Biodegradable Copolymer for Stimuli-Responsive Sustained Release of Doxorubicin

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Supporting Information

ABSTRACT: Pendent functionalization of biodegradable polymers provides unique importance in biological applications. In this work, we have synthesized a polymeric nanocarrier for the controlled release of the anticancer drug doxorubicin (DOXI). Inspired by the pH responsiveness of acylhydrazine bonds along with the interesting self-assembly behavior of amphiphilic copolymers, this report delineates the development of a PEG-SS-PCL-DOXI copolymer consisting of DOXI, PEG, and a caprolactone backbone. First, the inclusion of a PEG moiety in the copolymer helps to achieve biocompatibility and aqueous solubility as well as a prolonged circulation time of the nanocarrier. Second, an acid-sensitive acylhydrazine-based linkage is chosen to attach DOXI to trigger sustained drug release, whereas the inclusion of an enzymatically cleavable disulfide linkage in the backbone adds to the advantage of backbone biodegradability at the intracellular level.

INTRODUCTION

Polymeric nanoparticles show an enhanced permeability and retention (EPR) effect. 1 5 6 Because of this, their physical and chemical properties can be tuned so as to achieve the aspects of a drug carrier. In the field of drug delivery, the most commonly used biodegradable polymers are poly ε-caprolactone (PCL), poly(alkylcyanoacrylate) (PACA), poly(lactic acid) (PLA), poly(lactide-co-glycolide) (PGLA), and their copolymers. 5 7 8 Biodegradable polymers, because of their degradability into small molecules, are superior in biomedical applications as they are readily metabolized and can be excreted from the body. Besides, the presence of PEG chains in the copolymer helps reducing opsonization and slows the clearance by the immune system. 9 10 The ring-opening polymerization (ROP) of lactones is an emerging synthetic technique because of its versatility to produce a wide range of biomedical polymers in a controlled manner. 11 13 16 However, pendent functionalizations of polycaprolactones are still a promising area of research because of their potential synthetic challenges. 14 16

A polymer-based “prodrug” approach is a robust technique of drug modification, 18 19 for example, elimination of the burst mechanism in drug release and tuning of drug pharmacokinetics. Several doxorubicin (DOXI)-based prodrugs have been synthesized and evaluated. 17 19 The primary amino and keto groups of DOXI are used for covalent attachment to the polymer backbone. Now, there are many ways by which the drug can be covalently attached to the copolymer via hydrolytically labile bonds such as imines, 20 acetics, 21 oximes, 22 24 orthoesters, 25 and acylhydrazines. 23 24 26 Among these linkers, we are especially fascinated by acylhydrazine linkers for their subtle response in drug delivery. 5 17 23 25 28 37 The drugs attached to the acylhydrazine linkers are released rapidly under the acidic conditions as compared to the physiological media (pH 7.4). In addition to pH responsiveness, the reductive nature of disulfide bonds in polymer prodrugs has also been reported. 29 32 The disulfide bond, being stable under physiological conditions, gets reduced to the corresponding alcohol in the intracellular region because of the presence of a high concentration of glutathione (GSH, 10 mM). 33 36

Herein, we have designed a system that responds to both pH and enzymes for a better therapeutic efficiency. First, we have synthesized and thoroughly characterized the newly designed copolymer PEG-SS-PCL-DOXI. The presence of amphiphilicity in the design induces self-assembly to generate nanoaggregates. Electron microscopy (scanning electron microscopy [SEM] and transmission electron microscopy [TEM]) studies confirm the vesicular nature of the observed nanoaggregates. Dialysis studies on nanoaggregates against an acidic medium confirm the pH responsiveness of the acylhydrazine linker. The reductive behavior of the newly designed copolymer in the presence of dithiothreitol (DTT) suggests the biodegradable nature of the...
backbone. Once the nanocarriers are internalized into the tumor cells, the acylhydrazine linkages are exposed to the acidic condition because of the collapse of the nanocarrier. Because of this, the DOXI molecules are sustainably released. In addition, because of the presence of GSH, in the tumor tissue, the nanocarriers rapidly disaggregate and the DOXI-attached chains are more exposed to the acidic environment for the sustained release. The cell viability studies support the biocompatible nature of the system, whereas epifluorescence microscopy and flow cytometry analysis suggest the increased internalization of DOXI into the cells. We are expecting that our newly designed nanocarrier will have greater application in the field of cancer therapy.

EXPERIMENTAL SECTION

Materials. Lithium diisopropylamide (LDA), propargyl bromide, hexamethylphosphoramide (HMPA), sodium azide, copper(II) sulfate, tin(II)-trifluoromethanesulfonate (Sn(OTf)2), ε-caprolactone, anhydrous tetrahydrofuran (THF), monomethoxy poly(ethylene glycol) (molecular weight [Mn] = 1450 g/mol), doxorubicin hydrochloride, 4-aminobenzoic acid, tert-butyl carbazate, carbo-di-imidazole, 2-hydroxyethyl disulfide, 1,8-diazobicyclo[5,4,0]undec-7-ene (DBU), dicyclohexylcarbodiimide (DCC), 4-dimethylaminopyridine (DMAP), 1-hydroxybenzotriazole (HOBt), N-(3-dimethylaminopropyl)-N-ethylcarbodiimide hydrochloride (EDC-HCl), 1,3,5-trifluorobenzotriazol-1-yl 3-ethoxy-3-(N,N-dimethylamino)phenylphosphorane (TEA), and triﬂuoroacetic acid, deuterated methanol (CD3OD), deuterated chloroform (CDCl3), and dimethyl sulfoxide-d6 (DMSO-d6) were purchased from Sigma-Aldrich. Sodium nitrate, potassium bromide, sodium sulfate, ammonium chloride, sodium chloride, sodium bicarbonate, toluene, ethanol, methanol, dimethyl formamide (DMF), ethyl acetate, dioxane, hexane, chloroform, acetone, hydrochloric acid (HCl), acetonitrile, and dichloromethane were purchased from Merck and used as received without further purification. All other solvents were of highest purity and purchased from Sigma-Aldrich. ε-Caprolactone, ethanol, and THF were distilled over calcium hydride under an inert atmosphere before use.

Procedure for Polymerization. Polymerization was carried out under an inert atmosphere of nitrogen in a glovebox. The compound mixture 6a and 6b (150 mg, 0.98 mmol), ε-caprolactone (1.005 g, 8.82 mmol), PEG-SS-OH (196 mg, 0.098 mmol), Sn(OTf)2 (0.075 g, 0.181 mmol), and toluene (3 mL) were taken in a vial. The resulting reaction mixture was allowed to stir for 48 h at room temperature. The polymerization mixture was quenched by adding HCl (1 N, 2 mL). Then, it was poured into acetone (5 mL) and precipitated in cold hexane (20 mL). Then, copolymer was collected and dried under vacuum. The copolymer was a light yellow viscous liquid. 1H NMR (500 MHz, CDCl3): δ 5.03 (m, 1H), 4.01 (t, 2H), 3.4–3.67 (PEG protons) 2.28 (t, 2H), 1.97 (m, 2H), 1.62 (m, 4H), 1.35 (m, 2H). FT-IR: (KBr, cm⁻¹): 3459.60, 2925.01, 2853.64, 179.48, 1441.22, 1401.35, 1238.43, 1160.52, 638.13. The unimodal nature of the gel permeation chromatography (GPC) trace confirmed controlled polymerization.

Click Reaction Conditions for Polyesters of the Copolymer. Compound 4 (3.5 mg, 0.005 mmol) was taken in 100 mL round-bottom flask and dissolved in 2 mL of water. The PEG-SS-PCL (50 mg, 0.005 mmol) copolymer after dissolving in THF was added dropwise to the reaction mixture. Sodium ascorbate (0.128 mg, 0.00065 mmol) and copper sulfate pentahydrate (0.16 mg, 0.00065 mmol) were added to the resultant mixture and stirred for 24 h at room temperature. The molecular weight was obtained from GPC using polystyrene standards (Mn = 10 200 g/mol, polydispersity index [PDI] = 2.3). FT-IR (KBr, cm⁻¹): 3438.81, 2945.83, 2865.95, 1727.29, 1704.88, 1619.42, 1419.86, 1397.97, 1367.81, 1190.42, 1107.95, 1046.80, 961.69, 934.10, 801.66, 732.34, and 584.49.

RESULTS AND DISCUSSION

It is desirable that a drug delivery vehicle should increase the drug solubility and decrease the toxicity, releasing the drug in a sustained manner.5,17,23–28 Inspired by these requirements, this article reports on developing an efficient method to covalently attach DOXI and poly(ethylene glycol) (PEG) as pendant motifs to the biodegradable caprolactone with an S–S
linker in the backbone (Scheme 4), which is explored systematically in this work. Towards this goal, the functionalization of lactones was performed in two steps (Scheme 3). At first, compound 5 was synthesized from cyclohexanone in the presence of LDA and propargyl bromide at −78 °C. The crude product was first purified by column chromatography, followed by distillation under reduced pressure at 160 °C, which yielded compound 5 as a colorless, viscous liquid (62%). The formation of compound 5 was confirmed by observing a new signal at δ 1.95 ppm in 1H NMR spectroscopy corresponding to terminal acetylene protons (Figure S12). Next, m-chloroperbenzoic acid was added to the distilled product in dichloromethane solvent (Baeyer–Villiger oxidation). An isomeric mixture of 3-((prop-2-ynyl) oxepan-2-one), 6a, and 7-((prop-2-ynyl) oxepan-2-one), 6b, in 62% yield was obtained by distillation using a vacuum pump.

The mixture of lactones was characterized by 1H NMR and 13C NMR spectroscopy (Figures S14 and S15). Then, the S–S linker containing PEG-SS-OH was synthesized as shown in Scheme 2. At first, PEG-OH was reacted with carbodiimidazole (CDI) in the presence of THF, which gave PEG-CDI as a colorless solid. Product formation was confirmed by 1H NMR (Figure S8) and MALDI-TOF spectroscopy (Figure S9). The product was further reacted with 2,2-disulfanediyldiethanol and DBU in chloroform as the solvent. The purification of the crude
product was carried out by reprecipitation with diethyl ether to get pure PEG-SS-OH as a colorless solid. The formation of macro initiator PEG-SS-OH was confirmed by $^1$H NMR (Figure S10) and MALDI-TOF spectroscopy. The increase in the molecular weight by 88 units confirmed the formation of the product (Figure S11).

Next, the attachment of the DOXI component to the PEG-SS-PCL copolymer was performed (Scheme 4). Azide-functionalised doxorubicin with an acylhydrazine linker (DOXI-N₃) was synthesized as shown in Scheme 1 following the literature procedure³⁷ and thoroughly characterized (Figures S1−S7 and 3b). After isolating the mixture of compounds 6a and 6b,⁴²,⁴³ we explored the copolymerization condition with ε-caprolactone. We employed the ROP method to copolymerize the mixture of compounds 6a and 6b with ε-caprolactone at room temperature for 48 h under a nitrogen atmosphere. The macro initiator PEG-SS-OH initiated the reaction, and Sn(OTf)₂ was used as a catalyst in toluene. The copolymer PEG-SS-PCL was synthesized at M/I = 100 by adding 2 mol % catalyst relative to the initiator. The ratio of the reagent was ([6a and 6b]:[ε-CL]:[Sn(OTf)₂]:[PEG-SS-OH] = 10:90:5:1). The copolymer was analyzed by $^1$H NMR (Figure 1a), FT-IR spectroscopy (Figure 3a), and GPC techniques. The observed GPC chromatogram of the PEG-SS-PCL copolymer was unimodal with $M_n = 7700$ g/mol and PDI = 1.25 using polystyrene standards (Figure 2). From the PDI, it is evident that the polymerization was well controlled.

After successful synthesis of the copolymer PEG-SS-PCL, the attachment of DOXI-N₃ was performed using alkyne−azide click chemistry. The reaction was carried out by employing the alkyne-
functionalized copolymer, DOXI-N$_3$ (4), with sodium ascorbate, and copper(II) sulfate.40 Usually, the coupling reactions between azide and alkyne were carried out in water or mixtures of water and a polar solvent. However, we performed the click reaction in THF and water (1:1) and by stirring the reaction mixture for 24 h at room temperature. The pure PEG-SS-PCL-DOXI copolymer

Figure 2. GPC analysis of PEG-SS-OH and PEG-SS-PCL.

Figure 3. FT-IR data of (a) PEG-SS-PCL copolymer, (b) compound DOXI-N$_3$, and (c) PEG-SS-PCL-DOXI.

Figure 4. (a) Plot of the concentration of PEG-SS-PCL-DOXI vs intensity ratio of emissions at 371 and 396 nm from pyrene. The observed critical aggregation concentration (CAC) was 180 μg/mL. (b) Dynamic light scattering (DLS) of PEG-SS-PCL-DOXI measured in aqueous solution. The size of the aggregate was about 104 nm with PDI = 0.26.

Figure 5. (a−d) TEM images of PEG-SS-PCL-DOXI, (e) SEM image, (f) cryo-SEM images, and (g) cartoon representation of the self-assembly of PEG-SS-PCL-DOXI in phosphate buffer.

Figure 6. Comparison of the DOXI release profiles of PEG-SS-PCL-DOXI at 37 °C at pH values of 5, 6.5, and 7.4.
was obtained by the subsequent evaporation of water using a high-vacuum pump. The formation of the copolymer was confirmed by \(^1\)H NMR and FT-IR spectroscopy. In the \(^1\)H NMR spectroscopy of the copolymer PEG-SS-PCL-DOXI, all of the characteristic peaks of polyethylene oxide, DOXI, and polycaprolactone were present. Additionally, a new peak was observed at \(\delta 7.37\) ppm corresponding to the triazole protons (Figure 1b). It was further confirmed by FT-IR spectroscopy, where the azide stretching frequency at 2095 cm\(^{-1}\) disappeared (Figure 3c). GPC analysis was carried out to indicate the efficiency of the click reaction using polystyrene as the standard.

The \(M_n\) shifted from 7700 to 10 200 g/mol with PDI = 2.3 (Figure S19).

The richness of amphiphilicity in PEG-SS-PCL-DOXI prompted us to measure the CAC in water using pyrene as a fluorescent probe (Figure 4a). Towards this goal, we dissolved 4 \(\mu\)g of pyrene in methanol. Next, we prepared a few samples with several concentrations of PEG-SS-PCL-DOXI. We dissolved the copolymer in 1 mL of water, and it was stirred at room temperature. Then, we fixed the pyrene concentration at 0.2 \(\mu\)M and varied the PEG-SS-PCL-DOXI concentrations from 0.01 to 0.2 mg/mL. We set the excitation wavelength at 339 nm and monitored the emission intensities at 371, 382, and 396 nm. We varied the relative emission fluorescence intensities at 396/371 nm with respect to copolymer concentrations. The value of the CAC was calculated from the copolymer concentration where the ratio of relative fluorescence intensity started to vary. We observed the CAC at 180 \(\mu\)g/mL.

Next, we wanted to determine the size of the nanoaggregate. For this purpose, we dissolved 1 mg of the copolymer in 1 mL of water and stirred for 30 min. Then, 2.5 mL of the aliquot was
taken from the solution, and DLS analysis was carried out to determine the particle size (Figure 4b). The radius of the aggregate was about 104 nm with PDI = 0.26. The morphology of the aggregate was obtained by SEM (Figure 5e,f) and TEM analysis. From the SEM studies, it was observed that PEG-SS-PCL-DOXI in a polar medium produced a vesicle-type structure. The observed vesicular morphology was further supported by TEM analysis. The diameter of these aggregates was about 200 nm which was in accordance with the result obtained from DLS. Both SEM and TEM images revealed a uniform vesicular shape.

Next, the stimuli responsiveness of the delivery vehicle was investigated at varying pH. For the in vitro drug release study of PEG-SS-PCL-DOXI, the dialysis experiment was carried out at pH 7.4, 6.5, and 5. Towards this goal, the copolymer PEG-SS-PCL-DOXI (1 mg) was dissolved in phosphate buffer solution (1 mL) followed by loading in a dialysis tube (3500 Da cutoff). The solution was then dialyzed against 80 mL of buffer solution at pH 5 by gentle stirring. An aliquot was taken from the sample, and absorbance was measured at 480 nm, which indicated the
release of DOXI (Figure 6). The fluorescence spectrum of each aliquot was also recorded at an excitation wavelength of 510 nm (Figure S17). Emissions from the free drug were monitored at the wavelengths 560 and 588 nm. Then, the aliquot was added back to the solution to keep the volume consistent. A similar measurement was carried out every 1 h and continued for 48 h. After 10 h, the increase in the fluorescence intensity was not significant. The release profile of the drug at pH 6.5 and 7.4 was monitored by the same procedure. The DOXI release from PEG-SS-PCL-DOXI at pH 7.4 was around 4%, which clearly indicated that the PEG-SS-PCL-DOXI copolymer was stable in physiological media. Interestingly, we observed the maximum drug release in an acidic environment with respect to pH 7.4, anticipating the usefulness of incorporating the acylhydrazine linker into the polymer backbone.

The presence of a disulfide linkage between PCL and the PEG moiety makes the copolymer reductively degradable. The disulfide bond can be cleaved by the reducing agents GSH and DTT. For the investigation of the redox responsiveness, the PEG-SS-PCL-DOXI copolymer was treated with 10 mM GSH (intracellular GSH concentration of tumor cells). The particle sizes were monitored by DLS at regular time intervals. The average diameter of GSH-treated copolymers increased immediately, to 1000 nm within 10 min (Figure 7a). The rapid change of aggregate size in the presence of GSH could be attributed to the rupture of disulfide bonds, which resulted in the destabilization of vesicular architecture. We hypothesized that the increase in the size was due to the breaking of the polymer chains via disulfide bonds followed by the recombination of the chains. However, when PEG-SS-PCL-DOXI copolymer was treated with GSH, a change in the size of aggregates was not observed, which supported our hypothesis. Further, no change in the size of the aggregates also suggested the stability of the copolymer under physiological conditions. The disruption of the self-assembled aggregates with response to GSH confirmed our proposal of increased biodegradability of the polymeric backbone inside of the cell.

Next, we investigated the degradation of the copolymer by GPC analysis. Figure 7b shows how the molecular weight of the PEG-SS-PCL changed after DTT treatment in THF. As represented in Figure 7b, the copolymer peak shifted to a higher retention time, indicating the successful degradation of PEG-SS-PCL into smaller fragments represented by three peaks with different intensities. This disaggregation was further confirmed by SEM analysis, which showed the disruption of the self-assembled morphology (Figure S16).

For cellular uptake analysis, free DOXI (Figure 8) and the PEG-SS-PCL-DOXI (Figure 9) nanocarrier with MCF-7 cells were cultured in 24 well plates at an initial concentration of 2.5 × 10^5 cells per well for 24 h in a MEM (minimum essential medium) at 37 °C. Next, cells were treated with free DOXI and the PEG-SS-PCL-DOXI nanocarrier having drug concentrations of 25, 50, and 100 μg/mL at 37 °C for 24 h. After incubation, cover slips were washed with 1× PBS, fixed using 4% paraformaldehyde (PFA), and then mounted on a slide. Microscopic observations were carried out by epifluorescence microscopy. From the cellular uptake studies, it was observed that both free DOXI and the PEG-SS-PCL-DOXI nanocarrier entered into cells with increased concentrations (25, 50, and 100 μg/mL).

From the cytotoxicity assay results, the free DOXI viability was more from the 25 to 500 μg/mL compared with that of the PEG-SS-PCL-DOXI nanocarrier (Figure 10). However, the PEG-SS-PCL-DOXI nanocarrier showed a gradual change in cell viability, which implied the sustained and well-controlled release property for drug delivery applications.

Flow cytometry results (Figure 11) indicated that the mean fluorescence intensities of free DOXI and the PEG-SS-PCL-DOXI nanocarrier were 449.63 and 421.99 in 50 μg/mL concentration and 654.9 and 620.33 in 100 μg/mL concentration, respectively (Figures 12 and 13). In 50 μg/mL concentration, the fold changes were 5.64 and 5.29 for the free DOXI and PEG-SS-PCL-DOXI, respectively. However, in the case of 100 μg/mL concentration, the fold changes were 8.21 and 7.78 for the free DOXI and PEG-SS-PCL-DOXI, respectively (Figure 14).

From the flow cytometry result, it was evident that the mean fluorescence intensity and fold change for both free DOXI and PEG-SS-PCL-DOXI were almost the same. From the comparative biological experiments of free DOXI and PEG-SS-PCL-DOXI, it was obvious to note that free DOXI showed effective killing of cancer cells over PEG-SS-PCL-DOXI. However, free DOXI did not have the capability to reach the cancer cell site specifically. Because of this, the great side effect in normal cells was not taken care.47–51 It was very interesting to note that the nanocarrier disrupted through the S–S linker only in the cancer cell because of the presence of GSH. Also, the presence of the acylhydrazine linker demonstrated the sustained release of DOXI at the cancer site. Overall, the newly developed PEG-SS-PCL-DOXI demonstrated the efficiency in reaching the cancer cell site specifically and releasing the drug in a sustained fashion.

**CONCLUSIONS**

This article describes the efficacious synthesis of the copolymer PEG-SS-PCL-DOXI, which serves as an efficient nanocarrier, releasing the drug in stimuli-responsive sustained fashion. Because of the inherent amphiphilicity, this nanovehicle self-assembled into a vesicular architecture, which is well supported by SEM and TEM studies. Interestingly, we have effectively conjugated DOXI to the biodegradable caprolactone backbone via the acid-sensitive acylhydrazine linker. This has facilitated the well-controlled and pH-responsive drug release under an acidic environment. Cell viability studies clearly show the efficient internalization of the nanocarrier into the tumor cells because of the biodegradability present in the backbone. It is our belief that this newly designed copolymer possesses all of the prospects of an efficient nanocarrier for the effective cancer treatment.

**ASSOCIATED CONTENT**

3 Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsomega.6b00018.

H NMR, 13C NMR spectra, MALDI of the monomers, dialysis study, SEM images, and DLS study of all polymers (PDF)

Epifluorescence video showing both free DOXI and the PEG-SS-PCL-DOXI nanocarrier entering MCF-7 cells, with the red emission from DOXI indicating effective internalization of the nanocarrier (AVI)

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ACKNOWLEDGMENTS

S.B. thanks IISER, Kolkata, for research fellowship. M.N.G. thanks UGC, New Delhi, for research fellowship. H.D. thanks CSIR for research fellowship. R.S. thanks the Department of Science and Technology, New Delhi, for Ramanujan fellowship and the DBT for funding. R.S. and J.D.S. thank IISER, Kolkata, for providing the infrastructure and also the startup funding.

REFERENCES

(1) Singla, A. K.; Garg, A.; Aggarwal, D. Paclitaxel and its formulations. *Int. J. Pharm.* 2002, 235, 179–192.
(2) Stukel, J. M.; Li, R. C.; Maynard, H. D.; Caplan, M. R. Two-Step Synthesis of Multivalent Cancer-Targeting Constructs. *Biomacromolecules* 2010, 11, 160–167.
(3) Li, D.; Sun, H.; Ding, J.; Tang, Z.; Zhang, Y.; Xu, W.; Zhuang, X.; Chen, X. Polymeric topology and composition constrained polyether–polyester micelles for directional antitumor drug delivery. *Acta Biomater.* 2013, 9, 8875–8884.
(4) Gowerden, T.; Stolnik, S.; Garnett, M. C.; Illum, L.; Davis, S. S. PLGA nanoparticles prepared by nanoprecipitation: drug loading and release studies of a water soluble drug. *J. Controlled Release* 1999, 57, 171–185.
(5) Omayra, L.; Jesús, P. D.; Ihre, H. R.; Gagne, L.; Fréchet, J. M. J.; Szoka, F. C. Polymer Dendritic Systems for Drug Delivery Applications: In Vitro and In Vivo Evaluation. *Biomacromolecules* 2002, 13, 453–461.
(6) Ulrich, K. E.; Cannizzaro, S. M.; Langer, R. S.; Shakesheff, K. M. Polymeric Systems for Controlled Drug Release. *Chem. Rev.* 1999, 99, 3181–3198.
(7) Gref, R.; Minamitake, Y.; Peracchia, M. T.; Trubetskoy, V.; Torchilin, V.; Langer, R. Biodegradable long-circulating polymeric nanoparticles. *Science* 1994, 263, 1600–1603.
(8) Tong, R.; Cheng, J. Anticancer Polymeric Nanomedicines. *Polym. Rev.* 2007, 47, 345–381.
(9) Carothers, W. H.; Dorough, G. L.; van Natta, F. J. Studies of polymerization and ring formation. X. The reversible polymerization of six-membered cyclic esters. *J. Am. Chem. Soc.* 1932, 54, 761–772.
(10) van Natta, F. J.; Hill, J. W.; Carothers, W. H. Studies of Polymerization and Ring Formation. XXIII. ε-Caprolactone and its Polymers. *J. Am. Chem. Soc.* 1934, 56, 455–457.
(11) Carothers, W. H.; van Natta, F. J. Studies on polymerization and ring formation. Glycol esters of carbonic acid. *J. Am. Chem. Soc.* 1930, 52, 314–326.
(12) Mizutani, M.; Arnold, S. C.; Matsuda, T. Liquid, Phenylazide-End-Capped Copolymers of ε-Caprolactone and Trimethylene Carbonate: Preparation, Photocuring Characteristics, and Surface Layering. *Biomacromolecules* 2002, 3, 668–675.
(13) Nederberg, F.; Bowden, T.; Hildborn, J. Synthesis, Characterization, and Properties of Phosphoryl Choline Functionalized Poly ε-caprolactone and Charged Phospholipid Analogues. *Macromolecules* 2004, 37, 954–965.
(14) Córdova, A.; Iversen, T.; Hult, K. Lipase-catalyzed formation of end-functionalized poly (ε-caprolactone) by initiation and termination reactions. *Polymer* 1999, 40, 6709–6721.
(15) Yoo, H. S.; Oh, J. E.; Lee, K. H.; Park, T. G. Biodegradable Nanoparticles Containing Doxorubicin-PLGA Conjugate for Sustained Release. *Chem. Res. 1999*, 16, 1114–1118.
(16) Du, C.; Deng, D.; Shan, L.; Wan, S.; Cao, J.; Tian, J.; Achilefu, S.; Gu, Y. A pH-sensitive doxorubicin prodrug based on folate-conjugated BSA for tumor-targeted drug delivery. *Biomaterials* 2013, 34, 3087–3097.
(17) Zhou, L.; Cheng, R.; Tao, H.; Ma, S.; Guo, W.; Meng, F.; Liu, H.; Liu, Z.; Zhong, Z. Endosomal pH-Activatable Poly(ethylene oxide)-graft-Doxorubicin Prodrugs: Synthesis, Drug Release, and Biodistribution in Tumor-Bearing Mice. *Biomacromolecules* 2011, 12, 1460–1467.
(18) Gillies, E. R.; Fréchet, J. M. J. pH-Responsive Copolymer Assemblies for Controlled Release of Doxorubicin. *Bioconjugate Chem.* 2005, 16, 361–368.
(19) Gillies, E. R.; Fréchet, J. M. J. A new approach towards acid sensitive copolymer micelles for drug delivery. *Chem. Commun.* 2003, 14, 1640–1641.
(20) Lehn, J.-M.; Eliseev, A. V. Dynamic Combinatorial Chemistry. *Science* 2001, 291, 2331–2332.
(21) Lehn, J.-M. From supramolecular chemistry towards constitutional dynamic chemistry and adaptive chemistry. *Chem. Soc. Rev.* 2007, 36, 151–160.
(22) Jin, Y.; Song, L.; Su, Y.; Zhu, L.; Pang, Y.; Qiu, F.; Tong, G.; Yan, D.; Zhu, B.; Xu, Z. Oxime Linkage: A Robust Tool for the Design of pH-Sensitive Polymeric Drug Carriers. *Biomacromolecules* 2011, 12, 3460–3468.
(23) Gillies, E. R.; Fréchet, J. M. J. Development of acid-sensitive copolymer micelles for drug delivery. *Pure Appl. Chem.* 2004, 76, 1295–1307.
(24) Matson, J. B.; Stupp, S. I. Drug release from hydrazone-containing peptide amphiphiles. *Chem. Commun.* 2011, 47, 7962–7964.
(25) Bae, Y.; Fukushima, S.; Harada, A.; Kataoka, K. Design of Environment-Sensitive Supramolecular Assemblies for Intracellular Drug Change: Polymeric Micelles that are Responsive to Intracellular pH Change. *Angew. Chem., Int. Ed. Eng.* 2003, 42, 4640–4643.
(26) Hu, X.; Liu, S.; Huang, Y.; Chen, X.; Jing, X. Biodegradable Block Copolymer-Doxorubicin Conjugates via Different Linkages: Preparation, Characterization, and In Vitro Evaluation. *Biomacromolecules* 2010, 11, 2094–2102.
(27) Meng, F.; Hennink, W. E.; Zhong, Z. Reduction-sensitive polymers and bioconjugates for biomedical applications. *Biomaterials* 2009, 30, 2180–2198.
(28) Chen, W.; Zou, Y.; Jia, J.; Meng, F.; Cheng, R.; Deng, C.; Feijen, J.; Zhong, Z. Functional Poly(ε-caprolactone) via Copolymerization of ε-Caprolactone and Pyridyl Disulfide-Containing Cyclic Carbonate: Controlled Synthesis and Facile Access to Reduction-Sensitive Biodegradable Graft Copolymer Micelles. *Macromolecules* 2013, 46, 699–707.
(29) Li, J.; Huo, M.; Wang, J.; Zhou, J.; Mohammad, J. M.; Zhang, Y.; Zhu, Q.; Waddad, A. Y.; Zhang, Q. Redox-sensitive micelles self-assembled from amphiphilic hyaluronic acid-deoxycholic acid conjugates for targeted intracellular delivery of paclitaxel. *Biomaterials* 2012, 33, 2310–2320.
(30) Liu, J.; Pang, Y.; Huang, W.; Zhu, Z.; Zhu, X.; Zhou, Y.; Yan, D. Redox-Responsive Polyphosphate Nanosized Assemblies: A Smart Drug Delivery Platform for Cancer Therapy. *Biomacromolecules* 2011, 12, 2407–2415.
(31) Sun, H.; Meng, F.; Cheng, R.; Deng, C.; Zhong, Z. Reduction-sensitive degradable micellar nanoparticles as smart and intuitive delivery systems for cancer chemotherapy. *Expert Opin. Drug Delivery* 2013, 10, 1109–1122.
(32) Chan, N.; An, S. Y.; Oh, J. K. Dual location disulfide degradable interlayer-crosslinked micelles with extended sheddable coronas exhibiting enhanced colloid stability and rapid release. *Polym. Chem.* 2014, 5, 1637–1649.
(33) Yan, Y.; Wang, Y.; Heath, J. K.; Nice, E. C.; Caruso, F. Cellular Association and Cargo Release of Redox Responsive Polymer Capsules Mediated by Exosomial Thiols. *Adv. Mater.* 2011, 23, 3916–3921.
(34) Jones, D. P.; Carlson, J. L.; Mody, V. C., Jr.; Cai, J.; Lynn, M. J.; Sternberg, P., Jr. Redox State Of Glutathione In Human Plasma. *Free Radicals Biol. Med.* 2000, 28, 625–635.
(35) Mitchell, J. B.; Russo, A. The role of glutathione in radiation and drug induced cytotoxicity. *Br. J. Cancer, Suppl.* 1997, 8, 96–104.
(36) Saito, G.; Swanson, J. A.; Lee, K.-D. Drug delivery strategy utilizing conjugation via reversible disulfide linkages: role and site of cellular reducing activities. *Adv. Drug Delivery Rev.* 2003, 55, 199–215.
(37) Ganivada, M. N.; Rao, V. N.; Dinda, H.; Kumar, P.; Das Sarma, J.; Shunmugam, R. Biodegradable Magnetic Nanocarrier for Stimuli Responsive Drug Release. *Macromolecules* 2014, 47, 2703–2711.
(38) Rao, V. N.; Kishore, A.; Sarkar, S.; Das Sarma, J.; Shunmugam, R. Norbornene-derived poly-D-lysine copolymers as quantum dot carriers for neuron growth. *Biomacromolecules* 2012, 13, 2933−2944.

(39) Mane, S. R.; Rao, V. N.; Chatterjee, K.; Dinda, H.; Nag, S.; Kishore, A.; Das Sarma, J.; Shunmugam, R. A unique polymeric nano-carrier for anti-tuberculosis therapy. *J. Mater. Chem.* 2012, 22, 19639−19642.

(40) Tan, L.; Maji, S.; Mattheis, C.; Chen, Y.; Agarwal, S. Antimicrobial Hydantoin-grafted Poly(ε-caprolactone) by Ring-opening Polymerization and Click Chemistry. *Macromol. Biosci.* 2012, 12, 1721−1730.

(41) Jazkewitsch, O.; Ritter, H. Formation and Characterization of Inclusion Complexes of Alkyne Functionalized Poly(ε-caprolactone) with β-Cyclodextrin. Pseudo-Polyrotaxane-Based Supramolecular Organogels. *Macromolecules* 2011, 44, 375−382.

(42) Kim, S. Y.; Lee, Y. M.; Baik, D. J.; Kang, J. S. Toxic characteristics of methoxy poly(ethylene glycol)/poly(ε-caprolactone) nanospheres; in vitro and in vivo studies in the normal mice. *Biomaterials* 2003, 24, 55−63.

(43) Wang, F.; Bronich, T. K.; Kabanov, A. V.; Rauh, R. D.; Roovers, J. Synthesis and Evaluation of a Star Amphiphilic Block Copolymer from Poly(ε-caprolactone) and Poly(ethylene glycol) as a Potential Drug Delivery Carrier. *Bioconjugate Chem.* 2005, 16, 397−405.

(44) Albertsson, A.-C.; Varma, I. K. Recent developments in ring opening polymerization of lactones for biomedical applications. *Biomacromolecules* 2003, 4, 1466−1486.

(45) Zhang, W.; Li, Y.; Liu, L.; Sun, Q.; Shuai, X.; Zhu, W.; Chen, Y. Amphiphilic Toothbrushlike Copolymers Based on Poly (ethylene glycol) and Poly (ε-caprolactone) as Drug Carriers with Enhanced Properties. *Biomacromolecules* 2010, 11, 1331−1338.

(46) Chang, L.; Deng, L.; Wang, W.; Lv, Z.; Hu, F.; Dong, A.; Zhang, J. Poly(ethylene glycol)-b-Poly(ε-caprolactone-co-γ-hydroxy ε-caprolactone) Bearing Pendant Hydroxyl Groups as Nanocarriers for Doxorubicin Delivery. *Biomacromolecules* 2012, 13, 3301−3310.

(47) Kim, B. J.; Cheong, H.; Hwang, B. H.; Cha, H. J. Mussel-Inspired Protein Nanoparticles Containing Iron (III)—DOPA Complexes for pH-Responsive Drug Delivery. *Angew. Chem., Int. Ed.* 2015, 54, 7318−7322.

(48) Jin, Y.; Huang, Y.; Yang, H.; Liu, G.; Zhao, R. A peptide-based pH-sensitive drug delivery system for targeted ablation of cancer cells. *Chem. Commun.* 2015, 51, 14454−14457.

(49) Wang, W.; Zhang, L.; Liu, M.; Le, Y.; Lv, S.; Wang, J.; Chen, J.-F. Dual-responsive star-shaped polypeptides for drug delivery. *RSC Adv.* 2016, 6, 6368−6377.

(50) Chen, M.; Gao, C.; Lü, S.; Chen, Y.; Liu, M. Dual redox-triggered shell-shedding micelles selfassembled from mPEGylated starch conjugates for rapid drug release. *RSC Adv.* 2016, 6, 9164−9174.

(51) Volsi, A. L.; Aberasturi, D. J.; Lacey, M. H.; Giammona, G.; Licciardi, M.; Liz-Marzán, L. M. Insulin coated plasmonic gold nanoparticles as a tumor-selective tool for cancer therapy. *J. Mater. Chem. B* 2016, 4, 1150−1155.