Preparation and Characterization of pH-responsive Polyurethane Micelles

Tao Zhai, Ya-yuan Guan, Jian-bin Luo

Abstract—A new type of pH-responsive multi-blocked polyurethane for drug delivery applications was prepared and characterized by GPC, 1H-NMR and FT-IR. Then, polyurethane micelles was prepared by dialysis method, and the critical micelle concentration was 2.5×10³ g/L as measured by fluorescence technique. TEM and DLS analyses showed that the size of spherical micelles increased from 89.2nm at physiological pH 7.4 to 320 nm at an acidic environment, i.e. pH 5.5, the pH value of intracellular microenvironment of cancer cells. Finally, a hydrophobic anti-cancer drug methotrexate (MTX) was used as a model drug to study the drug release profiles of the drug loaded micelles at neutral and acid environment. The results showed that the drug release is much faster at pH 5.5 than that at pH 7.4. Therefore, the synthesized polyurethanes are promising to be used as pH-responsive drug carriers.

Index Terms—pH-responsive; polyurethane; micelles; drug delivery

I. INTRODUCTION

In the past decades, various chemotherapeutic drugs have been discovered for the treatment of cancer. However, use of these drugs is often associated with numerous problems including patient toxicity and poor tumor delivery. To address these problems, various drug carriers have been developed to propose new approaches for intracellular delivery of anticancer drugs [1]. One of the most widely studied drug delivery systems is the self-assembly amphiphilic blocked copolymer micelles due to their unique features, such as enhanced solubility of drugs, high loading capacity and reduced systemic adverse effects, and high tunability of chemical and physical characteristics [2-5]. Despite these advantages of polymeric micelles, insufficient drug release at tumor sites and leakage in healthy tissues decrease the therapeutic efficacy as well as cause negative side effects. To improve the anticancer efficacy, rapid drug release upon reaching the tumor site is expected. Stimuli responsive micelles, which response to external triggers, such as temperature, light, pH, oxidative, reductive and enzymatic have been synthesized and investigated in detail to meet the requirement of rapid release[8-17]. These micelles can stably encapsulate hydrophobic drugs and release them at the target site triggered by external stimuli. In particular, pH-responsive amphiphilic copolymers and their nanosized assemblies are of great interests in recent years due to the difference of pH value between the tumor microenvironment and the healthy tissue [18, 19]. The pH of the extra cellular matrix is slightly more acidic (pH<7.0) than that of normal tissues [20, 21]. The high metabolic rate of tumor cells leading to excess lactic acid and hydrolysis of ATP which results in lower pH at the tumor microenvironment. The pH-responsive micelles may dissociate and facilitate the drug release at more acidic tumor site. For example, copolymers with imidazole rings or tertiary amines with pKa values about 6.5 triggered by acidic endosomal pH conditions, leading to enhanced drug efficiency due to improved intracellular drug dose[22].

Polyurethane have been widely developed for biomedical applications, especially for tissue engineering and drug delivery[23-28], due to their good biocompatibility, attractive physical properties and the flexibility of molecular structure design which facilitate the introduction of various functional moieties into polyurethane[29-31]. The biodegradable polyurethane can be obtained by incorporation of biodegradable segments into either the hard segments or the soft segments of the polyurethane backbones [32-35]. Recently, stimuli-responsive polyurethanes for drug delivery system have gained increasing attention. In this research, we report a new kind of pH-responsive multiblocked polyurethane (PEG-MDEA-PCL) which is easily prepared by a facile one-pot approach by incorporation of tertiary amino groups into the polyurethane backbone. The polyurethane were synthesized by using poly(ethylene glycol) (PEG) and poly(ε-caprolactone) (PCL) as the soft segment, lysine diisocyanate (LDI) and N-methylidiethanolamine (MDEA) as hard segment. As is shown in Fig. 1, the polyurethane

![Fig. 1. Schematic illustration of MTX-loaded micelles dissociation and release of MTX under acid pH](image-url)
block copolymers self-assembled into stable micelles in aqueous medium at pH 7.4, which, however, disassociated and released the cargos at acid environment due to the protonation of the tertiary amines of MDEA. It is well known that PEG and PCL have been approved as biodegradable and biocompatible materials by US Food and Drug Administration (FDA), and they have been widely applied as biomedical materials. Lysine based diisocyanates (LDI) were used as diisocyanates to prepare the biodegradable polyurethane due to its nontoxicity which facilitate the clearance of the carriers. The hydrophilic PEG corona of the micelles provides an impermeable shell for enzymes and proteins to avoid recognition of the micelle by the reticuloendothelial system (RES) [6]. The small size of the micelles can also preferentially accumulate at the tumor site via the enhanced permeation and retention (EPR) effect [7]. In vitro study demonstrated that the size of the micelles significantly increased from 89.2 nm under physiological pH 7.4 to 320 nm at acidic pH 5.5. Methotrexte (MTX) was used as a model drug to test the responsiveness of PEG-MDEA-PCL micelles. 

II. EXPERIMENTAL

A. Materials

N, N-dimethyl formamide (DMF) was dried over CaO for 2 days at room temperature and vacuum distilled before use. Poly (ethylene glycol) (Mn=1000) and poly ε-caprolactone diol (Mn=2000) were dehydrated under reduced pressure at 100°C for 2h before use. LDI was obtained in our laboratory according to the method described by Nowick et al [36]. Methotrexte (MTX) was purchased from Asta Tech (98%, China). All other chemicals were reagent grade and used as received.

B. Synthesis of polyurethane PEG-MDEA-PCL

PEG-MDEA-PCL was synthesized by a typical two step polymerization procedure as is shown in Fig. 2 and their feed ratios are listed in Table 1. Briefly, anhydrous PEG and PCL were added to a three-neck flask, then LDI and 0.1 wt% Ditin n-butyl dilaurate (DBTDL) was added to the flask. The reaction mixture was stirred at 80°C for 1h before adding 20mL dry DMF to dissolve the prepolymer. Subsequently the chain extender MDEA was added and reacted at 80°C for another 15h. Finally, the reaction mixture was poured into water under stirring to extract unreacted reactants. The resulting precipitate was dissolved in DMF and poured into water and the procedure was repeat another two times to further extract the unreacted molecular. The purified precipitates were dried under vacuum at 80°C for 48hours, with a yield of about 75%.

Fig. 2. Synthetic route of PEG-MDEA-PCL

C. Measurements

1HNMR spectrum was recorded on an Agilent 400 MHz spectrometer using deuterated chloroform (CDCl3) as the solvent. Fourier transform infrared (FTIR) spectra were performed at room temperature on a Nicolet IR200 Fourier-transform infrared spectrometer (Thermo Electron Corporation). The samples for infrared analysis were prepared by casting 2% (wt/v) polymer solution in THF directly onto KBr plates and dried in oven at 70°C for 24h before characterization. Molecular weights were measured by gel permeation chromatography (GPC). GPC was performed on a PL-GPC 220 (Polymer Laboratories, UK) gel permeation chromatograph using N,N-dimethylformamide (DMF) as eluent at a flow rate of 1ml min−1 at 60°C. Ultraviolet spectrophotometric (UV) analysis was performed using UV-1800PC spectrophotometer. Transmission electron microscopy (TEM) studies were performed with a JEOL JEM-100CX microscope (Japan), operated at an accelerating voltage of 80 kv. The samples for TEM imaging was negatively stained by adding a droplet of 1% (w/v) phosphotungstic acid on carbon-coated copper grids and dried in the air before TEM observation. Dynamic light scattering (DLS) measurements were taken in aqueous solution using a Malvern Mastersizer 2000 particle size analyzer (Malvern ZEN3690, UK). Critical micelle concentrations (CMCs) of the copolymers were determined by fluorescence measurements using pyrene as a hydrophobic probe. Steady-state fluorescence spectra were performed using a 970 CRT fluorescence spectrophotometer (Shanghai precision & scientific instrument Co. Ltd., China) at an emission spectra of 372 nm.

D. Preparation of Self-Assembled micelles.

Polymer micelles were prepared by dialysis method. A total of 10mg PEG-MDEA-PCL was dissolved in 2mL of DMF with stirring at room temperature for 1h. Then the polymer solution was added dropwise into 5mL of deionized water under vigorous stirring for another 1h, then the solution was transferred into a dialysis bag (MWCO8000-14000) and dialyzed against deionized water for 24h. During the dialyzing process, the water was exchanged frequently.

E. pH-responsiveness of PEG-MDEA-PCL micelles

HCl was added into the micelle solutions to mimic the weak acidic (pH 5.5) within tumor tissue, the pH values were checked using a PHS-3C pH meter (Shanghai precision & scientific instrument Co. Ltd., China). The particle size and
distribution in weak acidic pH 5.5 and pH 7.4 were recorded using DLS measurement.

F. Preparation of MTX-Loaded micelles.

In brief, 10mg PEG-MDEA-PCL was dissolved in 2mL of DMF, followed by adding 2mg MTX under stirring at room temperature for 1h. The mixture was then added dropwise to 5mL of deionized water and stirred for another 1h. Subsequently, the mixture was dialyzed against deionized water for 24h (MWCO 8000–14000). To determine the drug loading content (DLC), the MTX-loaded micelle solution was lyophilized and dissolved in DMF again. The UV absorbance of the solution at 303nm was measured to determine the total loading of MTX.

Drug loading content (DLC) and drug loading efficiency (DLE) of PEG-MDEA-PCL were calculated according to the following formula:

\[
\text{DLC (wt %)} = \left( \frac{\text{weight of loaded drug}}{\text{weight of polymer}} \right) \times 100\%
\]

\[
\text{DLE (wt %)} = \left( \frac{\text{weight of loaded drug}}{\text{weight of drug in feed}} \right) \times 100\%
\]

G. pH-Triggered Drug Release

The in vitro release profiles of MTX from PEG-MDEA-PCL micelles were studied by dialyzing the drug-loaded micelles under different pH values. Typically, Lyophilized MTX-loaded nanoparticles (1mg/mL) were transferred into a membrane tubing (MWCO 8000–14000), then they were immersed into a glass bottle containing 20ml of PBS (50mM) with a pH value of either 5.5 or 7.4 at 37°C under stirring. At predetermined time intervals, 6ml of external buffer solution was removed for UV measurement, and replaced with 6ml of fresh PBS with the same pH value. MTX concentration was calculated based on the absorbance of 303nm using UV analysis. The release experiments were conducted in triplicate.

III. RESULTS AND DISCUSSION

Amphiphilic polyurethane PEG-MDEA-PCL was synthesized with PEG, PCL as the soft segment and LDI, MDEA as the hard segment. The chemical structure of the resulting products was first characterized by a 1HNMR spectrum. As is shown in Fig.3. The peaks at 4.39(-CH), 4.19(-CH2OCO), 3.15(O=C-NH-CH2), 1.51(-CH2CH2-) were the characteristic chemical shifts corresponding to LDI units, peaks at 1.26 ppm are ascribed to the -CH2CH2-CH2- and CH3 of LDI. The peaks at 4.06 (OCH2CH2O), 3.88 (OCH2CH2O), 2.31(-CH2COO), 1.65(OCH2CH2CH2-CH2) belong to the characteristic chemical shifts corresponding to PCL segment; the peaks appeared at 2.14 (CH3), 2.94(N-CH3), 4.29(N-CH2CH3) indicated the presence of the MDEA units in the resulting copolymers. The GPC profile of PEG-MDEA-PCL is shown in Fig.4. The samples showed moderate molecular weights (Mn=25697) and narrow molecular weight distribution (PDI=1.65).

The chemical structures of synthesized LDI and PEG-MDEA-PCL were also examined by FTIR, as shown in Fig. 5. The absorbance at 2265 cm⁻¹ assigned to the group of N=C=O in LDI. FTIR spectra of PEG-MDEA-PCL showed that the absorption bands at about 2200cm⁻¹ corresponding to N=C=O stretching vibration absolutely disappeared, and around 3400cm⁻¹ bands attributed to N-H vibration appeared, indicating that isocyanate groups of the LDI had been completely reacted with hydroxyl groups and formed urethane and urea groups. The C-O-C stretching vibration of the repeated unit OCH2CH2 of PEG was found at 1103cm⁻¹, while the stretching band at 1734 and 1653cm⁻¹ were assigned to the absorption of eeter carbonyl groups of PCL, free and hydrogen-bonded carbonyl of urethane groups. The band at 1653cm⁻¹ was ascribed to the hydrogen-bonded carbonyl of urea groups. The absorption peaks at 2940 and 2861cm⁻¹ were attributed to the stretching vibration of methyl and methylene, respectively. The peak at 1240 was corresponding to C-O-C symmetric. The peak at 1080cm⁻¹ was corresponding to stretching vibration of the repeated unit OCH2CH2 of PEG and C-O-C asymmetric stretching vibration of ester, respectively. FTIR results confirm the successful synthesis of the amphiphilic copolymer.
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The multiblock amphiphilic PEG-MDEA-PCL provide an opportunity to self-assemble into nanometer aggregates in aqueous solution. After dissolved in DMF and dialyzed against water, PEG-MDEA-PCL was able to form a core/shell structure in water. The shell of the micelles comprises the hydrophilic PEG and the inner core of the micelles consists of the hydrophobic PCL. The formation of PEG-MDEA-PCL micelles was first proved by fluorescence technique with pyrene as a probe. The critical micelle concentration (CMC) was determined with pyrene as a fluorescence probe. The total fluorescent intensity increased with an increasing concentration of pyrene in aqueous solution of pyrene. A red shift from 333 to 338nm was observed with the concentration of block copolymer varied from 1x10^{-6} to 0.18g/L, suggesting the pyrene molecules were transferred from the water into the hydrophobic core and micelles formed. To obtain the CMC of polyurethane micelles, the intensity ratios of I_{338}/I_{333} from the excitation spectra of pyrene were plotted against the log of polyurethane concentrations as shown in Fig.6. The CMC is obtained from the intersection of the two tangent lines shown by the arrows. The CMC is 2.5x10^{-4} g/L.

Fig. 5. FTIR spectra of LDI and PEG-MDEA-PCL

The size of micelles should be considered while preparing drug delivery vehicles. Micelles with a diameter less than 200nm are less susceptible to RES clearance, which can also preferentially accumulate in solid tumors via the enhanced permeation and retention (EPR) effect and are able to avoid renal clearance. In the study, different polyurethanes were synthesized with three different feed ratios of PEG and PCL, their feed ration, size and size distribution are listed in Table 1, the size and size distribution of PEG-MDEA-PCL micelles were determined by DLS measurement. As shown in Fig.7, with the increase of PEG content, the micelle size was increased and exhibit wider distributions, the reason is that the higher PEG content tend to exhibit high PEG density on the micelle shells, which significantly influence the aggregation to form large secondary clusters, leading to increased average micelle size and further broadening the overall distribution of the micelles. Size and size distributions is an important issue in drug delivery vehicles. Those micelles with smaller size are less susceptible to RES clearance, and size distribution of micelles should be uniform as much as possible to gain more power on the control of injection and disposal of drug vehicles, thus considering the size and size distribution, PEG-MDEA-PCL1 micelle with smaller size and size distribution was chosen for further study, and the polyurethane in the study is denoted as PEG-MDEA-PCL. DLS measurements showed that PEG-MDEA-PCL formed micelles with average sizes of 89.2 nm. The size and morphology of PEG-MDEA-PCL were also measured and TEM. TEM micrograph revealed that these micelles had a spherical morphology with nanoscale sizes of 70 nm. The size observed by TEM was smaller than that measured by DLS due to micelles dehydration caused by water evaporation under high vacuum during TEM imaging. These results indicate that the size of PEG-MDEA-PCL micelles is suitable for intracellular drug delivery.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Feed molar ration</th>
<th>Micelle Size (nm)</th>
<th>PDI</th>
</tr>
</thead>
<tbody>
<tr>
<td>PEG-MDEA-PCL1</td>
<td>0.3:0.7</td>
<td>89.2</td>
<td>0.136</td>
</tr>
<tr>
<td>PEG-MDEA-PCL2</td>
<td>0.5:0.5</td>
<td>124.1</td>
<td>0.250</td>
</tr>
<tr>
<td>PEG-MDEA-PCL3</td>
<td>0.7:0.3</td>
<td>156.3</td>
<td>0.321</td>
</tr>
</tbody>
</table>

Table 1. Size and size distribution (PDI) of micelles with different PEG in feed

Fig. 6. The critical micelle concentration of PEG-MDEA-PCL

Fig. 7. DLS curves of micelles with different molar ration of PEG and PCL
To prove the pH-sensitive property, DLS measurement was used to determine the micelles sizes change in responsive to tumor acidic pH 5.5 and normal cell pH 7.4. As shown in Fig. 9, the micelle increased significantly from 89.2 to 320 nm and exhibit wider distributions with decreasing pH from 7.4 to 5.5. The size and morphology of the nanoparticles at neutral and acid environment, i.e. pH 7.4 and pH 5.5, were observed by TEM and the corresponding images were shown in Fig. 10. As is shown in Fig 10, polymer micelles showed a spherical morphology with a size about 70 nm in the neutral environment. However, the micelles showed an enlarged sized and irregular mophorlogy in the acid solutions, indicating protonation of the ternary amino in MDEA segments in the acid environment which inturn cause the swollen or even dissolving of the PU micelles.

To investigate the drug loading and release properties of the pH-sensitive micelles based on amphiphilic polyurethane PEG-MDEA-PCL, methotrexate (MTX) was used as a model hydrophobic drug. MTX is an antineoplastic agent for osteosarcoma, lung and breast cancers [40]. The loading content and loading efficiency of MTX into micelles are 11.7% and 42.5%, respectively. After the loading of the drug, the average size of PCL-SS-MPEG was 92.1nm measured by DLS as is shown in Fig.8. Compared with the blank micelles size of 89.2 nm, the size change of drug-loading micelle was negligible.

It was interested to knowe whether the MTX relea release is facilitated in response to acidic pH. The release curves of MTX are displayed in Fig. 11. The results indicate that the total amount of drug release from the MTX-loaded PEG-MDEA-PCL micelles is 32.5% in PBS (50 mM, pH 7.4) after 24 h, and the MTX release is relatively slow under physiological conditions. Noticeably, as in acid environment (PBS, 50 mM, pH 5.5), the MTX release was obviously facilitated, and the amount of MTX released from micelles reached to 53.0% after 24 h. The pH-sensitive drug release phenomenon can be well explained based on the structural transitions of the PEG-MDEA-PCL micelles: acidic pH results in swelling of the drug-loaded micelles because of the protonation of the teriary amino groups in MDEA segments. The drug release in vitro indicates that the polyurethane PEG-MDEA-PCL micelles can response to lower pH and thus facilitate the MTX release.

To summarize, a new kind of novel multi-block polyurethane copolymer PEG-MDEA-PCL with pH responsiveness was successfully prepared. By varying the ratio of the PEG and PCL in the polymer, the size of the micelle changed. The polyurethane micelle can self-assembly into nanosize micelles in water media. The drug release of the micelle can be facilitated when triggered by acidic pH, which show promise as biomaterials for controlled drug release.

Fig. 8. DLS curves of micelles and micelles with drugs

Fig. 9. DLS curves of micelles under different pH

Fig. 10. TEM images of micelle under pH7.4 (A) and pH5.5 (B)

Fig. 11. In vitro release of MTX from micelles in PBS (50mM, pH 7.4) at 37 ℃ under different pH

IV. CONCLUSION

In summary, a new kind of novel multi-block polyurethane copolymer PEG-MDEA-PCL with pH responsiveness was successfully prepared. By varying the ratio of the PEG and PCL in the polymer, the size of the micelle changed. The polyurethane micelle can self-assembly into nanosize micelles in water media. The drug release of the micelle can be facilitated when triggered by acidic pH, which show promise as biomaterials for controlled drug release.

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