFT polymerizations

The microwave-assisted RAFT polymerization was first tested for NIPAM using ECTA as the CTA and AIBN as the initiator at 65–75 °C. The polymerization mixture was subjected to NMR measurements as a function of polymerization time to obtain the time-conversion curve. Figure 1a showed the change in NIPAM conversion with time for different reaction temperatures. The curve revealed that there was an induction period at first two minutes to activate the polymerization and subsequently, a linear in-

Figure 1. Kinetic plots (a–c) and evaluation (d) of the M_n (solid) and M_w/M_n (hollow) for microwave-assisted RAFT polymerization of NIPAM by using an ECTA CTA in toluene at [NIPAM]/[CTA]/[initiator] = 10/1/0.05 under 100 W pulsed microwave irradiation

crease of conversions up to 77–82% over the reaction time was observed after ten minutes. The induction phenomena is not completely clear so far, however, it might be related to the heating process that the applied microwave energy at the initial reaction stage was mostly consumed to raise the reaction temperature instead of fragmentation of the initial RAFT radical [38, 39]; or it might be due to the non-uniform superheating of the mixture causing a deviation from zero point. The logarithmic concentration ratio of the monomer ($\ln[M]_0/[M]$) versus the time plots for three temperatures (65, 70, and 75 °C) were also linear after the induction period (Figure 1b), indicating that the concentration of the propagating radicals was essentially constant throughout the polymerization. Therefore, the kinetic plots could be considered pseudo first order reaction and propagation rate constants k_{app} were determined using the steady state conversions after the induction period. For comparison, the corresponding polymerizations obtained from oil-bath heating were also tested under the same conditions. However, the same catalyst polymerized NIPAM with a similar molecular weight only up to 27% conversion after 6 h of oil-bath heating. The propagation rate constant k_{app} was obtained from the linear regression slope of the pseudo-first-order kinetic plots, and was estimated to be 150 times faster than the conventional heating method. With $[NIPAM]/[CTA]/[initiator] = 10/1/0.05$, the relative values of k_{app} for the polymerizations with microwave heating were 1.51×10^{-3} , 1.64×10^{-3} , and $1.81 \times 10^{-3} \text{ s}^{-1}$ at 65, 70, and 75 °C, respectively, whereas for the conventional heating, it was determined to be $1.17 \times 10^{-5} \text{ s}^{-1}$

at 75 °C. The activation energy, E_a , for the RAFT polymerization was estimated as 17.3 kJ/mol (Figure 1c), which is slightly higher than previously reported data [40]. The molecular weights also increased linearly with the monomer conversion to a dispersity ($\bar{D}$) of approximately 1.34 (Figure 1d), indicating that the polymerization remained controlled. However, a relatively high dispersity was observed at 65 °C, which might be attributable to the slow initiation caused by the inefficient decomposition of AIBN at this temperature.

To assess the livingness of the PNIPAM formed in a microwave-assisted system, PNIPAM (M_n = approximately 6000 g/mol) was used as a macromolecular RAFT agent for the diblock copolymer synthesis. Depicted in Figures 2, 3 and 4 are the ^1H NMR and FTIR spectra of PNIPAM and PNIPAM-*b*-MAA. The ^1H NMR spectrum of PNIPAM disclosed two typical signals for the methine protons ($-\text{NHCH}(\text{CH}_3)_2$) at $\delta = 3.99$ and methyl protons ($-\text{NHCH}(\text{CH}_3)_2$) at 1.13–1.33 ppm as two broad singlet in Figure 2. Other signals such as the methylene protons ($\delta = 1.51$ –1.97 ppm) and methine protons (2.03–2.50 ppm) in polymer backbone also agreed with the structure. The ethoxy proton signals from the terminal CTA were observed at $\delta = 4.62$ ppm ($-\text{OCH}_2\text{CH}_3$ of ECTA). In Figure 3, the copolymers also exhibited the characteristic methine protons of NIPAM (*g*), the acidic protons of MAA (*c*), and ethoxy proton signals of CTA (*i*). The peak at 3.33 is due to the presence of moisture in $\text{DMSO}-d_6$, which absorbs moisture very quickly during handling. Moreover, the IR absorptions illustrated in Figure 4

Figure 2. ^1H NMR spectra of PNIPAM

Figure 3. ^1H NMR spectra of PNIPAM-*b*-MAA

Figure 4. FTIR spectra of PNIPAM and PNIPAM-*b*-MAA

Table 1. Reaction conditions and characterization data of microwave-assisted synthesis (Mixture was irradiated at 75 °C at 100 W for 10 min.)

Entry	Monomer	CTA	$[\text{M}]_0/[\text{CTA}]/[\text{I}]_0$	$m:n$	M_n^a	M_w	D	LCST [°C]
1	NIPAM	ECTA	10:1:0.05	—	8 157	10 900	1.3	32
2	MAA	PNIPAM	100:1:0.05	1:1.91	13 890	21 200	1.5	42–44

^aPrior to the GPC analysis, the polymers were modified through methylation of the carboxylic acid groups by using trimethylsilyldiazomethane in DMF at room temperature

at 1717 and 1651 cm^{-1} can be attributed to the C=O stretchings of the carboxylic acid in MAA and the amide in NIPAM, respectively. The molar composition of the NIPAM to MAA was approximately 1:1.91, as determined by NMR and titration; the results are summarized in Table 1.

Figure 5 depicts the kinetic plots and molecular weight analysis for the polymerization of PNIPAM-*b*-MAA in DMF and toluene. A slightly longer induction period was observed in Figure 5a, revealing a lower reactivity for the macro-CTA. However, the induction

time is still much less than the conventional RAFT polymerization of MAA [41]. After the induction period, linear pseudo-first-order kinetic behavior was observed, indicating that a constant number of radical concentrations were maintained throughout polymerization process. The kinetic plots (Figure 5a and 5b) were consistent with a living polymerization mechanism, and the linear increase in the molecular weight with conversion (Figure 5c) indicated that the polymerization was in a reasonably controlled manner with the M_w/M_n values ranging from 1.4 to 1.6

Figure 5. Kinetic plots (a–c) and evaluation (d) of the M_n (solid) and M_w/M_n (hollow) for microwave-assisted RAFT polymerization of MAA by using PNIPAM as the macro-CTA in toluene

(Figure 5d). Additionally, the relative values of k_{app} for the PNIPAM-*b*-MAA polymerizations with microwave heating were 2.62×10^{-4} , 5.7×10^{-4} , and $8.22 \times 10^{-4} \text{ s}^{-1}$ at 65, 70, and 75 °C, respectively. The average dispersity of the diblock copolymer (1.46 at 75 °C) was slightly higher than that of the PNIPAM (1.35 at 75 °C), suggesting that some inefficient initiation and termination reactions of the macro-CTA at high conversions cannot be entirely avoided.

3.2. Stimulus-responsive transition of polymers

Figures 6 and 7 portray the temperature- and pH-dependent turbidity of the micelle solution through the UV–VIS spectrophotometer results at 600 nm. The transmittance values decreased with increasing temperature, reaching 52% at 44 °C; furthermore, the transparency of the solution decreased by 40% when the pH was adjusted from 8.5 to 3.0. The aggregation was also evidenced by dynamic light scattering data. Figure 8 illustrates the compared particle size distribution of PNIPAM-*b*-MAA as observed at diverse pH levels and temperatures; notably, the hydrodynamic diameter increased significantly with the temperature, from 25 to 44 °C at either pH 7 or 4. In addition, the particle diameter increased as the pH was decreased from 7 to 4 at different temperatures. The

dual-responsive behavior of this polymer is promising for the design of a novel antitumor drug carrier, because it indicates that thermal targeting to the pathological area could occur when tumor tissue is selectively heated to 40 to 44 °C [42]. Incorporating the MAA component not only resulted in an increase of the lower critical solution temperature (LCST) from 32 to 44 °C, but also induced aggregations in the acidic environment, which responded according to the property required for the extracellular environment of solid tumors and endocytosis conditions [43, 44]. The ability of PNIPAM-*b*-MAA to form micelles in an aqueous solution was tested through fluorescence spectroscopy with pyrene used as a probe. The pyrene was also mixed with polymer solutions at various concentrations, and the emission spectra were recorded [45, 46]. The intensity ratio of the first vibrational band at 373 nm to the third vibrational band at 393 nm (I_{373}/I_{393}) was plotted as a function of the polymer concentrations, as presented in Figure 9. At concentrations above the critical micelle concentration (CMC), I_{373}/I_{393} decreased sharply, indicating micelle formation and partitioning of the pyrene inside the hydrophobic cores of the micelles. Thus, the CMC was estimated to be 0.1 mg/mL from the sharp drop in the I_{373}/I_{393} ratios.

Figure 6. Plot of transmittance [%] for the aqueous polymer solutions (2 mg/mL) as a function of the temperature at 600 nm for PNIPAM-*b*-MAA

Figure 7. Plots of transmittance [%] of aqueous polymer solutions (1 mg/mL) as a function of pH at 600 nm for PNIPAM-*b*-MAA

3.3. Preparation of the cisplatin-loaded polymer conjugate

The conjugation of cisplatin to PNIPAM-*b*-MAA was achieved through a ligand exchange between the chloride ion and carboxylate groups in either a mono-functional, bifunctional, or interchain crosslinking manner, as depicted in Figure 10. The FTIR spectra verified the conjugation between the polymer and cisplatin, as indicated in Figure 11. The spectra also exhibited two pronounced peaks at 3292 and 1385 cm^{-1} , revealing the absorptions of N–H stretching from

cisplatin ligands (Pt-NH_2), and the chelation of the COO-Pt stretch, respectively [47, 48]. In addition, a reduction in absorbance of the carbonyl (C=O) stretch of COOH at 1704 cm^{-1} relative to the amide C=O band at 1633 cm^{-1} (the difference between relative intensity in (a) and (b) in Figure 11) is consistent with the prediction that some of the $-\text{COOH}$ groups would react with the Pt atoms in a ligand exchange reaction [49]. Dynamic light scattering was also employed to confirm size changes in the polymer induced by the conjugation (Figure 12). The average

Figure 8. Number-averaged particle size distributions of the PNIPAM-*b*-MAA in an aqueous solution with a concentration of 0.1 mg/mL

Figure 9. Relationship between the intensity ratio (I_{373}/I_{393}) of pyrene emission spectra ($\lambda_{ex} = 339$ nm) and the concentration of PNIPAM-*b*-MAA ($\blacktriangle$) and Pt@PNIPAM-*b*-MAA ($\blacksquare$) at 25°C in an aqueous solution with pH 7.5

hydrodynamic diameter of the polymer increased from approximately 210 to 280 nm after Pt conjugation, indicating an interchain crosslinking of polymer chains by Pt within the polymer chains. Figure 13a shows the TEM images of micelles obtained by self-assembly of Pt@PNIPAM-*b*-MAA in aqueous solution. The particle sizes of the Pt@PNIPAM-*b*-MAA at 25°C were approximately 100–200 nm with almost spherical core-shell structures. The cisplatin-loaded polymer conjugate self-assembled into almost spherical micelles without any particular formulation. We believe that the intrachain cross-linking forming a short rigid block might help generating the hydrophobic core structure of PMAA and leave the hydrophilic PNIPAM on outer surface at room temperature (below LCST). Figure 13b shows an Energy-dispersive X-ray

Figure 10. Schematic description of the polymer-cisplatin prodrug conjugate synthesis

Figure 11. FTIR spectra of PNIPAM-*b*-MAA (a) and its corresponding drug conjugate Pt@ PNIPAM-*b*-MAA (b)

spectroscopy (EDS) spectrum which displayed a platinum content of 12.2 wt%. ICP-MS also confirmed that the content of Pt in polymer conjugate was 11.2 wt% which indicated a strong drug-loading

capacity, considering that the feed ratio of platinum to copolymer was only 12.6 wt%. The cisplatin-entrapped polymer conjugate retained adequate solubility at normal physiological pH condition and

Figure 12. Number-averaged particle size distributions of the PNIPAM-*b*-MAA at 25 °C (blue line), and the Pt@PNIPAM-*b*-MAA (red line) in an aqueous solution with a concentration of 0.1 mg/mL after cisplatin conjugation

formed micelles at a CMC value of 0.25 mg/mL, as shown in Figure 9. The cytotoxicity of the diblock copolymer, Pt@PNIPAM-*b*-MAA, and free cisplatin was measured using an MTT assay. The effect of PNIPAM-*b*-MAA concentrations on cell viability of 7F2 osteoblast-like cells is shown in Figure 14. Polymers reduced cell viability by approximately 10–20% from 1 to 70 $\mu\text{g/mL}$ comparing to the control sample whereas a significant viability reduction at 140 $\mu\text{g/mL}$ was observed. Therefore, the polymer concentration 70 $\mu\text{g/mL}$ was chosen for the cytotoxicity against 7F2 osteoblast-like cells. Figure 15 showed that the blank copolymer did not exhibit appreciable cytotoxicity, indicating that its biocompatibility is suffi-

Figure 13. The TEM images of Pt@PNIPAM-*b*-MAA prepared by the solvent evaporation method at polymer concentration of 0.3 mg/mL in aqueous solution (a, b) and EDS analysis of the prepared core-shell micelle (c)

Figure 14. The effect of polymer concentrations on cell viability of 7F2 osteoblast-like cells. Data are expressed by the mean of percent cell viability compared to control $\pm$ standard deviation ($n = 6$). * $p < 0.05$, compared to control.

Figure 15. The cell viability of 7F2 osteoblast-like cells treated with polymers, polymer-Cisplatin conjugates and free drugs. Data are expressed by the mean of percent cell viability compared to control $\pm$ standard deviation ($n = 6$). * $p < 0.05$, compared to the cells treated with polymers alone.

ciently high for in vivo applications. On the other hand, the cisplatin conjugated polymer and free cisplatin drug reduced cell viability by approximately 36.1 and 42.8%, respectively. Considering the Pt metal entrapped in polymer conjugate was only 12.6 wt%, the result suggested that the efficacy of the Pt@PNIPAM-*b*-MAA was comparable to that of the free cisplatin and the conjugation with the amphiphilic copolymer did not compromise its cytotoxic activity.

4. Conclusions

The use of various pharmaceutical nanocarriers has become one of the most studied areas of nanomedicine. In the present study, we examined the synthesis of a fast microwave-assisted polymeric drug carrier featuring both pH and temperature responsiveness through RAFT polymerization. Through the use ECTA as the CTA and AIBN as the initiator, the polymerizations exhibited living polymerization features such as a linear relationship between M_n and the conver-

sion, a controlled molecular weight, and a relatively narrow molecular weight distribution. Furthermore, the values of k_{app} demonstrated that our microwave-assisted process was approximately 150 times faster than those observed under conventional heating conditions, where $[NIPAM]/[CTA]/[initiator] = 10/1/0.05$. Reactivating the PNIPAM as a macro-RAFT agent for MAA copolymerization produced a diblock copolymer PNIPAM-*b*-MAA that did not lose its ‘living’ nature under the microwave irradiation. Increasing the temperature above 40 °C or reducing the pH of the solution can generate various degrees of aggregations, which are ideal for drug accumulation control in the design of new antitumor drug delivery systems. Theoretically, various anticancer polymeric drug conjugates could be accessible through the replacement of different drug molecules. These polymeric micelles are expected to possess high potential for antitumor drug delivery because of a strong combination of passive targeting characteristics (i.e., the enhanced permeability and retention effect) and the sensitivity to either temperature or acidic microenvironments.

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