Photo- and thermo-responsive multicompartiment hydrogels for synergistic delivery of gemcitabine and doxorubicin

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A B S T R A C T
Hydrogels have found promising applications in drug delivery due to their biocompatibility, high drug loading capability, and tunable release profiles. However, hydrogel-based carriers are primarily employed for delivering hydrophilic payloads while hydrophobic drugs cannot be efficiently delivered due to the lack of hydrophobic domains within conventional hydrogel matrices. Herein, we report that thermo- and photo-responsive hydrogels could be constructed from amphiphilic triblock copolymers, poly(N-isopropylacrylamide)-b-poly(4-acryloyl morpholine)-b-poly[(2-(((2-nitrobenzyl)oxy)carbonyl) amino) ethyl methacrylate] (PNIPAM-b-PNAM-b-PNBOC), and the resulting hydrogels could be further engineered as a new carrier for both hydrophilic gemcitabine (GCT) and hydrophobic doxorubicin (DOX). PNIPAM-b-PNAM-b-PNBOC triblock copolymers were first self-assembled into micelles with hydrophobic photosensitive PNBOC cores, hydrophilic PNAM inner shells, and thermoresponsive PNIPAM coronas below the lower critical solution temperature (LCST), while hydrogels of physically cross-linked micellar nanoparticles were achieved at elevated polymer concentrations and high temperatures above the critical gelation temperature (CGT). Rheological experiments revealed that the CGT was highly dependent on polymer compositions and concentrations, that is, a longer hydrophobic PNBOC block or a higher polymer concentration led to a decreased CGT. However, the CGT prior to UV irradiation (CGT0) could be drastically elevated after UV irradiation (CGTUV) as a result of UV irradiation-induced concurrently cross-linking and hydrophobic-to-hydrophilic transition within PNBOC cores. As such, gel-to-sol transition could be accomplished by either temperature decrease or exposure to UV irradiation at a fixed temperature lower than the CGTUV. Note that both GCT and DOX could be simultaneously encapsulated into the hydrogels due to the coexistence of extramicellar aqueous phase and hydrophobic micellar cores. Intriguingly, the subsequent co-release of GCT and DOX could be regulated by taking advantage of either temperature or UV irradiation-mediated gel-to-sol transitions.

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1. Introduction

The development of novel drug carriers on the basis of stimuli-responsive polymers is of considerable interest. These smart drug vehicles can, at least in part, conquer the drawbacks of small molecule counterparts such as systemic toxicity, insufficient circulation time, and lack of long-term stability [1–6]. Previously, supramolecular aggregates with diverse self-assembled morphologies have been engineered as smart nanovehicles for site-specific delivery of therapeutic agents, exhibiting improved therapeutic efficiency [7–15]. Further, recent studies have suggested that combinatorial therapy that integrates several different therapeutic agents into one system is of superior efficiency in treating formidable diseases [16,17]. For instance, chemotherapeutical drugs, hydrophilic gemcitabine (GCT) and hydrophobic doxorubicin (DOX), show synergistic anticancer efficacy due to their non-overlapping toxicities. However, it is difficult to incorporate two categories of therapeutic drugs with distinct water-solubility into one nanocontainer since conventional nanovehicles (e.g., micelles and nanorods) only possess hydrophobic domains that can solely be employed for delivery of hydrophobic payloads. Of these, polymersomes also referred to as vesicles, are distinguished by the coexistence of both aqueous interiors and hydrophobic bilayer membranes that can simultaneously encapsulate both hydrophilic and water-immiscible drugs [6,18–20]. Nevertheless, conventional polymersomes suffer from poor permeability of bilayer membranes, rendering the release of encapsulated payloads uncontrollable, although the development of stimuli-responsive polymersomes has remarkably alleviated this cumbersome issue [21–23].

In comparison with polymersomes, hydrogels are three-dimensional, cross-linked networks, which have been widely used for drug delivery application because of their excellent biocompatibility, high drug loading efficiency, and programmable release profiles [24–34]. Although hydrogels could be fabricated through either covalent or noncovalent bonds,

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hydrogels are generally composed of hydrophilic polymers that can only be used for loading hydrophilic payloads [34–42], preventing them from delivering hydrophobic therapeutic drugs. To resolve this issue, multicompartiment hydrogels were fabricated in a stepwise manner from amphiphilic triblock copolymers [43–49], which formed micellar nanoparticles and then subsequently underwent gelation process as a result of ‘cross-linking’ of micellar nanoparticles. Consequently, hydrogels comprising both hydrophilic and hydrophobic domains could be achieved [43]. Notably, the fabrication of hydrogels with multi-domains was of promising potential in delivering multiple encapsulants. For example, the Duvall group explored the drug delivery application of thermo- and reactive oxygen species (ROS)-responsive hydrogels from poly(propylenesulfide)-b-(N,N-dimethylacrylamide)-b-(N-isopropylacrylamide) (PPS-b-PDMA-b-PNIPAM) amphiphilic triblock copolymers, revealing that the release of hydrophobic model drug, Nile red (NR), could be regulated by ROS [50]. This study clearly demonstrated that water-immiscible payloads could be incorporated into hydrogel matrices consisting of hydrophobic domains originating from preformed micellar cores. However, the co-delivery of both hydrophobic and hydrophilic payloads in this hydrogel system was not assessed. Given that hydrogels have been proved to be potent in delivering hydrophilic drugs, we surmised that both hydrophilic and hydrophobic drugs could be incorporated into multicompartiment hydrogel-based carriers with built-in hydrophobic domains [51]. Moreover, hydrogel-based carriers with multi-domains should exhibit unique advantages in terms of scale-up products, increased drug loading contents, and extended release durations as compared to vesicle-based nanovehicles [24–28].

To verify our hypothesis, herein, photo- and thermo-responsive amphiphilic triblock copolymers, poly(N-isopropylacrylamide)-b-poly(4-acryloylmorpholine)-b-poly(2-(((2-nitrobenzyl)oxy)carbonyl)amino ethyl methacrylate) (PNIPAM-b-PNAM-b-PNBOC), were synthesized via consecutive RAFT polymerizations. Photoresponsive PNBOC block can not only serve as the hydrophobic building block to facilitate the formation of PNBOC-cored micelles but also provide an additional opportunity to tune the phase transition temperatures of resulting triblock copolymers by UV irradiation [52–56]. The as-synthesized triblock copolymer self-assembled into three-layered micellar nanoparticles with photoresponsive PNBOC cores and hydrophilic PNAM inner shells and thermoresponsive PNIPAM coronas when the temperature was lower than the lower critical solution temperature (LCST) at a relatively low concentration (e.g., 2.5 g/L). Upon elevating the polymer concentrations to higher than 10.0 wt%, opaque sol solutions were formed at ambient temperature and the sol solutions experienced gelation subjected to a temperature rise to higher than the critical gelation temperature (CGT) of triblock copolymers (Scheme 1). Notably, PNBOC moieties can produce primary amine groups under UV irradiation from decaged carbamate linkages, which further implemented aminolysis reactions and thus cross-linked nanoassemblies and concurrently rendered the cross-linked micellar cores hydrophilic, [23] thereby elevating the CGT (defined as CGTUV) of irradiated triblock copolymers. As such, at an intermediate temperature (CGTUV < T < CGT), a gel-to-sol transition was observed, whereas the irradiated sol solutions could be further transformed to hydrogels upon a further temperature increase (T > CGTUV). Moreover, the dual-responsive hydrogels could be engineered as a new drug carrier and the synergistic release of GCT and DOX drugs could be triggered by UV irradiation and temperature-induced gel-to-sol transitions. This work demonstrated, for the first time, that the co-delivery of chemotherapeutic drugs with synergistic efficacy but distinct water-solubility could be achieved, exhibiting promising application in site-specific drug delivery and selective release of drugs for efficient therapy.
2. Materials and methods

2.1. Materials

N-isopropylacrylamide (NIPAM) was recrystallized twice from toluene/n-hexane mixture (v/v = 1:3). 4-Acryloylmorpholine (NAM) was purified by distillation prior to use. 2,2-Azobisobutyronitrile (AIBN) was purified by recrystallization from 95% ethanol prior to use. Nile red (NR), gemcitabine (GCT), and 4-cyano-4-((phenylcarbonothioylthio)pentanoic acid (CPADB) were purchased from Sigma-Aldrich and used as received. 2-Isoynatoethyl methacrylate was purchased from TCI Co., Ltd. and used as received. 2-Nitrobenzyl alcohol, dibutyltin dilaurate (DBTL), and doxorubicin hydrochloride (DOX·HCl) were purchased from Sinopharm Chemical Reagent Co., Ltd. and used without further purification. Water was deionized with a Milli-Q SP reagent water system (Millipore) to a specific resistivity of 18.4 MΩ cm. All the reaction solvents were purified by a solvent purification system (Pure Solv™). All other reagents were purchased from Sinopharm Chemical Reagent Co., Ltd. and used as received unless otherwise specified. 2-((((2-Nitrobenzyl)oxy)carbonyl) amino)ethyl methacrylate (NBOC) [24] was prepared according to reported procedures.

2.2. Sample preparation

Synthetic routes employed for the preparation of dual-responsive PNIPAM60-g-b-PNAM307-g-b-PNBOCx triblock copolymers (TP1-TP3) via sequential RAFT polymerizations are shown in Scheme 2.

2.2.1. Synthesis of PNIPAM60 macroRAFT agent

Typically, NIPAM (1.0 g, 8.8 mmol, 100.0 equiv.), CPADB (25.0 mg, 0.09 mmol, 1.0 equiv.), AIBN (2.8 mg, 0.017 mmol, 0.2 equiv.), and DMSO (8.0 mL) were added into a reaction tube equipped with a magnetic stirring bar. The tube was carefully degassed by three freeze-pump-thaw cycles and then sealed under vacuum. After being thermostated at 70 °C in an oil bath and stirred for 2h, the reaction tube was quenched into liquid nitrogen, opened, diluted with THF, and precipitated into an excess of diethyl ether. The above dissolution-precipitation cycle was repeated three times and the final PNIPAM block macroRAFT agent had an Mn of 7.6 kDa and a polydispersity index (Mw/Mn) of 1.11 (Table 1).

2.2.2. Synthesis of PNIPAM60-g-b-PNAM307 diblock precursor using PNIPAM60 as the chain transfer agent (Scheme 2)

PNIPAM60-g-b-PNAM307 diblock precursor was synthesized via RAFT polymerization using PNIPAM60 as the macroRAFT agent. Briefly, NAM (0.3 mg, 2.0 μmol, 0.1 equiv.), AIBN (0.3 mg, 2.0 μmol, 0.1 equiv.), and DMSO (4.0 mL) were added into a reaction tube equipped with a magnetic stirring bar. The tube was carefully degassed by three freeze-pump-thaw cycles and then sealed under vacuum. After being thermostated at 70 °C in an oil bath and stirred for 0.5 h, the reaction tube was quenched into liquid nitrogen, opened, diluted with THF, and precipitated into an excess of diethyl ether. The above dissolution-precipitation cycle was repeated three times and the final PNIPAM60-g-b-PNAM307 copolymer was obtained as pale reddish powder after being dried in a vacuum oven at room temperature overnight. The DP of PNAM block was determined to be ~307 by 1H NMR spectroscopy (Fig. S1b) and the resultant diblock polymer was thus denoted as PNIPAM60-g-b-PNAM307. GPC analysis revealed that the obtained PNIPAM60-g-b-PNAM307 had an Mn of 51.9 kDa and a polydispersity index (Mw/Mn) of 1.15 (Table 1).

2.2.3. Synthesis of PNIPAM60-g-b-PNAM307-g-b-PNBOCx triblock copolymers (Scheme 2)

PNIPAM60-g-b-PNAM307-g-b-PNBOCx triblock copolymers were synthesized via RAFT polymerization using the PNIPAM60-g-b-PNAM307 diblock copolymer precursor as the macroRAFT agent. Using the preparation of PNIPAM60-g-b-PNAM307-g-b-PNBOCx (TP2) as an example, in a typical run, NBOC (1.0 g, 3.25 mmol, 50.0 equiv.), PNIPAM60-g-b-PNAM307 macroRAFT agent (3.27 g, 65.0 μmol, 1.0 equiv.), AIBN (1.1 mg, 6.5 μmol, 0.1 equiv.), and DMSO (8.0 mL) were added into a reaction tube equipped with a magnetic stirring bar. The tube was carefully degassed by three freeze-pump-thaw cycles and then sealed under vacuum. After being thermostated at 70 °C in an oil bath and stirred for 24 h, the reaction tube was quenched into liquid nitrogen, opened, diluted with THF, and then precipitated into an excess of diethyl ether. The above dissolution-precipitation cycle was repeated three times.
and the final PNIPAM_{60}^{-b}-PNAM_{307}^{-b}-PNBOC_{34} copolymer was obtained as pale reddish powder after being dried in a vacuum oven at room temperature overnight. The DP of PNBOC block was determined to be -34 by 1H NMR spectroscopy (Fig. S1c). GPC analysis revealed TP2 triblock copolymer had an M_n of 61.8 kDa and a polydispersity index (M_w/M_n) of 1.24 (Table 1).

Using a similar protocol, PNIPAM_{60}^{-b}-PNAM_{307}^{-b}-PNBOC_{12} (TP1) and PNIPAM_{60}^{-b}-PNAM_{307}^{-b}-PNBOC_{55} (TP3) were also synthesized using PNIPAM_{60}^{-b}-PNAM_{307} diblock copolymer precursor as the macroRAFT agent. The structural parameters of TP1 and TP3 are outlined in Table 1.

2.3. Self-assembly of triblock copolymers

Typically, 2 mg triblock copolymer was dissolved in 1 mL THF, stirred and maintained at a predetermined temperature in a water bath for 25 min. Then, 9 mL PBS buffer (pH 7.4, 10 mM) was slowly injected at an addition rate of 1 mL/h via a syringe pump. After stirring for another 2 h, THF was removed by dialysis (cellulose membrane, MWCO: 3.5 kDa) against PBS buffer (pH 7.4, 10 mM). Fresh PBS buffer was replaced approximately every 4 h.

2.4. Preparation of NR-loaded triblock copolymer micelles

Hydrophobic NR dye was loaded into the hydrophobic cores of TP2 micelles during the self-assembly process as detailed above to probe the polarity changes within the micellar cores under UV light irradiation. Typically, TP2 and NR were dissolved in THF (1 mL) with the concentrations being 2 g/L and 0.02 g/L, respectively. Following the above-mentioned self-assembly protocol, NR-loaded micelles were obtained and the fluorescence evolution of NR-loaded micelles under UV irradiation was recorded at predetermined time intervals (λ_ex = 550 nm).

2.5. Preparation of triblock copolymer hydrogels

The triblock copolymers were directly dissolved in PBS buffer (pH 7.4, 10 mM) at four different concentrations (10.0, 12.5, 15.0, and 20.0 wt%) to test the capability of the triblock polymers to form hydrogels. The vial inversion experiment was used to demonstrate the hydrogel formation.

2.6. Preparation of GCT/DOX Co-loaded hydrogels and controlled release of GCT/DOX payloads

Typically, 20 mg DOX-HCl was dissolved in 2 mL DMSO and 4 mL triethylamine was then added and stirred at room temperature overnight. After that, different amounts of TP2 polymer was added and stirred at ambient temperature for 2 h. The mixtures were then subjected to dialysis against PBS buffer (pH 7.4, 10 mM) for 24 h to remove unloaded DOX and DMSO solvent. The dialysate was adjusted to predetermined volumes (pH 7.4, 10 mM) to obtain varying TP2 concentrations (10.0, 12.5, 15.0, and 20.0 wt%) by adding PBS buffer. Finally, GCT stock solution (10 μL, 1.0 g/L) was then added. The sol solutions were then heated up to specific temperatures to allow for gelation. According to a standard calibration curve, in all four polymer concentrations, the DOX and GCT loading efficiencies (LE) were determined to be 80.5 and 100.0% and the DOX and GCT loading contents (LC) were estimated to be approximately 15.9 wt% and 14.0 wt%.

For the triggered release of DOX and GCT, in a typical release experiment, GCT/DOX-loaded TP2 hydrogels (300 μL) were transferred to a dialysis cell with a molecular weight cut-off (MWCO) of 2.0 kDa and then dialyzed against 9 mL of PBS buffer (pH 7.4, 10 mM) at different temperatures. The released GCT and DOX concentrations in the dialysate were quantified by measuring the absorption intensities at 276 nm for GCT and at 480 nm for DOX against corresponding standard calibration curves [57].

3. Results and discussion

3.1. Synthesis and self-assembly of amphiphilic triblock copolymer gelators

Amphiphilic thermo- and photo-responsive triblock copolymers (TP1–TP3), PNIPAM-b-PNAM-b-PNBOC, were synthesized via sequential RAFT polymerizations, starting from commercially available CPADB RAFT agent (Scheme 2). The intermediate polymer precursors and the target triblock copolymers were characterized by 1H NMR spectroscopy (Fig. S1) and the structural parameters of the synthetic polymers are summarized in Table 1. It is well-documented that thermoresponsive PNIPAM exhibits a coil-to-globule transition at around ~32 °C (i.e., LCST) in aqueous solution [58], while PNBOC copolymers bearing 2-nitrobenzyl ester moieties have been manifested to be photosensitive to light (i.e., UV) and that the irradiation can trigger the gelation of the polymers [23]. As such, the introduction of PNIPAM and PNBOC blocks was expected to impart the resulting triblock copolymers with unique thermo- and photo-responsive behavior.
In the next step, the self-assembly behavior of resulting triblock copolymer was examined at first. For instance, TP2 copolymer can be readily dissolved in THF, a follow-up water addition triggered the aggregation of hydrophobic PNBOC blocks with the formation of micellar nanoparticles at ambient temperature. Dynamic light scattering (DLS) measurements of the colloidal solution (0.2 g/L) revealed that the as-assembled nanoparticles had an intensity average hydrodynamic diameter, $D_h$, of 68 nm (Fig. 1a) and the formation of spherical nanoparticles was confirmed by TEM observation (Fig. 1c). Further, a micelle-to-unimer transition of the as-assembled micelles was observed once the colloidal solution was diluted with a large amount of good solvent of PNBOC block (e.g., DMSO), as evidenced by the DLS measurement (Fig. 1a). Specifically, in a DMSO/PBS mixture (v/v = 9/1), the $D_h$ of TP2 triblock copolymer dropped to <10 nm, in consistence with the formation of unimers (Fig. 1a). This result clearly demonstrated that the formation of micellar nanoparticles was dominated by noncovalent hydrophobic association of PNBOC blocks in a selective solvent. Besides TP2 with a medium PNBOC block, TP1 and TP3 bearing shorter and longer PNBOC blocks also self-assembled into micellar nanoparticles under an identical self-assembly condition, as evidenced by TEM observations (Fig. S2a, c).

### 3.2. Study on the photo- and thermo-responsive behavior of triblock copolymer assemblies

Given the presence of photoresponsive PNBOC block and thermo-responsive PNIPAM block within the triblock copolymers, we started our experiments with the examination of photoresponsive behavior of the triblock copolymer micelles in aqueous solution by irradiating the micellar solution with a hand-held UV lamp ($\lambda_{\text{max}} = 365 \text{ nm}$; 1 mW/cm²). After UV irradiation for 10 min at ambient temperature (e.g., 25 °C), although we did not observe significant changes in either $D_h$ or assembled morphology (Fig. 1b, d), an increased $D_h$ was achieved for the irradiated micellar solution after dilution with DMSO, as opposed to the micellar disassembly prior to UV irradiation. Similarly, the micellar aggregates were retained for TP1 and TP3 assemblies as well after UV irradiation (Fig. S2b, d). To understand this discrepancy, we examined the UV–vis absorption of TP2 micelles subjected to UV irradiation, which has been proved to be a robust technique to monitor the photoresponsive behavior of o-nitrobenzyl ester derivatives. Upon UV irradiation, the absorption peak centered at 246 nm underwent gradual increase and then leveled off after 10 min irradiation, while a constant drop of the absorbance at 273 nm was discerned, in line with the release of o-nitrosobenzaldehyde (o-NSBA) moieties under UV irradiation (Fig. 2a). Further, quantitative analysis of released o-NSBA using UV–vis spectroscopy manifested that over 97% of o-nitrobenzyl ester moieties were cleaved after 10 min UV irradiation (Fig. S3). Simultaneously, DLS analysis revealed a negligible change in $D_h$ and a slight decrease in corresponding scattering intensity (Fig. 2b). Notably, these results were not remarkably affected by altering the chain lengths of PNBOC blocks, and similar UV–vis absorbance spectra and DLS results were obtained for TP1 and TP3 colloidal solutions subjected to UV irradiation (Fig. S4). Specifically, there were no significant changes in $D_h$ but scattering intensities underwent steady decreases upon UV irradiation for both TP1 and TP3 copolymers (Fig. S4b, c). Nevertheless, irradiation time-dependent evolution of fluorescence spectra of NR loaded within the micellar cores exhibited a progressive fluorescence drop and then reached a plateau after 5 min irradiation (Fig. 2c, d), indicative of a significant polarity transition within the micellar cores after UV irradiation.

It is worth noting that, in our previous work regarding the photoresponsive behavior of NBOC moieties, we found that NBOC moieties experienced photo-cleavage under UV irradiation and were transformed to 2-aminoethyl methacrylate (AEMA) moieties, accompanied with the release of o-NSBA and CO₂. The newly generated primary amine groups mainly underwent spontaneous intra/interchain aminolysis reactions with partial primary amine groups being protonated, cross-linking the assemblies and simultaneously rendering the initially hydrophobic domains hydrophilic [23]. Thus, the increased $D_h$ in the DMSO/PBS mixture, slightly decreased scattering intensity, along with significantly dropped polarity within the micellar cores potently elaborated that the previously proposed traceless cross-linking mechanism took effect again in the present triblock copolymer system after UV irradiation.

In addition to photoresponsive behavior, the thermoresponsive performance of micellar nanoparticles were also appraised. It is well-established that PNIPAM undergoes thermo-induced collapse when the temperature is higher than the LCST [58]. Temperature-dependent...
transmittance at the wavelength of 800 nm of TP2 micelles revealed an abrupt decline when the temperature was higher than 34 °C at a TP2 concentration of 2.5 g/L, and the transmittance further decreased to approximately 0 when the temperature was higher than 43 °C (Fig. 3a), in good agreement with the temperature-responsive behavior of PNIPAM blocks. However, an evident rise in the scattering light intensity was only observed upon heating the TP2 micellar solution to higher than 40 °C. We attributed these distinct transition temperatures to the concentration-dependent temperature-responsive behavior of PNIPAM derivatives, which has been examined in previous studies [59]. Further, after UV irradiation for 10 min, the onset temperature of transmittance decrease and scattering intensity increase rose to ~38 °C and 43 °C, respectively (Fig. 3b), which should be ascribed to UV irradiation-induced hydrophobic-to-hydrophilic transition within the micellar cores, thereby elevating the phase transition temperature. Again, similar transitions were observed for TP1 and TP3 triblock copolymers (Fig. S5), although the transition temperatures varied from case to case and a lower hydrophilic PNBOC block led to lower LCST prior to UV irradiation but higher LCST after UV irradiation (Table 1). It was rather reasonable that triblock copolymers bearing longer hydrophobic PNBOC blocks had decreased LCST. Nevertheless, after UV irradiation, more hydrophilic moieties would be generated for the triblock copolymers having longer PNBOC blocks, resulting in higher LCST. Therefore, we can conclude that the thermoresponsive behavior of triblock copolymers was retained and, more importantly, the temperature-responsive performance could be further regulated by UV irradiation.

3.3. Rheological studies on stimuli-triggered sol-gel transitions

Building on the previous studies on the thermoresponsive poly(ethylene-alt-propylene)-b-poly(ethylene oxide)-b-poly(N-isopropylacrylamide) triblock copolymer, pre-organized micellar nanoparticles at an increased concentration (e.g., >5.0 wt%) could be further physically cross-linked due to the collapse and aggregation of PNIPAM coronas upon heating, resulting in the formation of hydrogels with local hydrophobic domains [43]. We hypothesized that the present dual-responsive triblock copolymers could also probably form hydrogels through a similar stepwise mechanism, given that the formation of micellar nanoparticles have been approved at a relatively low concentration (Fig. 1). To testify

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whether the dual-responsive triblock copolymers could form free-standing hydrogels, rheological experiments were then conducted at varying concentrations and the critical gelation temperature (CGT) was defined as the crossover point of shear storage (G′) and loss moduli (G″). As shown in Fig. 4a–c, all three triblock copolymers can form hydrogels before UV irradiation and the CGTs gradually decreased with increasing the hydrophobic PNBOC block lengths at the same concentrations. For example, the CGT0 of TP1 with the shortest PNBOC block was determined to be 48.3 °C while it decreased to 44.0 °C for TP2 with a medium PNBOC block and to 40.4 °C for TP3 with the longest PNBOC block at an identical concentration of 10.0 wt% (Fig. 4a–c). In addition to hydrophobic block length, the CGT was heavily influenced by the triblock copolymer concentrations as well and a higher concentration led to lower CGTs. Specifically, the CGT0 of TP2 triblock copolymer constantly dropped from 44.0 °C to 27.3 °C when the polymer concentrations increased from 10.0 wt% to 20.0 wt% (Figs. 4b, d–f and S6). This result was consistent with the LCST results (Figs. 3 and S5), although much lower triblock copolymer concentrations were applied for the LCST measurements (e.g., 2.5 g/L).

Interestingly, temperature-induced gelation process could be directly discerned by naked eye (Fig. 5). Although no hydrogels were formed at 25 °C for TP2 gelator, regardless of the concentrations in the range of 10.0–20.0 wt%, free standing hydrogels were achieved upon temperature increase, in line with the rheological experiments (Figs. 4 and S6). Notably, when TP2 concentration was higher than 12.5 wt%, hydrogel could be readily achieved at physiological temperature (i.e., 37 °C), implying potential applications in a biologically relevant scenario.

Notably, upon UV irradiation, the hydrophobic NBOC moieties were transformed to 2-aminoethyl methacrylate (AEMA) residues, cross-linking the initially hydrophobic cores and concurrently rendering them hydrophilic [23]. As a result, the UV irradiation-induced hydrophobic-to-hydrophilic transition within the micellar cores may play a critical role in the gelation process. Rheological measurements after UV irradiation were then performed to investigate the gelation performance of irradiated triblock copolymers. Interestingly, although hydrogels were still achieved for all three triblock copolymers, increased CGTs (termed as CGTUV) were observed at all the tested polymer concentrations (Fig. 4a–c). For example, at a fixed polymer concentration of 10.0 wt%, the CGTUV was determined to be 58.2, 54.4, and 51.2 °C for UV-irradiated TP1, TP2, and TP3, respectively, which were in sharp contrast to the CGT0 of 48.3, 44.0, and 40.4 °C without UV irradiation (Fig. 4). Notably, at 37 °C TP2 copolymer at concentrations higher than 12.5 wt% could form free standing hydrogels (Figs. 4 and 5). However, after UV irradiation, the CGTUV increased to over 37 °C thereby eliciting a gel-to-sol transition if the hydrogels were kept at 37 °C. Indeed, the UV irradiation-mediated gel-to-sol transition could be facilely discerned by naked eye (Fig. 5). Moreover, the subsequent sol-to-gel transition was observed subjected to a further temperature increase, in accordance with the rheological studies (Fig. 4). Taken together, photo- and thermo-responsive PNBOC-b-PNAM-b-PNIPAM triblock copolymers self-assembled into micellar nanoparticles at a relatively low concentration (e.g., 2.5 g/L), whereas micelles with PNBOC cores experienced further aggregations at elevated concentrations and high temperatures with the formation of hydrogels due to thermo-induced collapse and physical cross-linking of PNIPAM coronas. The resulting hydrogels could be transformed to a sol solution due to increased CGTs resulting from UV irradiation-induced cross-linking and hydrophobic-to-hydrophilic transition of micellar cores, while free-standing hydrogels were reformed upon a further temperature increase to above corresponding CGTUV. It should be noted that, besides UV-triggered gel-to-sol transition, the gel-to-sol transition could be also reversibly regulated by temperature variation by taking advantage of thermoresponsive phase transition of PNIPAM blocks. Therefore, we postulated that embedded payloads within the hydrogels could be regulated by either UV irradiation- or temperature-induced gel-to-sol transitions.

3.4. UV irradiation- and temperature-triggered synergistic release of GCT and DOX from hydrogels

Subsequently, the dual-responsive triblock copolymers were engineered as a new drug carrier and the controlled release of drug payloads were then studied. Note that both hydrophilic and hydrophobic payloads should be able to be incorporated due to the coexistence of hydrophobic domains and extramicellar hydrophilic gel networks. Firstly, the controlled release profiles of hydrophilic gemcitabine (GCT) and hydrophobic doxorubicin (DOX) from TP2 hydrogels were investigated. As

Fig. 4. (a–c) CGT values determined for (a) TP1, (b) TP2, and (c) TP3 micellar solutions before (black bars, CGT0) and after (red bars, CGTUV) UV irradiation for 10 min at varying concentrations. (d–f) Temperature-dependent storage moduli (G′) and loss moduli (G″) recorded for TP2 micellar solutions (pH 7.4 buffer) with concentrations of (d) 12.5 wt%, (e) 15.0 wt%, and (f) 20.0 wt% before and after UV irradiation; all rheological measurements were conducted with a heating rate of 1 °C/min at small strain (1%) and a frequency of 10 rad/s. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

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detailed above, at a TP2 concentration of 20.0 wt% and 37 °C, physical hydrogels of micellar networks were formed, allowing for loading hydrophobic DOX within the micellar cores and hydrophilic GCT within extramicellar aqueous phase. Prior to UV irradiation, both GCT and DOX were released very slowly and DOX was released even slower than GCT, presumably due to the drugs being trapped within hydrogel matrices and the slower release rate of DOX being ascribed to that DOX was confined within the hydrophobic micellar cores but GCT was located within the extramicellar hydrophilic gel networks. Therefore, the release of DOX was indeed implemented in a stepwise manner involving the diffusion into the extramicellar gel networks as the first step, exhibiting a slower release kinetics (Fig. S7). However, the release rates of both GCT and DOX were significantly accelerated after UV irradiation due to UV irradiation-induced concomitant gel-to-sol transition and hydrophobic-to-hydrophilic transition within micellar cores. Interestingly, albeit faster, the release rate of DOX was constantly slower than that of GCT even after UV irradiation, further confirming the distinct spatial locations of DOX and GCT within hydrogel matrices. Contrariwise, upon diluting the TP2 hydrogels to a concentration of 10.0 wt% at 37 °C, the hydrogels were transformed to a sol solution. Notably, the release of GCT was no longer controllable, regardless of with or without UV irradiation. We attributed this result to that the gel-to-sol transition released encapsulated GCT into the aqueous media and GCT small molecules can freely diffuse into dialysate (Fig. S8a). Conversely, the release of DOX can still be regulated by UV irradiation-triggered hydrophobic-to-hydrophilic transition within the micellar cores due to that DOX was retained within hydrophobic micellar cores even after dilution, despite the release rate being slightly faster than for releasing from hydrogels (Fig. S8b).

As detailed above, both hydrophilic and hydrophobic drugs can be controlled release independently from dual-responsive hydrogel-based vehicles. In the next step, we further investigated the co-release of GCT and DOX from TP2 hydrogels by means of UV irradiation-triggered gel-to-sol and hydrophobic-to-hydrophilic transitions within the micellar cores (Fig. 6). Similar to the release profiles of hydrogels encapsulating a single drug (Fig. S7), the release rates of GCT and DOX from TP2 hydrogels at a concentration of 12.5 wt% were remarkably improved by UV irradiation yet much slower release was obtained without UV irradiation (Fig. 6b, c). Moreover, a further study revealed that the release rates of GCT and DOX were negatively correlated to the increased TP2 concentrations, as reflected by the decreased cumulative drug release extents within the first 10 h against increased TP2 concentrations, irrespective of with or without UV irradiation (Fig. 6e, f). However, at a fixed TP2 concentration, extended UV irradiation led to faster release of both GCT and DOX, benefiting from the gel-to-sol phase transition and the formation of hydrophilic cores (Fig. 6e, f). Therefore, the
UV irradiation-induced concurrent gel-to-sol transition at physiological temperature and hydrophobic-to-hydrophilic transition within micellar cores could simultaneously trigger the release of both hydrophilic and hydrophobic payloads with synergistic effects from hydrogel matrices, exhibiting promising application for combinatorial therapy, which to our knowledge has not been achieved previously in the hydrogel-based drug delivery systems.

Building on the above results, both the polymer concentrations and UV irradiation had significantly effects on the release profiles of GCT and DOX. Note that the gel-to-sol transition could also be regulated by temperature variations that directly dominated the phase transition behavior of PNIPAM chains. Finally, the influence of temperature on the controlled co-release of GCT and DOX was evaluated. Specifically, at physiological temperature (37 °C) with a TP2 concentration of 15.0 wt%, the release of GCT and DOX was remarkably inhibited without UV irradiation and ~20% GCT and 10% DOX were released after 70 h incubation, respectively, in agreement with the release profiles at other TP2 concentrations (e.g., 12.5 wt% and 20.0 wt%). Again, the release rates of GCT and DOX were remarkably elevated after UV irradiation for 10 min by taking advantage of concurrent gel-to-sol transition and hydrophobic-to-hydrophilic transition within micellar cores at first, followed by a further aggregation of micellar nanoparticles at elevated concentrations and temperatures. We found that the gelation process could be dramatically tuned by a number of parameters including polymer compositions and concentrations, temperature variations, and UV

By sharp contrast, if heating the hydrogel solution to 45 °C (higher than CGTUV of TP2), no gel-to-sol transition would occur after UV irradiation, although UV-triggered hydrophobic-to-hydrophilic transition would still take place. Interestingly, the release of both GCT and DOX was substantially retarded even after UV irradiation. For example, ~20% GCT and 10% DOX were released within 70 h incubation both before and after UV irradiation (Fig. S10). This result clearly demonstrated that the gel-to-sol transition exerted more significant influence on the drug release performance from hydrogels as compared to hydrophobic-to-hydrophilic transition within micellar cores since no enhanced GCT and DOX release was achieved without gel-to-sol transition. Taken together, the release profiles of DOX and GCT can be delicately tuned by both light irradiation and temperature variations, in conjugation with the hydrophobic-to-hydrophilic transition within micelle cores and gel-to-sol transition of hydrogels, which could be of promising application in co-delivery of drugs with distinct solubilities.

4. Conclusions

In summary, we have demonstrated that thermo- and photo-responsive triblock copolymers, PNBOC-b-PNAM-b-PNIPAM, were successfully synthesized through sequential RAFT polymerizations and the resulting triblock copolymers could form hydrogels through a stepwise manner with the formation of PNBOC-cored micelles at first, followed by a further aggregation of micellar nanoparticles at elevated concentrations and temperatures. We found that the gelation process could be dramatically tuned by a number of parameters including polymer compositions and concentrations, temperature variations, and UV
light irradiation. The as-assembled hydrogels comprising both hydrophilic domains and hydrophobic aqueous phase can concomitantly encapsulate water-soluble GCT and water-insoluble DOX drugs. The following co-release of GCT and DOX could be regulated by either UV irradiation or temperature variations. The stepwise self-assembly strategy provides a convenient way to fabricate hydrogels capable of delivering two kinds of drugs with distinct water-solubilities, which is quite advantageous over hydrogel-based vehicles that can solely deliver hydrophobic payloads. Notably, although UV light was applied for the present study to trigger the gel-to-sol transition and drug release, nitrobenzyl ester derivatives have been approved to be responsive to near infrared (NIR) irradiation as well possessing better tissue penetration, [60,61] presaging promising applications of the current dual-responsive hydrogel platform in a truly physiological environment for site-specific drug release.

**Conflict of interest**

The authors declare no conflict of interest.

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**Appendix A. Supplementary data**

Supporting information Available: Additional characterization data of 1H NMR, TEM, absorption spectra, optical transmittance, and conformational Research Funds for the Central Universities (WK3450000001), and Specialized Research Fund for the Doctoral Program of Higher Education (SRFDP, 20123402130010) is gratefully acknowledged.

**References**


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