Macromolecules

Volume 42, Number 4 February 24, 2009

Communications to the Editor

Synthesis of PEG-Armed and Polyphosphoester Core-Cross-Linked Nanogel by One-Step Ring-Opening Polymerization

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Received December 1, 2008
Revised Manuscript Received January 4, 2009

Polymeric nanogels are cross-linked polymeric nanoparticles possessing high water content in aqueous environment.[1−3] Water-soluble drug molecules including anticancer drugs (e.g., doxorubicin hydrochloride) and bioactive molecules (e.g., antisense oligomer, protein) can be incorporated into the nanogel network, rendering the nanogel potential as drug carrier for biomedical applications.[4−8] Ideal nanogels as nanocarriers for drug delivery should be efficient and convenient for loading drug molecules. The nanogel materials alone are also expected to be biocompatible, and they should be available with simple synthesis. On the other hand, for systemic drug administration, PEGylated nanoparticles are known to extend their blood circulation,[9,10] and in this context, nanogels with PEG arms will be advantageous in drug delivery.

Various synthetic strategies for nanogel preparation have been reported.[3] Typically, nanogels can be synthesized by radical heterogeneous polymerization in an inverse mini- or micro-emulsion.[11−13] They can also be prepared by a process of precipitation polymerization,[14,15] or by cross-linking biopolymers,[16,17] or by supramolecular assembly,[18,19] and so on. The preparation and development of nanogels for drug delivery have been detailed reviewed recently.[1−3]

In this Communication, we report the convenient synthesis of PEG-armed nanogels with cross-linked biodegradable polyphosphoester core via one-step ring-opening polymerization. We took the advantage of a method of synthesizing core-cross-linked star polymers, called "arm first" procedure.[20−23] Such a procedure in general uses a living linear polymer (arm) to initiate the polymerization of a difunctional or multifunctional monomer.[23] The polymerization used in such a procedure can be through atom transfer radical polymerization,[20,24] group transfer polymerization,[21,25] or ring-opening polymerization.[22,23] As shown in Scheme 1, on the basis of well-established method for ring-opening polymerization of five-membered cyclic phosphate monomer under initiation of $Sn(Oct)_2$ and alcohol,[26] we used poly(ethylene glycol) monomethyl ether (M_w = 5000, $mPEG_{5000}$) as the arm to polymerize a difunctional phosphate monomer, namely 3,6-dioxaoctan-1,8-diyl bis(ethylene phosphate) (TEGDP), to obtain the core-cross-linked star polymer, which substantially swelled in aqueous solution to form PEG-armed nanogels. This synthesis procedure is surfactant-free, and the core material is constituted of polyphosphoester, which has been demonstrated to be biodegradable[27,28] and used widely in drug delivery and tissue engineering applications.[29−31] The obtained PEGylated nanogels showed convenient and high capacity of doxorubicin hydrochloride loading, while release of doxorubicin from the nanogels was found to be accelerated in the presence of phosphodiesterase I, which is present in many mammals' cells and known to catalyze the hydrolysis of phosphoester linkages.[32]

TEGDP monomer was synthesized using a method similar to the synthesis of ethylethylene phosphate (2-ethoxy-2-oxo-1,3,2-dioxaphospholane, EEP), as previously reported.[26] The synthesis procedure and characterization are described in detail in the Supporting Information (Figure S1). As reported, core-cross-linked star polymers can be prepared by arm-first, core-first, and in−out procedures.[23,33,34] In this study, to obtain the core-cross-linked star polymer consisting of PEG arms and cross-linked polyphosphoester core, we mixed $mPEG_{5000}$, $Sn(Oct)_2$, and TEGDP at a molar ratio of 1:0.5:10 in dioxane and performed the polymerization at 80 °C for 12 h (Scheme 1). Our previous kinetic studies on the ring-opening polymer-

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10.1021/ma802688y CCC: $40.75
Published on Web 01/21/2009

ization of EEP in solution with co-initiation of stannous octoate and dodecanol suggest the formation of active center of stannous alkoxide.[26] Therefore, it is believed that an active stannous alkoxide center formed at the end of $mPEG_{5000}$ molecule in the above mixture, which further initiated the polymerization of TEGDP. A similar procedure has been applied by Wiltshire et al. for the synthesis of poly(ε-caprolactone) armed and degradable polyester core-cross-linked star polymers.[22]

It is noteworthy that in this study no macrogelation took place throughout the synthesis procedure when the monomer concentration in dioxane was 1.0 M. The conversion of TEGDP monomer was monitored by HPLC according to a method similar to that for EEP conversion determination in our previous study.[26] It shows in Figure S2 (Supporting Information) that after 6 h reaction 93.5% of TEGDP monomer (with an elution volume at 28.2 mL) have been consumed. Extending the reaction time to 12 h resulted in 98.9% TEGDP monomer conversion. It also shows a fraction of oligomers with M_n slightly higher than $mPEG_{5000}$, which is most likely a linear block copolymer of $mPEG_{5000}$ with short polyphosphoester chain. At the same time, it must be mentioned that nanogels have been filtered off using 100 nm membranes before the measurements. To remove the fraction of oligomers and unreacted monomer, the reaction mixture was concentrated, resuspended in water, and dialyzed (Spectra/Por, MWCO = 15 000) against Milli-Q water for 72 h. The nanogel was obtained by lyophilization.

Elemental analyses of the obtained nanogel revealed that the contents of C, H, and P were 44.5%, 7.82%, and 4.21%, respectively. Based on these results, the calculated molar ratio of transformed $mPEG_{5000}$ to TEGDP was 1.00:5.01. The chemical structure was further analyzed by NMR measurements. As shown in Figure 1A, the ^{1}H NMR spectrum of the nanogel showed resonances at 4.10−4.40 ppm (a and b), which are the characteristic signals of methylene protons conjunct to the phosphoester linkages (−POCH_2CH_2O−, −P−OCHH_2CH_2−OCH_2−). Resonance at 3.50−3.80 ppm (d + e + f) is assigned to PEG methylene protons and protons of the triethylene glycolyl group not conjunct to phosphoester linkages (−OCH_2CH_2O−, −P−OCHH_2CH_2−OCHH_2CH_2−OCHH_2CH$_2$O−P−). The resonance at 3.38 ppm (g) is due to protons of the end methoxyl group of $mPEG_{5000}$ (−OCH$_2$CH$_2$OCH_3). It must be noted that signals from the protons of polyphosphoester core are significantly suppressed in its ^{1}H NMR spectrum. Based on the integration of resonances at 4.10−4.40 and 3.50−3.80 ppm, the molar ratio of transformed $mPEG_{5000}$ to TEGDP is only 1.00:2.37, which is significantly lower than that calculated from the elemental analyses. It is possible that highly cross-linked polyphosphoester core has less mobility, leading to decreased and broaden NMR signals. A similar phenomenon has also been observed when characterizing core-cross-linked miktoarm star copolymers by Matyjaszewski et al.[35] Figure 1B shows the ^{13}C NMR spectrum of the nanogel, which is in agreement with the chemical structure. The ^{31}P NMR spectrum of the nanogel shown in Figure 1C also demonstrated the polymerization of TEGDP monomer, showing a strong resonance at −5.30 ppm,

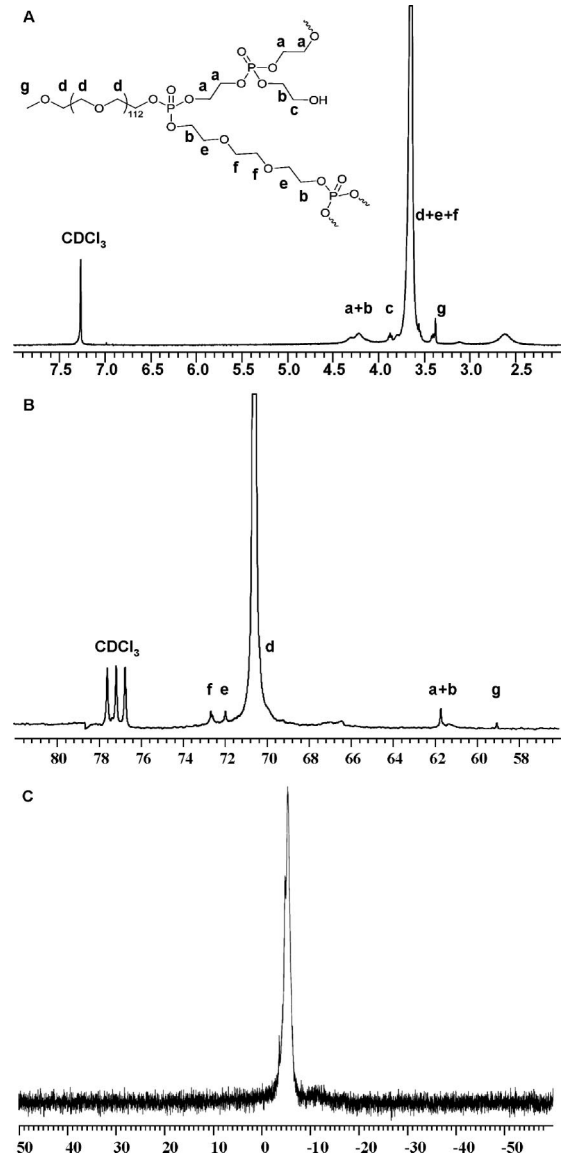
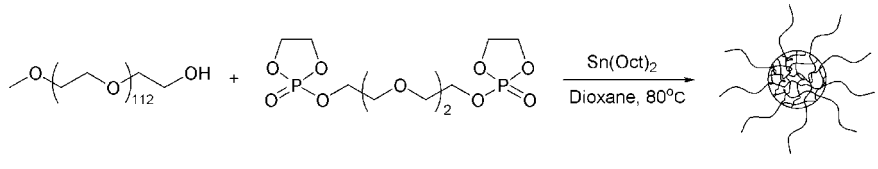


Figure 1. ^{1}H (A), ^{13}C (B), and ^{31}P NMR (C) spectra of the nanogel in $CDCl_3$ (ppm).

instead of a resonance at 13.84 ppm observed in the monomer's spectrum.

Transmission electron microscopy (TEM) observation showed that nanogel took a spherical morphology with a clear core−shell structure with an average diameter around 220 nm (Figure 2A). However, the distribution of nanogels were around 140−460 nm with an average diameter of 255 nm when measured by dynamic light scattering in Milli-Q water (Figure 2B). The smaller value from TEM observation should be due to the dehydration of nanogel under TEM measurement. On the other hand, the nanogel in water is slightly larger and broader distributed than that measured in dioxane (Figure S3, Supporting Information). The ring-opening polymerization of TEGDP may

Scheme 1. Synthesis Scheme of PEG-Armed Polyphosphoester Core-Cross-Linked Nanogel



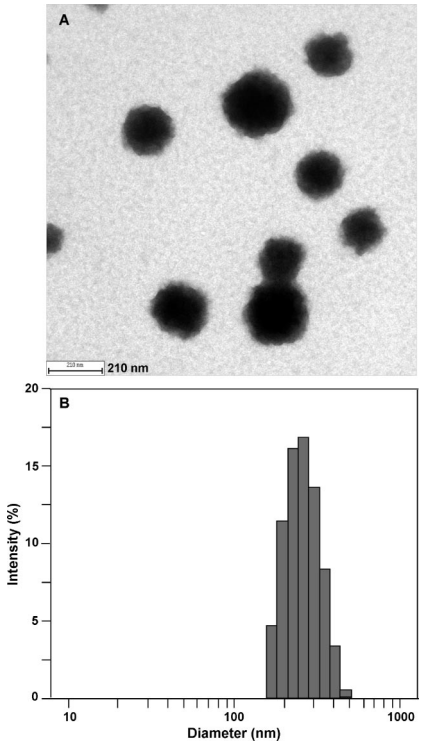


Figure 2. TEM image (A) and the size and size distribution of the nanogels in water measured by dynamic light scattering carried out on a Malvern Zetasizer Nano ZS90 (B).

Table 1. Drug Loading Efficiency (DLE) and Drug Loading Content (DLC) of the Nanogel

entry	feed weight ratio of DOX to nanogel	DLE (%)	DLC (%)
1	0.10:1	91.00 ± 5.82	9.10 ± 0.59
2	0.50:1	57.79 ± 5.13	28.90 ± 2.60

lead to a relatively broad distribution of polyphosphoester and uneven core-cross-linking density of the nanogel; thus, the broader distribution of nanogel in water may be attributed to swelling of the nanogel under stronger solvation by water than by dioxane. To further determine whether the polyphosphoester core is hydrophilic, we measured the fluorescence spectra of pyrene probe in the presence of different concentrations of the nanogel. Pyrene inserts into the hydrophobic domain and shows a significant red shift in its excitation spectra with increased hydrophobic materials.[36] In this study, no nanogel concentration-dependent red shift was observed in the excitation spectra of pyrene, as shown in Figure S4 of the Supporting Information. This result is similar to the observation with chitosan-based nanogels,[16] which infers the hydrophilic core of polyphosphoester of the nanogel.

Many nanogels such as PEG-based and chitosan-based nanogels have been used as drug carriers to load doxorubicin hydrochloride (DOX),[13,16] one of the most commonly used chemotherapeutic agents with side effects such as myelosuppression and cardiotoxicity.[37,38] It has been shown that the virus-mimetic nanogel exhibited as high as 91.6% DOX loading efficiency.[5] To test the potential of the PEG-armed polyphosphoester core-cross-linked nanogel for drug delivery, we incubated the nanogel with DOX at various weight ratios and determined the free DOX not loaded into the nanogel to calculate the drug loading efficiency (DLE) and drug loading content (DLC). As shown in Table 1, the DLE of the nanogel reached 91.00% when the initial ratio of DOX to nanogel was

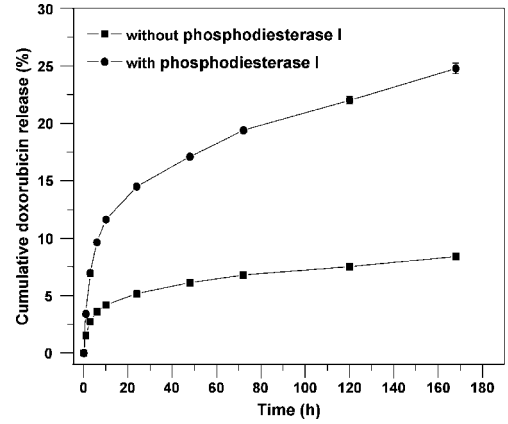


Figure 3. Time-dependent doxorubicin release from drug-loaded nanogel with and without phosphodiesterase I.

0.1:1 (w/w). Increasing the feed weight ratio of DOX to nanogel to 0.5:1 resulted in a lower drug loading efficiency, but the DLC increased to 28.90%, as summarized in Table 1.

The release of DOX from the nanogel was determined by incubation of drug-loaded nanogel (with 9.10% drug loading) in phosphate buffered saline (0.02 M, pH 7.4). The release profile was compared with that of nanogel incubated with the enzyme phosphodiesterase I (5 units L^{-1}). This enzyme is known to catalyze the degradation of polyphosphoester in aqueous solution.[27] As shown in Figure 3, in the absence of phosphodiesterase I, following the 4% burst release of doxorubicin in 10 h, only another 4.4% of total DOX release was observed in the rest tested 158 h. However, in the presence of phosphodiesterase I, DOX release was accelerated, showing around 12% of total DOX release in the first 10 h followed by continuous DOX release. The cumulative DOX release reached 24.8% of total DOX amount in 168 h. In the case of systemic administration of a nano drug delivery system for cancer therapy, it is expected that there is minimal drug leakage from the carrier during the circulation in blood, but drug molecules should be released and function in the tumor tissue or tumor cells. Therefore, this nanogel with less DOX release in normal phosphate buffered saline might be advantageous in systemic administration, since the drug release can be accelerated in cells if considering the presence of phosphodiesterase I enzyme in mammalian cells.[32]

To evaluate the biocompatibility of the nanogel, the in vitro cytotoxicity to A549 cells was determined by MTT and live/dead staining assays. As shown in Figure 4, at all tested concentrations up to 10 mg mL^{-1}, the viabilities of the A549 cells were close to 100% after 72 h incubation, while cells did not tolerate the treatment with SDS when the dose is above 0.1 mg mL^{-1}. Correspondingly, live/dead staining results also demonstrate the cell compatibility of the nanogel. Only a very few A549 cells incubated with the nanogel (10 mg mL^{-1}) for 72 h exhibited red fluorescence after both "live" staining with 1 μM calcein-AM and "dead" staining with 4 μM EthD-1, indicating that most of the cells were viable.

In conclusion, we have synthesized PEG-armed polyphosphoester core-cross-linked nanogel by one-step ring-opening polymerization, which can be used as drug carriers. The nanogel is biocompatible to cells as evaluated by MTT and live/dead assays. The nanogel shows a core−shell structure with average diameter of 255 nm and exhibited efficient and convenient doxorubicin drug loading. The kinetics of doxorubicin release from the nanogel indicated that drug release is accelerated in the presence of phosphodiesterase I, likely due to the catalyzed degradation of polyphosphoester core of the nanogel, while the

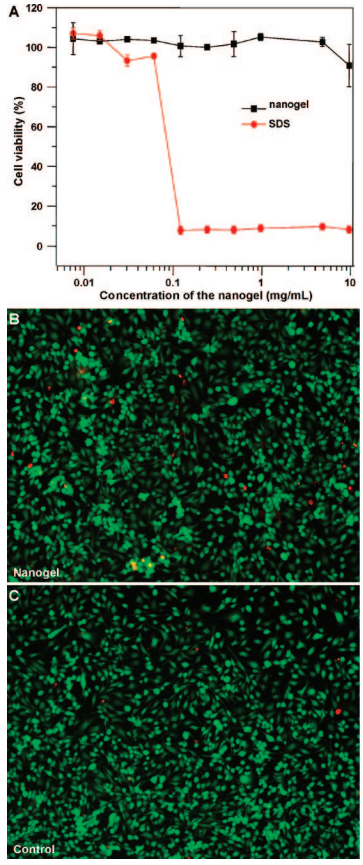


Figure 4. Cell viability of A549 cells incubated with the nanogel or SDS for 72 h (A). Overlaid fluorescence images of A549 cells after 72 h incubation with (B) or without (C) 10 mg mL^{-1} of the nanogel.

drug release is relatively retarded in the absence of the enzyme, rendering the nanogel potential as the drug carrier for systemic drug delivery.

Acknowledgment. This work was supported by grants from the National Natural Science Foundation of China (50733003), the Ministry of Sciences and Technology of the People's Republic of China (2006CB933300, 2009CB930300), and "Bairen" Program of Chinese Academy of Sciences.

Supporting Information Available: Experimental, characterization of monomer, HPLC determined monomer conversion, distribution of nanogel in dioxane. This material is available free of charge via the Internet at http://pubs.acs.org.

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MA802688Y