Self-assembling methoxypoly(ethylene glycol)-b-poly(carbonate-co-\(\ell\)-lactide) block copolymers for drug delivery

Michael Danquah\textsuperscript{a}, Tomoko Fujiwara\textsuperscript{b}, Ram I. Mahato\textsuperscript{a,*}

\textsuperscript{a}Department of Pharmaceutical Sciences, University of Tennessee Health Science Center, 19 South Manassas (Room 224), Memphis, TN 38103-3308, USA
\textsuperscript{b}Department of Chemistry, The University of Memphis, Memphis, TN 38152, USA

\textbf{A R T I C L E  I N F O}

Article history:
Received 11 September 2009
Accepted 24 November 2009
Available online 17 December 2009

\textbf{Keywords:}
Micelles
Copolymers
Poly(lactic acid)
Poly(carbonate-co-\(\ell\)-lactide)
Polyethylene glycol
Bicalutamide

\textbf{A B S T R A C T}

Bicalutamide is the most widely used non-steroidal antiandrogen for treating early stage prostate cancer, but suffers variable oral absorption due to its limited aqueous solubility. Thus, our objective was to synthesize novel biodegradable copolymers for the systemic micellar delivery of bicalutamide. Flory–Huggins interaction parameter (\(\chi_{FH}\)) was used to assess compatibility between bicalutamide and poly(lactide) or poly(carbonate-co-lactide) polymer pairs. Polyethylene glycol-b-poly(carbonate-co-lactide) [PEG-b-P(CB-co-LA)] copolymers were synthesized and characterized by NMR and gel permeation chromatography. These micelles had average diameter of 100 nm and had a smooth surface and distinct spherical shape. Drug loading studies revealed that adding the carbonate monomer could increase bicalutamide loading. Among the series, drug loading of micelles formulated with PEG-b-P(CB-co-LA) copolymer containing 20 mol\% carbonate was about four-fold higher than PEG-b-PLLA and aqueous solubility of bicalutamide increased from 5 to 4000 \(\mu\)g/mL. CMC values for PEG-b-P(CB-co-LA) copolymers was up to 10-fold lower than those of PEG-b-PLLA. In vitro release experiments showed PEG-b-P(CB-co-LA) copolymers to be more efficient in sustaining the release of bicalutamide compared to PEG-b-PLLA. Bicalutamide-loaded PEG-b-P(CB-co-LA) micelles showed significant inhibition of LNCaP cell growth in a dose-dependent manner which was similar to the methanol solution of free drug.

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1. Introduction

Androgens are known to play a pivotal role in the development and maintenance of the prostate by interacting with the androgen receptor (AR)\textsuperscript{[1]} \cite{1}. Consequently, androgen ablation, especially the use of antiandrogens, has been used as a standard treatment for men with prostate cancer. Among various antiandrogens, bicalutamide (Casodex\textsuperscript{\textregistered}) is the most widely used non-steroidal antiandrogen for treating early stage prostate cancer due to its relatively long half life and tolerable side effects\textsuperscript{[2]} \cite{2}. However, bicalutamide exhibits poor aqueous solubility (5 \(\mu\)g/mL), which results in poor and variable drug absorption across the gastro intestinal tract. Furthermore, bicalutamide cannot be administered systemically, as traditional approaches to increase its aqueous solubility using solubilizing agents such as dimethyl sulfoxide (DMSO) and Cre mophor EL is not practical in humans due to hemolysis, acute hypertensive reactions and neuropathies\textsuperscript{[3]} \cite{3}. One way of improving the solubility of hydrophobic drugs and in particular bicalutamide is by using polymeric micelles. Micelles are attractive drug delivery vehicles primarily because they can solubilize hydrophobic drugs in their core leading to improved bioavailability and drug stability. Furthermore, micelles are capable of preventing drug degradation, minimizing the adverse effects of the drug on visceral organs and have the possibility of being made site-specific\textsuperscript{[4]} \cite{4}. A number of amphiphilic diblock copolymers composed of polyethylene glycol (PEG) and various biodegradable hydrophobic cores capable of forming micelles have been reported in the literature. Examples include: poly(ethylene glycol)-b-poly(aspartic acid) [PEG-b-PAA]\textsuperscript{[5]} \cite{5}, poly(ethylene glycol)-b-poly(lactic-co-glycolic acid) [PEG-b-P(PLGA)]\textsuperscript{[6]} \cite{6}, poly(ethylene glycol)-b-poly(caprolactone) [PEG-b-PCL]\textsuperscript{[7]} \cite{7} and poly(ethylene glycol)-b-poly(n\(_{\alpha}\)-lactide) [PEG-b-PDLLA]\textsuperscript{[8]} \cite{8}. Key properties of these micelle systems such as size, stability, drug release kinetics and drug loading have also been well studied.

We have recently demonstrated the feasibility of using PEG-b-PDLLA micelles to increase the aqueous solubility of bicalutamide\textsuperscript{[9]} \cite{9}. Although we were able to increase the aqueous solubility of bicalutamide, we observed only moderate drug loading levels...
Furthermore, polycarbonate degrades into carbon dioxide, exhibit low toxicity and possess tunable mechanical properties which may provide adequate stability to the micelle system and is known for its application as a drug delivery material [10–12]. For the carbonate block we selected the cyclic 5-methyl-5-benzyloxy-carbonyl-1,3-dioxane-2-one carbonate monomer. 5-methyl-5-benzyloxy carbonyl-1,3-dioxane-2-one is a modification of 5-benzyloxy carbonyl-1,3-dioxane-2-one which is an intermediate in the synthesis of numerous antiviral compounds [13]. This carbonate monomer was chosen since polycarbonates are biodegradable, exhibit low toxicity and possess tunable mechanical properties [14–16]. Furthermore, polycarbonate degrades into carbon dioxide and benzyl alcohol, which unlike the degradation products of poly(ε-lactide) [e.g. lactic acid] are less acidic, has less effect on microenvironment and pH and as such will not result in local inflammation. We hypothesize that the introduction of the carbonate monomer would provide additional degrees of freedom to tailor a micelle delivery system that is relatively stable, exhibits improved sustained release and has a hydrophobic core that is more compatible with bicalutamide leading to enhanced drug loading.

Improvement in the extent of compatibility between a drug and the core-forming block of the micelle may translate into superior encapsulation efficiency [17–20]. A number of groups have explored the possibility of predicting drug solubilization in micelles based on thermodynamics and found their predictions to closely approximate experimental results [18,21–23]. Polymer/drug compatibility may be characterized by the Flory–Huggins interaction parameter ($\chi_{Dh}$) which accounts for the forces of interaction between the polymer and the drug; and low $\chi_{Dh}$ values suggest that the polymer is thermodynamically a good solvent for the drug. To design a micelle system with improved drug loading, we first performed an in silico study using the Molecular Pro Software to assess the Flory–Huggins interaction parameter ($\chi_{Dh}$) between bicalutamide and poly(ε-lactide) (PLLA) and a series of poly(carbonate-co-lactide) copolymer with varying carbonate to lactide ratios based on group contribution method. In this study, a series of poly(ethylene glycol)-b-poly(carbonate-co-lactide) copolymers were synthesized and characterized. Also, the influence of carbonate content on key micelle properties such as size, drug loading, stability and release kinetics was investigated. Furthermore, the microstructure of the micelle core block was analyzed to examine the influence of co-monomer sequence distribution on the physicochemical properties of the copolymer and thermal analysis was used to elucidate the impact of carbonate introduction on the crystalline or amorphous nature of the hydrophobic core. Finally, the efficacy of bicalutamide-loaded micelles was assessed in lymph node adenocarcinoma (LNCaP) human prostate cancer cell lines.

2. Materials and methods

2.1. Materials

A mixture of 2,2-bis(hydroxymethyl)propionic acid (22.5 g, 0.16 mol), potassium hydroxide (888 mg, 15.7 g, 0.169 mol), and dimethylformamide (DMF) (125 mL) was heated to 100 °C for 1 h with stirring at which point homogenous potassium salt solution was formed. Benzyl bromide (34.5 g, 0.202 mol) was added dropwise to the warm solution, and stirring was continued at 100 °C for 15 h. Upon completion of the reaction, the mixture was cooled and the solvent was removed under vacuum. The residue was dissolved in ethyl acetate (150 mL), hexanes (150 mL), and water (100 mL). The organic layer was retained, washed with water (100 mL), dried (Na2SO4), and evaporated. The resulting solid was recrystallized from toluene to give pure benzyl 2,2-bis(methylol)propionate, as white crystals (20 g, 58%).

Benzyl 2,2-bis(methylol)propionate (11.2 g, 0.05 mol) was dissolved in pyridine (25 mL, 0.3 mol) and CHCl3 (150 mL), and the solution was chilled to – 78 °C under Na2. A solution of triphenylphosphine (7.5 g, 25.0 mmol) in CH2Cl2 was added dropwise over 1 h, after which the reaction mixture was stirred at room temperature for 2 h. The reaction was quenched by the addition of saturated aqueous NH4Cl (75 mL). Subsequently, the organic layer was washed with 1 N aqueous HCl (3 × 100 mL), saturated aqueous Na2SO3 (1 × 100 mL), dried (Na2SO4), filtered and evaporated to give 5-methyl-5-benzyloxy carbonyl-1,3-dioxane-2-one as a white solid (10.6 g, 95%). The ensuing solid was recrystallized from ethyl acetate prior to polymerization.

2.4. Synthesis of PEg-Poly(carbonate-co-lactide)

Stannous 2-ethylhexanoate (10 mol% relative to mPEG) was added to the mixture of prescribed quantities of PEG, lactide and base monomer in a dried polymerization flask under the protection of nitrogen atmosphere. The reaction mixture was heated to 130 °C for 24 h with stirring. Afterward, the product was cooled to room temperature to terminate the reaction. The copolymer was dissolved in chloroform and precipitated in a large amount of diethyl ether and hexane (1:2), filtered and dried under vacuum at room temperature.

2.5. Polymer characterization

2.5.1. Nuclear magnetic resonance (NMR)

1H NMR spectra were recorded on a JOEL (270 MHz, T – 25 °C) and 13C spectra were recorded with a Varian (500 MHz, T – 25 °C) using deuterated chloroform (CDCl3) and deuterated dimethyl sulfoxide (DMSO-d6) as solvents. The chemical shifts were calibrated using tetramethylsilane as an internal reference and given in parts per million.

2.5.2. Gel permeation chromatography (GPC)

A Shimadzu 20A GPC system equipped with two Jordi Gel DVB/500 columns and a differential refractive index detector was used to determine the molecular weight and polydispersity index (PD) of the copolymers. Tetrahydrofuran (THF) was used as eluent at a flow rate of 1 mL/min at 35 °C. A series of narrow polystyrene standards (900–100,000 g/mol) were used for calibration and the data was processed using a LC Solution ver.1.21 GPC operation software.

2.5.3. Differential scanning calorimetry (DSC)

A TA Instruments DSC Q 2000 module was used to determine the thermal properties of the synthesized copolymers. Samples were placed in aluminum pans under nitrogen heated from 25 °C to 100 °C, cooled to – 90 °C to remove thermal history and heated to 100 °C at a rate of 10 °C/min.
2.6. Critical micelle concentration (CMC)

Fluorescence spectroscopy was used to estimate the critical micelle concentration (CMC) of PEG-b-PLLA and PEG-b-(PCB-co-LA) copolymer using pyrene as a hydrophobic fluorescent probe. Twelve samples of PEG-b-PLLA or PEG-b-(PCB-co-LA) dissolved in methanol with concentrations ranging from $1 \times 10^{-7}$ to $1 \mu$L were prepared and allowed to equilibrate with a constant pyrene concentration of $6 \times 10^{-8}$ M for 48 h at room temperature. The fluorescence spectra of pyrene were recorded with a Molecular Devices SpectraMax M2/M2e spectrofluorometer (Sunnyvale, CA). An excitation wavelength of 390 nm was used and the emission spectra were recorded from 320 to 450 nm with both bandwidths set at 2 nm. Peak height intensity ratio (I$_{338}$/I$_{328}$) of the third peak (I$_3$ at 338 nm) to the first peak (I$_1$ at 333 nm) was plotted against the logarithm of polymer concentration. The value of the CMC was obtained as the point of intersection of two tangents drawn to the curve at high and low concentrations, respectively.

2.7. Preparation of bicalutamide-loaded micelles

Bicalutamide-loaded micelles were prepared using the film sonication method as previously described with slight modifications [9]. In brief, 5 mg of bicalutamide and 95 mg of PEG-b-PLLA or PEG-b-(PCB-co-LA) were dissolved in 5 mL chloroform. The mixture was vortexed for 5 min to ensure homogeneity and the solvent evaporated under N2 flow. The resulting film was hydrated in 10 mL phosphate buffered saline (PBS) to yield a final concentration of 10 mg/mL and sonicated for 10 min using a Misonix ultrasonic liquid processor (Farmingdale, NY) at an amplitude of 50. The ensuing formulation was then centrifuged at 5000 rpm for 10 min to separate micelles from residual free drug. Subsequently, the supernatant was filtered using a 0.22 µm nylon filter. The micelle preparation was lyophilized for 48 h and stored at 4°C to prolong shelf-life and avoid untimely release of the drug.

2.8. Drug loading and encapsulation efficiency

Drug loading was determined as follows: 100 mg lyophilized bicalutamide-loaded micelles were dissolved in chloroform and the drug present in solution measured by ultraviolet spectroscopy. The weight of drug loaded in the micelles was calculated using a calibration curve and background absorbance interference from PEG-b-PLLA or PEG-b-(PCB-co-LA) copolymer was accounted for by measuring the absorbance of blank PEG-b-PLLA or PEG-b-(PCB-co-LA) under the same conditions. Drug loading content and encapsulation efficiency were then determined using equations 3 and 4 as follows:

$$\text{drug loading density} = \frac{\text{weight of drug in micelles}}{\text{weight of micelles}} \times 100\% \quad (3)$$

$$\text{drug encapsulation efficiency} = \frac{\text{weight of drug in micelles}}{\text{weight of drug originally fed}} \times 100\% \quad (4)$$

2.9. Particle size distribution

Mean particle size and size distribution of drug-loaded micelles were determined by dynamic light scattering (DLS) using a Zetasizer (Malvern Instruments, Worcestershire, UK) at a 1 mg/mL polymer concentration. Samples were analyzed at room temperature with a 90° detection angle and the mean micelle size was obtained as a Z-average. All measurements were performed seven times and reported as the mean diameter ± SD for triplicate samples.

2.10. Transmission electron microscopy

Micelles prepared using PEG-b-(PCB-co-LA) copolymer were visualized using a JEM-1000 (Japan) transmission electron microscope (TEM). 5 µL of micelle suspension was loaded on a copper grid, followed by blotting of excess liquid and air-dried before negative staining with 1% uranyl acetate. The grid was visualized under the electron microscope at 60 kV and magnifications ranging from 50,000× to 100,000×.

2.11. Bicalutamide release from micelles

The dialysis technique was employed to study the release of bicalutamide from the various copolymer micelles in PBS (pH 7.2). Bicalutamide-loaded micelles with a final bicalutamide concentration of 0.2 mg/mL were placed into a dialysis membrane with a molecular weight cut-off of 8000 Da and dialyzed against 50 mL PBS (pH 7.2) in a thermostatically controlled shaker with a rotation speed of 100 rpm. The samples were withdrawn at specified times for a period of seven days and assayed with a validated UV spectrophotometer by measuring the absorbance of the solution at 270 nm. The samples taken for measurement were replaced with fresh media and the cumulative amount of drug released into the media at each time point was evaluated as the percentage of total drug released to the initial amount of the drug. All experiments were performed in triplicate and the data reported as the mean of the three individual experiments.

2.12. In vitro cytotoxicity of bicalutamide-loaded micelles

The ability of bicalutamide and bicalutamide-loaded micelles to inhibit cell proliferation was evaluated using LNCaP human prostate cell line. Cells were cultured in RPMI 1640 media supplemented with 2 mM glutamine, 10% fetal bovine serum (FBS) and 1% antibiotic-antimycotic at 37°C in humidified environment of 5% CO2 and subcultured every 3 days to ensure exponential growth. The cells were then seeded in 96-well plates at a density of $5 \times 10^3$ viable cells/well and incubated for 48 h to permit cell attachment. The cells were exposed to bicalutamide-dissolved in methanol or bicalutamide-loaded micelles at concentrations ranging from 1 to 100 µg for 48 h. At the end of treatment, 20 µL of MTT solution (5 mg/mL) was added to each well and incubated for 4 h. The plates were then centrifuged at 1500g for 2 min and the medium aspirated. The residual formazan crystals were solubilized with 100 µL DMSO and the plate analyzed using a microplate reader. The absorbance values were recorded at a test wavelength of 560 nm. Cell viability for a given concentration was expressed as a percentage of the intensity of controls. The data was reported as the mean of triplicate experiments.

3. Results

3.1. Design of diblock copolymer based on enhanced compatibility between bicalutamide and hydrophobic core

Using PEG-b-PLLA copolymer as a template, a series of diblock copolymers were designed by modifying the PLLA hydrophobic core with a carbonate monomer (i.e. 5-methyl-5-benzoxycarbonyl-1,3-dioxane-2-one). The goal here was to enhance the compatibility between bicalutamide and the hydrophobic core of the micelle. Since the Flory–Huggins interaction parameter ($\chi_{FH}$) has been shown to be a good indicator of polymer–drug compatibility [18,20,23], we determined $\chi_{FH}$ for PLLA and P(CB-co-LA) using equation (2). The Hansen partial solubility parameters for bicalutamide, PLLA and P(CB-co-LA) were obtained based on the group contribution method using the Molecular Modeling Pro software (Table 1). The partial solubility parameters which account for dispersive forces between atoms, permanent dipole interactions between molecules and the tendency of molecules to hydrogen bond were used to calculate the solubility parameter which was subsequently used to compute the interaction parameter. Generally, as $\chi_{FH}$ approaches zero, compatibility between the polymer and the drug progressively increases since the polymer increasingly becomes a better thermodynamic solvent for the drug, resulting in improved drug solubilization. The interaction parameter between bicalutamide and PLLA was calculated to be 11.06 while the interaction parameter between bicalutamide and P(CB-co-LA) was computed to be 7.34. Hence, by introducing a carbonate monomer (5-methyl-5-benzoxycarbonyl-1,3-dioxane-2-one) into the PLLA hydrophobic core, our design suggested a potential increase in compatibility between bicalutamide and the micelle core and provided a logical justification for the synthesis of the poly(ethylene glycol)-b-poly(carbonate-co-lactide) [PEG-b-(PCB-co-LA)] copolymers.

3.2. Synthesis and characterization of 5-methyl-5-benzoxycarbonyl-1,3-dioxane-2-one

The cyclic carbonate monomer (5-methyl-5-benzoxycarbonyl-1,3-dioxane-2-one) was synthesized as described by Pratt et al. in [9].

Table 1

<table>
<thead>
<tr>
<th>Drug/polymer</th>
<th>$\delta$ polarity ($\text{J/cm}^3\text{cm}^{-2}$)</th>
<th>$\delta$ dispersion ($\text{J/cm}^3\text{cm}^{-2}$)</th>
<th>$\delta$ hydrogen ($\text{J/cm}^3\text{cm}^{-2}$)</th>
<th>Total solubility parameter* $\chi_{FH}$ $\text{b}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bicalutamide</td>
<td>8.89</td>
<td>19.66</td>
<td>12.43</td>
<td>24.90</td>
</tr>
<tr>
<td>PLLA</td>
<td>2.48</td>
<td>19.73</td>
<td>20.92</td>
<td>28.68</td>
</tr>
<tr>
<td>P(CB-co-LA)</td>
<td>1.43</td>
<td>21.62</td>
<td>8.48</td>
<td>11.06</td>
</tr>
</tbody>
</table>

* Total solubility parameters computed using partial solubility parameters and equation (1).

$\text{b}$ Flory–Huggins interaction parameters between bicalutamide and PLLA/P(CB-co-LA) were calculated using equation (2).
with slight modifications (Scheme 1, Stage 1) [24]. Briefly, 2,2-
bis(hydroxymethyl)propionic acid was used to obtain benzyl 2,2-
bis(methylol)propionate as an intermediate compound which was 
subsequently reacted with triphosgene to obtain the cyclic 
carbonate monomer. The chemical structure of the monomer was 
confirmed by mass and \(^1\)H NMR spectroscopy. From the mass 
spectrum, the molecular ion peak agreed with the calculated 
molecular weight of the synthesized monomer (250 g/mol). Also, as 
depicted in Fig. 1A, the signal d at 7.5 ppm is characteristic of the 
phenyl ring protons in the monomer; while the signal b at 4.2 and 
4.7 ppm was assigned to the methylene protons present in the 
carbonate ring. Other peak assignments include signals a and c at 
1.3 and 5.2 ppm, representing methyl and benzyl CH\(_2\) protons, 
respectively in the monomer.

\[
\text{H}_2\text{C}=\text{CH}_2\text{O}_{x}\text{H} + \]

\[
\text{OH} \quad \text{OH} \\
\text{O} \quad \text{O} \\
\text{+ Br} \quad \text{O} \\
\text{OH} \quad \text{OH} \\
\text{O} \quad \text{O} \\
\text{STAGE 1} \\
\]

\[
\text{stannous} \\
\text{2-ethylhexanoate} \\
\]

\[
\text{STAGE 2} \\
\]

\[
\text{H}_3\text{CO} \quad \text{CH}_2\text{CH}_2\text{O}_{x} \text{H} \\
\text{O} \quad \text{O} \\
\text{O} \\
\text{O} \\
\text{O} \\
\]

Scheme 1. Synthesis of PEG-b-P(CB-co-LA) copolymers. Stage 1: 5-Methyl-5-benzyloxycarbonyl-1,3-dioxane-2-one. Conditions: (i) KOH, DMF, 100 °C, 15 h (ii) Triphosgene, pyridine, CH\(_2\)Cl\(_2\), -78-0 °C. Stage 2: Ring opening polymerization of L-Lactide and 5-Methyl-5-benzyloxycarbonyl-1,3-dioxane-2-one initiated by poly(ethylene glycol).
3.3. Synthesis and characterization of PEG-b-P(carbonate-co-lactide) copolymer

The synthesis route of PEG-b-P(CB-co-LA) is delineated in Scheme 1, Stage 2. A series of PEG-b-P(CB-co-LA) copolymers with varying carbonate (10, 20 and 40 mol%) content were synthesized by a one pot ring opening polymerization of L-lactide and 5-methyl-5-benzyloxycarbonyl-1,3-dioxane-2-one using methoxy-PEG as the macroinitiator and stannous 2-ethylhexanoate as a catalyst. Among the series, the percent conversion of PEG-b-P(CB-co-LA) copolymer from carbonate was the highest for the 10 mol% series and was estimated to be 87% based on $^1$H NMR spectroscopy. Further $^1$H NMR analyses were used to verify the composition of PEG-b-P(CB-co-LA) copolymers. From Fig. 1B, the following resonance peaks were observed for the copolymers at $\delta$: 1.24 (CH$_3$ in CB unit, s, 3H); $\delta$: 1.55–1.57 (CH$_3$ in LA unit, s, 3H); $\delta$: 3.65 (CH$_2$ in PEG, m, 4H); $\delta$: 4.25–4.35 (CH$_2$ in CB main chain m, 4H); $\delta$: 5.12 (CH in LA unit q, 1H, and CH$_2$ in CB side group, s, 2H); $\delta$: 7.3 (phenyl, m, 5H). All signals are assigned as methoxy poly(ethylene glycol) [mPEG], polymerized LA and CB units. The presence of the 7.3 ppm multiplet peak indicates the existence of the carbonate group in the copolymer structure. In addition, the disappearance of the signal at 4.2 and 4.7 ppm in the carbonate monomer (Fig. 1A) and the emergence of the multiplet peak at $\delta$: 4.25–4.35 ppm in the copolymer (Fig. 1B) confirms the
successful ring opening polymerization of the carbonate monomer and the formation of PEG-b-P(CB-co-LA) copolymers.

Table 2 summarizes some characteristics of the synthesized PEG-b-PLLA and PEG-b-P(CB-co-LA) copolymers. The block copolymer molecular weight was calculated based on a comparative analysis of the four methylene protons of PEG ($\delta = 3.65$ ppm), one methylene proton of lactide ($\delta = 5.12$ ppm) and the five protons associated with the phenyl ring in the carbonate monomer observed in the $^1$H NMR spectrum. It is worth mentioning that since the lactide proton peak at 5.12 ppm is confounded with the signal from the two protons of the carbonate monomer, two-fifth of the carbonate signal at 7.3 ppm was subtracted from the 5.12 ppm signal to obtain the actual lactide peak intensity used for molecular weight calculations.

For the three copolymers studied: PEG$_{114}$-b-P(C$_{5}$-CO-LA$_{32}$), PEG$_{114}$-b-P(C$_{8}$-CO-LA$_{24}$) and PEG$_{114}$-b-P(C$_{8}$-CO-LA$_{24}$): the molecular weight were 8604 g mol$^{-1}$, 8679 g mol$^{-1}$ and 7510 g mol$^{-1}$, respectively. Hence, the calculated molecular weight from $^1$H NMR spectroscopy, $M_n,_{NMR} = M_n,_{PEG} + M_n,_{Carbonate} + M_n,_{L,Lactide}$.

$$\text{CMC (g/L) normalized with } M_n \text{ from GPC. Critical micelle concentration (CMC) was determined at 25}^\circ \text{C using pyrene as hydrophobic probe.}$$

### Table 2

<table>
<thead>
<tr>
<th>Block copolymer</th>
<th>Carbonate (mol%)</th>
<th>Theoretical mol. wt. (g/mol)</th>
<th>$M_n$ (GPC)</th>
<th>$M_w$ (GPC)</th>
<th>$M_w/M_n$ (GPC)</th>
<th>CMC (g/L)</th>
<th>CMC $\times 10^3$ (mol$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PEG$_{114}$-b-PLLA</td>
<td>0</td>
<td>10,000</td>
<td>9460</td>
<td>9104</td>
<td>1.07</td>
<td>0.03</td>
<td>32.95</td>
</tr>
<tr>
<td>PEG$<em>{114}$-b-P(C$</em>{5}$-CO-LA$_{32}$)</td>
<td>10</td>
<td>10,000</td>
<td>8604</td>
<td>6228</td>
<td>8.53</td>
<td>0.005</td>
<td>8.03</td>
</tr>
<tr>
<td>PEG$<em>{114}$-b-P(C$</em>{8}$-CO-LA$_{24}$)</td>
<td>20</td>
<td>10,000</td>
<td>8679</td>
<td>6384</td>
<td>8.925</td>
<td>0.002</td>
<td>3.13</td>
</tr>
<tr>
<td>PEG$<em>{114}$-b-P(C$</em>{9}$-CO-LA$_{24}$)</td>
<td>40</td>
<td>10,000</td>
<td>7510</td>
<td>5694</td>
<td>8.573</td>
<td>0.004</td>
<td>7.02</td>
</tr>
</tbody>
</table>

* Subscripts reflect degree of polymerization of each monomer obtained from $^1$H NMR spectroscopy.

a Mol% indicates carbonate content in P(CB-co-LA) block copolymer. Molecular weight calculated from $^1$H NMR spectroscopy, $M_n,_{NMR} = M_n,_{PEG} + M_n,_{Carbonate} + M_n,_{L,Lactide}$.

b CMC (g/L) normalized with $M_n$ from GPC. Critical micelle concentration (CMC) was determined at 25 $^\circ$C using pyrene as hydrophobic probe.

3.4. Preparation and characterization of PEG-b-P(CB-co-LA) copolymer micelles

The film sonication method described in a previous publication [9] was used to prepare both unloaded and bicalutamide-loaded polymeric micelles from the synthesized PEG-b-PLLA and PEG-b-P(CB-co-LA) copolymers [9]. The size distribution for both unloaded and bicalutamide-loaded polymeric micellar formulations as determined by dynamic light scattering (DLS) was relatively broad with a PDI of approximately 0.2, reflecting the possible presence of a population of micellar size distribution and surface morphology. (A) Dynamic light scattering histogram; and (B) transmission electron micrograph of micelle formulated with PEG-b-P(CB-co-LA) copolymer obtained using uranyl acetate staining.

![Fig. 2. Gel permeation chromatograms of (A) PEG-b-PLLA [$M_n = 9460$ g/mol, $M_w/M_n = 1.07$] and (B) PEG$_{114}$-b-P(C$_{8}$-CO-LA$_{24}$) [$M_n = 9720$ g/mol, $M_w/M_n = 1.40$] copolymers, both acquired in THF.](image)

![Fig. 3. Micelle size distribution and surface morphology. (A) Dynamic light scattering histogram; and (B) transmission electron micrograph of micelle formulated with PEG-b-P(CB-co-LA) copolymer obtained using uranyl acetate staining.](image)
aggregates. Fig. 3A shows a typical size distribution plot obtained from DLS of PEG-b-P(CB-co-LA) micelles using a polymer concentration of 10 mg/mL. The size distribution is bimodal with a smaller size population ~53 nm (58%) and a larger population ~162 nm (42%). Effective hydrodynamic diameter for both unloaded and bicalutamide-loaded PEG-b-PLLA and PEG-b-P(CB-co-LA) micelles reported as the z-average based on mean intensity was determined by DLS. In the case of the unloaded micelles, the average diameter was found to range from 107 to 135 nm, while the average diameter of bicalutamide-loaded micelles ranged from 99 to 123 nm (Table 3). In both cases the 20 mol% carbonate copolymer had the largest average diameter whilst the 40 mol% carbonate copolymer had the smallest average diameter.

Particle size and surface morphology of these micelles were also determined by transmission electron microscopy (TEM). The micelles have a broad size distribution with mean number average diameter below 50 nm (Fig. 3B) which is lower than the z-average diameters obtained from DLS. TEM images also reveal a tendency for micellar aggregation and confirm that PEG-b-P(CB-co-LA) formed true spherical micelles in water with distinct boundaries as anticipated.

The polymer micelles were further characterized by fluorescence spectroscopy to determine the critical micelle concentration (CMC). The CMC of the synthesized block copolymers decreased upon introduction of the carbonate monomer from ~3.3 to ~0.3 μM for 0 and 20 mol% carbionate, respectively (Fig. 4 and Table 2). The lower CMC values suggest that the inclusion of the carbonate moiety results in block copolymers thermodynamically more favored to self-assemble.

The amount of bicalutamide loaded into micelles was calculated using equation (3) based on 5% theoretical loading (i.e., 5 mg drug/100 mg polymer). PEG-b-P(CB-co-LA) copolymers exhibited higher drug loading compared to PEG-b-PLLA copolymer (Table 3). Among PEG-b-P(CB-co-LA) copolymer series, the highest loading (4.10%) was observed for PEG114-b-P(CB8-co-LA24) copolymers which was at least four-fold better than PEG-b-PLLA copolymer with regards to drug loading and had an encapsulation efficiency of approximately 82%. For PEG114-b-P(CB5-co-LA32) the drug loading was 3.36% which is about three times higher than that of PEG-b-PLLA and its encapsulation efficiency was roughly 73%. PEG114-b-P(CB9-co-LA5) copolymer had a drug loading of 1.38% and an encapsulation efficiency of 28% which is only slightly better than PEG-b-PLLA which had a drug loading of 1% and an encapsulation efficiency of 22%. Since the degree of polymerization of P(CB-co-LA) hydrophobic core varied across the series, the drug loading was also assessed on a molar basis with respect to just the hydrophobic core (i.e., mol bicarbonato/mol hydrophobic core) to normalize the data. Here again, PEG114-b-P(CB9-co-LA5) had the highest loading (1097%), followed by PEG114-b-P(CB5-co-LA32) and then PEG114-b-P(CB9-co-LA5) which had 876% and 779% loading, respectively. In all cases, the drug loading on a molar basis in PEG-b-P(CB-co-LA) copolymer series was several fold higher than that of PEG-b-PLLA copolymer.

To establish that PEG-b-P(CB-co-LA) micelles are capable of forming core–shell structures, the 1H NMR spectra of micelle samples in deuterated water (D2O) and DMSO-d6 were compared. Typical spectra for PEG114-b-P(CB8-co-LA24) micelles are shown in Fig. 5. In D2O, only the PEG proton peaks can be detected while the signals for PLLA and polycarbonate are not evident. On the contrary, distinct peaks for PLLA and polycarbonate were observed in DMSO-d6 in addition to the PEG signal, suggesting that PEG-b-P(CB-co-LA) micelle form core–shell structures capable of encapsulating bicalutamide. This result is consistent with what is reported in the literature since the PEG forms the corona of the micelle and enhances its solvation in D2O while PLLA, polycarbonate and the encapsulated bicalutamide are in the core of the micelle and have restricted mobility in D2O.

### 3.5. Sequence analysis

Sequence distribution in a copolymer is frequently analyzed in terms of monomer triads. Considering that the hydrophobic core of the synthesized PEG-b-P(CB-co-LA) copolymers is made up of two monomers: carbonate (C) and lactide (L), this system has eight possible theoretical triads. Prior to the analysis of triads in the copolymer, the complete assignment for the carbonyl peaks in PEG-b-P(CB-co-LA) copolymer based on 13C NMR spectra of homopolymers: PLLA and polycarbonate was first determined and the results are shown in Fig. 6A. To elucidate the arrangement of triads in PEG-b-P(CB-co-LA) copolymer, 13C NMR spectrum was expanded for clarity (Fig. 6B): expansion of peak “a” (upper) and peak “d” (lower). The resonance peak at 169.6 ppm is assigned to the central carbonyl of PLLA and LLC triads. Further upfield the resonance peak occurring at 169.45 ppm is due to the central carbonyl in the CLC and CLL triads. The resonance peak at 154.35 ppm is assigned to

### Table 3

<table>
<thead>
<tr>
<th>Block copolymera</th>
<th>Drug loading (g C/g) ± SDb</th>
<th>Encap. efficiency (nm) ± SD</th>
<th>Bicalutamide/polymer core (mole%)</th>
<th>Mean diameter ± SD (nm)c</th>
</tr>
</thead>
<tbody>
<tr>
<td>PEG114-b-PLLA24</td>
<td>1.0 ± 0.31</td>
<td>22.04 ± 3.82</td>
<td>22</td>
<td>110 ± 0.9</td>
</tr>
<tr>
<td>PEG114-b-P(CB5-co-LA32)</td>
<td>3.36 ± 0.18</td>
<td>72.68 ± 4.27</td>
<td>876</td>
<td>101 ± 1.0</td>
</tr>
<tr>
<td>PEG114-b-P(CB9-co-LA5)</td>
<td>4.10 ± 0.23</td>
<td>81.96 ± 2.54</td>
<td>1097</td>
<td>123 ± 1.5</td>
</tr>
<tr>
<td>PEG114-b-P(CB9-co-LA5)</td>
<td>1.38 ± 0.46</td>
<td>27.56 ± 2.91</td>
<td>779</td>
<td>99 ± 1.4</td>
</tr>
</tbody>
</table>

a Subscripts reflect degree of polymerization of each monomer obtained from 1H NMR spectroscopy.
b Percentage of drug loaded into micelles based on 5% theoretical loading.
c Mean particle size was determined by dynamic light scattering.
the central carbonyl of CCC and CCL triads, while the resonance peak at 154.15 ppm is due to the central carbonyl of the LCL and LCC triads. The above data together with the observed splitting of the carbonyl peaks strongly suggests that the hydrophobic core PEG-b-P(CB-co-LA) is a random copolymer. Having confirmed the formation of random copolymers, the influence of carbonate monomer on the distribution of L–L and C–C sequence was assessed by comparing the 13C NMR spectra of 10, 20 and 40 mol% carbonate copolymers. From Fig. 7, increasing CB contents from 10 to 40 mol% decreases L–L sequence and increases C–C sequence (particularly for 40%).

3.6. Thermal analysis

The thermal properties of the synthesized block copolymers were examined using differential scanning calorimetry (DSC) and the thermograms of PEG-b-PLLA and PEG-b-P(CB-co-LA) copolymers are shown in Fig. 8. Within the temperature range examined (−90 to 175 °C), two endotherm peaks at 51.5 and 155 °C were observed for PEG-b-PLLA copolymer. An exotherm peak was also observed occurring at 88.5 °C. To the contrary, only one endotherm peak, the fusion of PEG block, was observed for all the PEG-b-P(CB-co-LA) copolymers which appeared to shift slightly to higher
temperatures with increasing carbonate content: 52.1, 52.9 and 53.8 °C for 10, 20 and 40 mol%, respectively.

3.7. In vitro release studies of bicalutamide from micelles

The in vitro bicalutamide release study was performed in phosphate buffered saline (PBS, pH 7.4) at 37 °C and 100 rpm. Fig. 9 shows the cumulative percentage of bicalutamide released from PEG-b-PLLA (0 mol% carbonate) and PEG-b-P(CB-co-LA) [10, 20 and 40 mol% carbonate] micelles. From the data, the release of bicalutamide is rapid from the micelles with 0 mol% carbonate content compared to micelles formulated using copolymers containing carbonate monomer. About 60% of the total drug was released within 6 h for 0 mol% carbonate content while the 10, 20 and 40 mol% carbonate content had a burst release of 40%. The 0 mol% carbonate copolymer used in this study had a GPC molecular weight of 5200 g mol⁻¹ to ensure that all copolymers used in the release study had similar molecular weights Fig. 10.

3.8. Evaluation of in vitro cytotoxicity

To determine inhibitory effect of bicalutamide-loaded micelles formulated with PEG-b-P(CB-co-LA) copolymer on cell proliferation, the cytotoxicity of free bicalutamide dissolved in methanol and that of bicalutamide formulated in micelles was evaluated in LNCaP human prostate cancer cell line for 48 h. From Fig. 10, micelle-formulated drug showed significant inhibition of LNCaP cell growth in a dose-dependent manner, which was similar to that obtained
using free drug. The IC_{50} of bicalutamide-loaded micelles was 74 \mu M while that of free drug was 80 \mu M. However, no direct comparison can be made since we had to dissolve free drug in methanol, whereas we prepared micelles in PBS.

4. Discussion

In spite of the numerous advantages associated with polymeric micelles as drug delivery systems, their application as therapeutics has been limited by low loadings and stability. The equilibrium partitioning of a hydrophobic drug into the hydrophobic core of polymeric micelles at a specific temperature is governed by the molar free energy of the drug, which depends on the mixing entropy and the enthalpy interaction between the drug and the polymer, as well as the amount of pressure–volume work required.
in putting a drug molecule into the lipophilic core [25]. It has been shown for polymeric micelles that lowering the enthalpy interaction contribution of the free energy improves drug loading. This may be achieved by carefully selecting the core-forming block such that its solubility parameters and that of the drug are essentially of the same order, indicating superior drug–polymer compatibility. The Flory–Huggins interaction parameter ($\chi_{FH}$) is a good measure of the compatibility between the core-forming block of the copolymer and the drug, with lower values pointing to enhanced compatibility and superior loading.

The purpose of this study was to increase bicalutamide loading into micelles by designing polymers based on improved drug–polymer solubility determined using the Flory–Huggins interaction parameter. Inclusion of carbonate monomer which contains a bulky phenyl ring resulted in decreased $\chi_{FH}$ values. In the strictest sense, $\chi_{FH}$ values above the 0.5 critical value allude to immiscibility between drug and polymer since phase separation of the polymer–drug mixture commences, while $\chi_{FH}$ values below 0.5 suggest that the drug and polymer are soluble in each other. The Flory–Huggins theory is based on the assumption that the orientation of molecules within the polymer–drug system is completely random and that upon mixing the polymer and drug no specific interactions are created or destroyed. However, since these assumptions are not entirely true for our system, the $\chi_{FH}$ values obtained were merely used as a guide in predicting enhanced compatibility in relative terms based on the observed trend of lower $\chi_{FH}$ values. It also provided a reasonable estimate of the closeness of the solubility parameters of P(CB-co-LLA) hydrophobic core and that of bicalutamide in the Hansen partial solubility three dimensional space compared to PLLA and bicalutamide.

A two-step reaction from 2,2-bis[hydroxymethyl]propionic acid was used to obtain the cyclic carbonate monomer (5-methyl-5-benzoxycarbonyl-1,3-dioxane-2-one) which was subsequently employed in the synthesis of PEG-b-P(CB-co-LLA) copolymer (Scheme 1). All copolymers were obtained through ring opening polymerization of PEG, lactide and carbonate. Since our objective was to vary hydrophobic group to increase drug loading into micelles, we did not vary the molecular of PEG in our copolymer to determine the effect of PEG molecular weight on micelle number and diameter.

Various synthetic schemes have been reported for synthesizing 5-methyl-5-benzoxycarbonyl-1,3-dioxane-2-one [24,26–28]. However, we employed the method described by Pratt et al. since the yield of the cyclic carbonate monomer is higher for this method. The yield of the carbonate monomer (95%) we obtained was similar to that obtained by Pratt et al. [24] and our characterization data matched the literature [27,29]. The ring opening polymerization of Lactide (LA) and the carbonate monomer was performed in bulk at 130 °C with Sn(Oct)$_2$ as catalyst (10 mol% relative to mPEG) (Scheme 1). We chose Sn(Oct)$_2$ as a catalyst since it is FDA approved and Sn(Oct)$_2$-catalyzed polymerization does not result in racemization of lactide at temperatures below 200 °C [30]. Also, Sn(Oct)$_2$ is a strong transesterification agent yielding copolymers with a randomized microstructure. Guan and coworkers reported synthesizing and characterizing a similar copolymer, however, they used Et$_2$Zn as the catalyst and a reaction temperature of 100 °C [28]. While a 7 h reaction time was sufficient for complete polymerization of PEG-b-PLLA, it resulted in incomplete polymerization of PEG-b-P(CB-co-LLA) copolymers. A reaction time of 24 h was used for PEG-b-P(CB-co-LLA) copolymers since complete polymerization was observed after 24 h. The observed lower DP of P(CB-co-LLA) core compared to the predicted DP suggests a lowering of the carbonate and lactide monomer reactivity with increasing carbonate content. From $^1$H NMR spectroscopy, the carbonate monomer was observed to have higher reactivity compared to lactide. Also, increasing carbonate content from 10 to 40 mol% resulted in lower reactivity for both carbonate and lactide monomers: carbonate reactivity decreased from 87% to 61% while lactide reactivity decreased from 66% to 24%. Overall, the molecular weight decreases while the molecular weight distribution increases with increasing carbonate content. This may be due to progressively higher levels of intra- (back-biting) and intermolecular (redistribution) transesterification side reactions resulting in the formation of cyclic oligomers as the carbonate content increases.

PEG-b-P(CB-co-LLA) copolymers self assembled into micelles with a core–shell structure (Fig. 5). The average diameter of micelles formulated from PEG-b-P(CB-co-LA) copolymers was in the range of 99–123 nm and appeared to be independent of carbonate content but correlated well with molecular weight. The introduction of carbonate monomer enhanced bicalutamide loading (Table 3) which confirmed our prediction based on the Flory–Huggins interaction parameter of PEG-b-P(CB-co-LLA) having superior bicalutamide loading to PEG-b-PLLA copolymer. However, this increase was not linear with carbonate content and seemed to peak at the rather low drug loading observed for the 40 mol% carbonate series. It is well known, that drug loading is related to the molecular weight observed with increasing carbonate content. As the feed carbonate content increases, the reactivity of both the lactide and carbonate decreases. Lactide reactivity decreases from 86% to 24% while carbonate reactivity decreases from 82% to 64% for 0 mol% to 40 mol% carbonate content. This coupled with the polymer degradation effect associated with intramolecular transesterification leads to the decreased molecular weight observed with increasing carbonate content. It is well known, that drug loading is related to the molecular weight of the hydrophobic core. Large hydrophobic blocks form large micelle cores and require less amount of work to put a molecule of drug into the hydrophobic core due to relatively lower internal pressure. Drug loading is also influenced by the interaction between the drug and the copolymer as well as the solubility of the copolymer. As the carbonate content increases...

![Fig. 10. Effect of free and PEG-b-P(CB-co-LA) micellar formulation of bicalutamide on inhibition of LNCaP cell proliferation 48 h post treatment. Cell viability was estimated by MTT assay and expressed as percent of 1% methanol or blank micelle controls. All data represent the mean ± S.D. of triplicates.](image-url)
the interaction between bicalutamide and the copolymer increases, however, the solubility of the copolymer decreases. Hence, the trade-off between drug–polymer interaction and polymer solubility influences the extent of drug loading. At carbonate content up to 20 mol% the effect of drug–polymer interaction predominates resulting in the observed improved drug loading, while at 40 mol% carbonate content the effect of decreased copolymer solubility dominates contributing to the observed reduction in drug loading. As such, the PEG-b-P(CB-co-LA) 20 mol% carbonate series exhibit optimum drug loading. It is worth mentioning that we also synthesized PEG-b-Polycarbonate (PEG-b-PCB) using the same polymerization conditions for PEG-b-P (CB-co-PLA) copolymer. However, the solubility of this polymer in tetrahydrofuran (THF) and commonly used organic solvents was poor; making characterization using GPC and NMR difficult. From Table 2, drug solubility appears to correlate with the CMC. Among the series of copolymers, 0 mol% carbonate has the highest CMC value of 32.95 × 10⁻³ mol⁻¹ and the lowest drug loading of 1.0% ± 0.31, while the 20 mol% carbonate series has the lowest CMC value 3.13 × 10⁻³ mol⁻¹ and the highest drug loading 4.1% ± 0.23. The decrease in the CMC upon inclusion of the carbonate monomer clearly demonstrates that they have higher thermodynamic stability compared to PEG-b-PLLA. A high thermodynamic stability is desirable for in vivo stability of polymeric micellar systems. Sequence distribution of random copolymers can influence fundamental polymer properties such as solubility, mechanical and thermal properties. The observed decrease in L–L sequence and increase in C–C sequence as carbonate content increases can be used to elucidate the behavior of the PEG-b-P(CB-co-LA) copolymers. When the ratio of lactide to carbonate monomer is small, the lactide segments in the random copolymer are sparsely distributed and have a lower probability of interacting with the ethylene oxide segments since the lactide is buried in the carbonate segments hence preventing intermolecular interactions. This may explain the observed sharp decrease in lactide reactivity (from 66 to 24%). Conversely, at high lactide to carbonate ratios, lactide segments tend to undergo intramolecular transesterification and lead to smaller molecular weight polymers due to degradation. Hence, these two competing phenomena affect polymer molecular weight depending on which factor is more dominant and the optimum molecular weight is obtained when they are balanced. In the present study, the 20 mol% carbonate series exhibited optimum molecular weight which translated into superior drug loading and thermodynamic stability. Thermal analysis of the copolymers by differential scanning calorimetry confirmed the semicrystalline nature of PLLA. The two endotherm peaks observed for PLLA reflect the melting temperature \( T_m \). Also the exotherm peak occurring at 88.5 °C was also observed which may signify the onset of cold crystallization. More importantly, the data revealed that the introduction of the carbonate monomer into the poly (l-lactide) chain resulted in a reduction in crystallinity as demonstrated by the absence of the cold crystallization and melting peaks in the copolymers. These results suggest that the carbonate monomer randomly incorporated into the PLLA chain and resulted in a shortening of lactide sequences thereby hindering the crystallization process. Our data is consistent with the literature and other groups have reported similar trends [28,31]. In vitro release studies clearly showed the introduction of the carbonate monomer to result in better sustained release of bicalutamide from the micelle core compared to PEG-b-PLLA. The improved sustained release of the carbonate modified copolymers can be attributed to better compatibility between bicalutamide and the hydrophobic block of the copolymer as well as the they being more amorphous compared to PEG-b-PLLA. Both PEG-b-PLLA and PEG-b-P(CB-co-LA) copolymers exhibited fast release during the first 6 h. This burst release is assumed to be due to drug trapped with the PEG corona and at the corona-core interface. Increasing carbonate content from 10 to 40 mol% does not appear to alter the release profile of bicalutamide over the 150 h period studied. The release behavior of hydrophobic drugs from spherical micelles is known to depend on the properties of the hydrophobic core. The inhibitory effect of bicalutamide-loaded micelles formulated with PEG-b-P(CB-co-LA) copolymer on cell proliferation was comparable to free drug solubilized in methanol in LNCaP cells. This result suggests that bicalutamide-loaded micelles formulated with PEG-b-P(CB-co-LA) copolymer did not diminish the inhibitory effect of bicalutamide on cell proliferation since the PEG-b-P(CB-co-LA) copolymers themselves were not toxic in LNCaP cells. Although methanol is a toxic reagent, we do not anticipate its intrinsic toxicity to significantly alter our results since we used the equivalent of 15% v/v methanol and had appropriate controls. Moreover, we have previously used dimethyl sulfoxide (DMSO) which was found to kill tumor cells.

5. Conclusion

We have established that chemical tailoring of the PEG-b-PLLA micelle core through the introduction of carbonate moieties enhanced the solubilization of the highly lipophilic drug bicalutamide. This result was consistent with predictions made based on the Flory–Huggins interaction parameters computed for polymer/bicalutamide pairs using the group contribution method. Bicalutamide loading in PEG-b-P(CB-co-LA) micelles was four times better than in PEG-b-PLLA. PEG-b-P(CB-co-LA) copolymers were also shown to significantly solubilize bicalutamide as aqueous micellar solutions had drug concentrations approximately 800-fold higher than the saturated solubility of bicalutamide in water. In water, PEG-b-P(CB-co-LA) copolymers formed spherical micelles with CMC values up to 10 times lower than PEG-b-PLLA copolymers (i.e., 0.31 μM for 20 mol% carbonate and 3.3 μM for 0 mol% carbonate), indicating that introduction of carbonate monomer results in copolymers which are more thermodynamically favored for self-assembly and exhibit better thermodynamic stability. Finally, PEG-b-P(CB-co-LA) copolymers were found to result in slower bicalutamide release compared to PEG-b-PLLA copolymers. Even though the inhibitory effect of bicalutamide-loaded PEG-b-P(CB-co-LA) micelles on LNCaP cell proliferation was similar to free drug these results are quite significant since drug dissolved in methanol would not be suitable for clinical application. In conclusion, these studies highlight the effect of chemically modifying the PEG-b-PLLA micelle core with a carbonate monomer on drug delivery vehicles. In vivo testing of bicalutamide-loaded PEG-b-P(CB-co-LA) micelles for tumor treatment is ongoing and would be reported in a separate paper. 

Acknowledgement

The authors thank Davita Watkins for her help in obtaining 13C NMR and GPC spectra.

Appendix

Figure with essential color discrimination. Figs. 1–3, 5–7 in this article have parts that are difficult to interpret in black and white. The full colour images can be found in the on-line version, at doi:10.1016/j.biomaterials.2009.11.081.
References


