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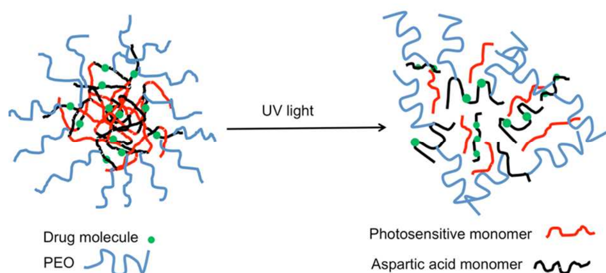

A photodegradable poly(ester amide) was developed. An amphiphilic graft copolymer derivative with paclitaxel conjugated via ester linkages formed micelles that released paclitaxel in response to UV light.

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ARTICLE TYPE

Photodegradable poly(ester amide)s for indirect light-triggered release of paclitaxel

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Stimuli-responsive micelles formed from amphiphilic copolymers are promising materials for the delivery of drugs and can potentially lead to enhanced biological properties and efficacies. Among the available stimuli, light is particularly attractive, as it can be highly localized in time and space. Described here is the development of a new fully photodegradable poly(ester amide) (PEA) backbone. Degradation in response to UV light was demonstrated by UV-vis spectroscopy, NMR spectroscopy, and size exclusion chromatography. Upon the incorporation of an L-aspartic acid-based monomer, providing carboxylic acid functional handles along the PEA backbone, the anticancer drug paclitaxel (PTX) was conjugated by an ester linkage and poly(ethylene oxide) was conjugated via an amide linkage to impart amphiphilicity. Micelles were prepared from the resulting amphiphilic copolymer and were demonstrated to break down in response to UV irradiation. This led to accelerated release of PTX, which is believed to result from the increased exposure of the ester linkages to water upon micelle disruption. The *in vitro* toxicities of both UV irradiated and non-irradiated micelles were also evaluated and compared to PTX in Cremophor EL/ethanol and to micelles without drug.

Introduction

In recent years, a wide variety of drug delivery systems have been developed to address the problematic properties of drug molecules, such as low aqueous solubility, short plasma circulation time, rapid *in vivo* degradation and systemic toxicity. The incorporation of therapeutics into polymeric systems such as nanoparticles, micelles, liposomes, or polymersomes has been shown to result in enhanced drug solubility/dispersibility, increased plasma half-life, reduced toxicity, and even enhanced therapeutic efficacy through targeting to the therapeutic site via the enhanced permeation and retention (EPR) effect or through the conjugation of active targeting ligands.[1-4] In an optimized case, a drug delivery system would retain its payload during systemic circulation and release it selectively at the therapeutic target.[5] With the aim of achieving this, stimuli-responsive polymeric materials have been developed for controlled-release drug delivery systems.[6-9]

Among the various polymeric systems that have been investigated for drug delivery, micelles are particularly attractive and have been widely investigated.[10-12] They are typically formed via the self-assembly of amphiphilic copolymers to form sub-100 nm structures comprising hydrophobic core regions that can serve as reservoirs for hydrophobic drugs, and hydrophilic shells that stabilize the micellar assembly. Their small size and hydrophilic surface help avoid recognition and uptake by macrophages of the reticuloendothelial system (RES) after intravenous administration, a crucial requirement for achieving prolonged residence time in the blood.[5] This feature allows accumulation in tumors and other diseased tissues due to the EPR effect.[13]

Polymer micelles that are responsive to environmental changes in pH,[14-16] temperature,[17,18] redox potential[19] or external stimuli such as ultrasound[20-22] or light[23-25] have been developed. Among the available stimuli, light is a particularly attractive trigger for use in controlling the behavior of biomaterials.[25] It can be applied externally and does not require specific chemical reagents or environmental conditions. In addition, many parameters such as light intensity and wavelength can be easily controlled and exposure areas with a resolution as small as 1 μm can be achieved under optimal conditions.[26] Polymeric micelles have been designed through the use of photochemical reactions to induce solubility changes in the hydrophobic block that lead to disintegration of the micelles and the release of physically encapsulated cargo in response to visible[27-30] or infrared (IR)[23,31] light. Alternatively, photocleavable *o*-nitrobenzyl moieties were incorporated into main chain of the hydrophobic block to achieve micelle disruption upon irradiation.[32,33]

Poly(ester amide)s (PEAs), polymers containing both ester and amide linkages in their backbones are promising materials for a wide range of biomedical applications as they can undergo degradation under a variety of enzymatic and non-enzymatic conditions and their monomers can be selected from a wide range of non-toxic metabolic intermediates.[34-42] We have demonstrated that PEA-poly(ethylene oxide) (PEO) graft copolymers can self-assemble in aqueous solution to form micelles, suggesting that these materials have potential as drug delivery vehicles.[43]

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Paclitaxel (PTX) is one of the most effective anti-cancer drugs used in clinical practice and exhibits strong cytotoxic activity against a variety of cancers, especially breast and ovarian cancer.[44] However, because of its low water-solubility[45] (0.25 µg/mL) PTX is currently formulated in a 50:50 mixture of Cremophor EL (CrEL) and ethanol, called Taxol, which has been found to result in hypersensitivity reactions.[44] This limitation has motivated an intensive search for better PTX delivery systems.[46-49]

We report here the development of a backbone photodegradable PEA and its application in a photosensitive micellar carrier with covalently conjugated PTX. Following the preparation and study of a model photodegradable PEA, L-aspartic acid moieties were incorporated into the PEA backbone and the resulting pendant carboxylic acid groups were used to covalently conjugate PEO by an amide linkage as well as the 2'-hydroxyl group of PTX via an ester linkage. It was anticipated that covalent immobilization of PTX should prevent an undesirable burst release of the drug, thus decreasing the background rate of drug release. As shown in Fig. 1, it was proposed that upon light-induced degradation of *o*-nitrobenzyl moieties in the hydrophobic PEA backbone, the micellar assemblies would be disrupted, resulting in increased exposure of the ester linkages between PTX and the PEA backbone to water, and thus accelerated hydrolytic release of PTX. Synthesis of the photodegradable polymers, their self-assembly into micelles, and their degradation were investigated. Studies were performed to evaluate the effect of photoirradiation on PTX release and the effect of photoinduced release on the *in vitro* toxicity of the micelles.

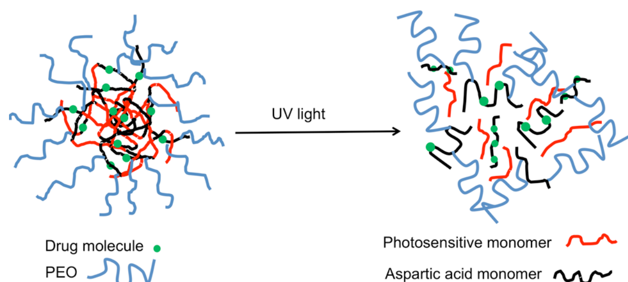
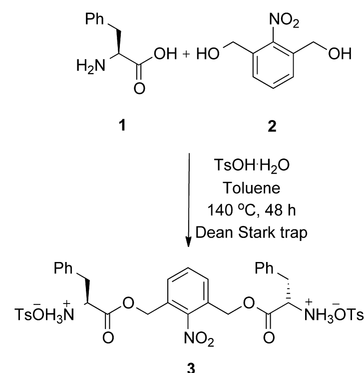


Fig. 1. PEA backbone degradation by UV light leads to micelle disruption and increased exposure of PTX-PEA ester linkages to water.

Results and discussion

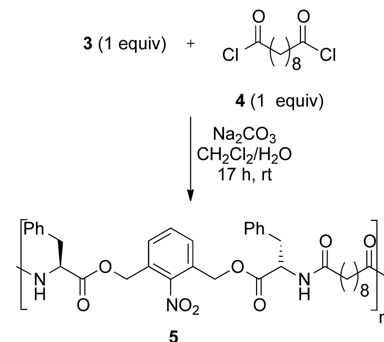
Synthesis of a photodegradable PEA

Prior to preparing the target photodegradable PTX delivery system, a model photodegradable PEA was synthesized and studied to confirm its degradability. To accomplish this, *o*-nitrobenzyl moieties were selected as the photodegradable units as they have been widely studied for a variety of applications.[25] An α-amino acid-based PEA backbone was selected due to the possibility to readily incorporate amino acids with pendant functional groups for the grafting of PTX as well as hydrophilic chains for micelle formation. First, as shown in Scheme 1, L-phenylalanine (**1**) was condensed with 2-nitro-1,3-benzenedimethanol (**2**) in toluene, in the presence of *p*-toluenesulfonic acid (TsOH) to provide the diester **3**. While in principle any amino acid could have been used, L-phenylalanine was ideal as the di-*p*-toluenesulfonic acid salt of this monomer is easily purified by recrystallization from water.



Scheme 1. Synthesis of monomer **3**.

As shown in Scheme 2, an interfacial polymerization was then performed by the addition of a solution of sebacoyl chloride (**4**) in CH_2Cl_2 to a solution of monomer **3** in an aqueous Na_2CO_3 to provide polymer **5**. In this design, photodegradable moieties were inserted at each monomer unit throughout the polymer. This should ensure that upon UV irradiation, it is possible to completely degrade the polymer. The structure of polymer **5** was confirmed through ^{1}H NMR and IR spectroscopic methods. Based on size exclusion chromatography (SEC) in DMF, the polymer had a weight average molecular weight (M_w) of 32500 g/mol and a dispersity (*Đ*) of 3.8. The high *Đ* was attributed to the step-growth polymerization mechanism, but was not a concern in this model system.



Scheme 2. Synthesis of photodegradable PEA **5**.

Photodegradation of the model polymer 5

The photodegradation of polymer **5** was studied by ultraviolet-visible (UV-vis) spectroscopy and NMR spectroscopy. For the UV-vis study, a solution of polymer in dioxane was irradiated using a medium pressure mercury lamp for 120 min and the UV-vis spectra were recorded every 20 min. As shown in Fig. 2a, over the degradation period, a decrease in absorbance was observed for the band at 260 nm while an increase at 310 nm was observed. This is consistent with previously reported results for this photodegradable moiety.[50-52]

To study the photodegradation by NMR spectroscopy, a solution of polymer **5** in DMSO-d_6 was irradiated with a medium pressure mercury lamp in a quartz NMR tube for 240 min and spectra were collected every 60 min. As shown in Fig. 2b, the multiplet at 5.15-5.24 ppm corresponding to the methylene

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groups of the *o*-nitrobenzyl ester in the polymer backbone decreased in intensity. Concomitantly, a new peak emerged at 8.08 ppm, corresponding to the expected *o*-nitrosobenzaldehyde product. The increased irradiation time required to effect complete cleavage of the polymer in the NMR experiment relative to the UV-vis study described above can be attributed to the higher concentration of the NMR sample. An SEC trace of the degraded sample showed no polymer peak, demonstrating complete degradation of the material (ESI†). Overall, these data demonstrated that polymer **5** degraded in the expected manner.

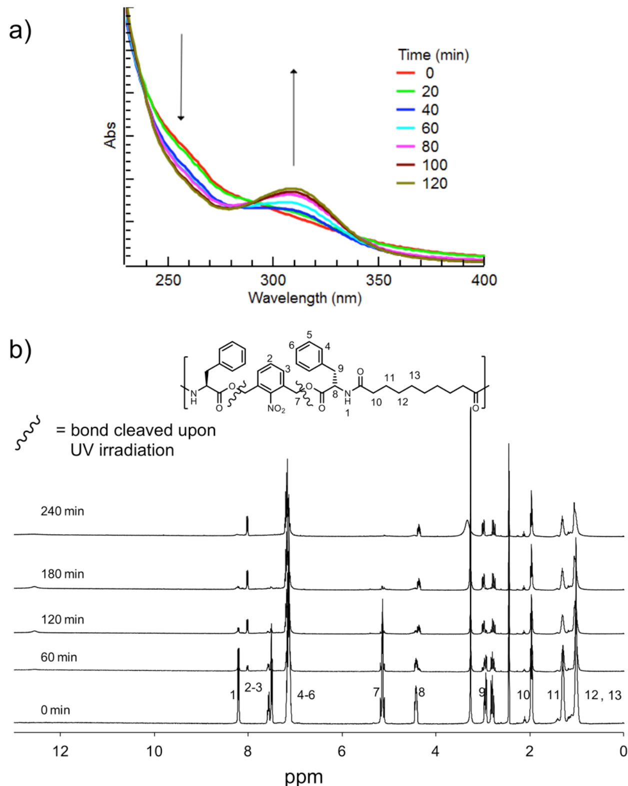
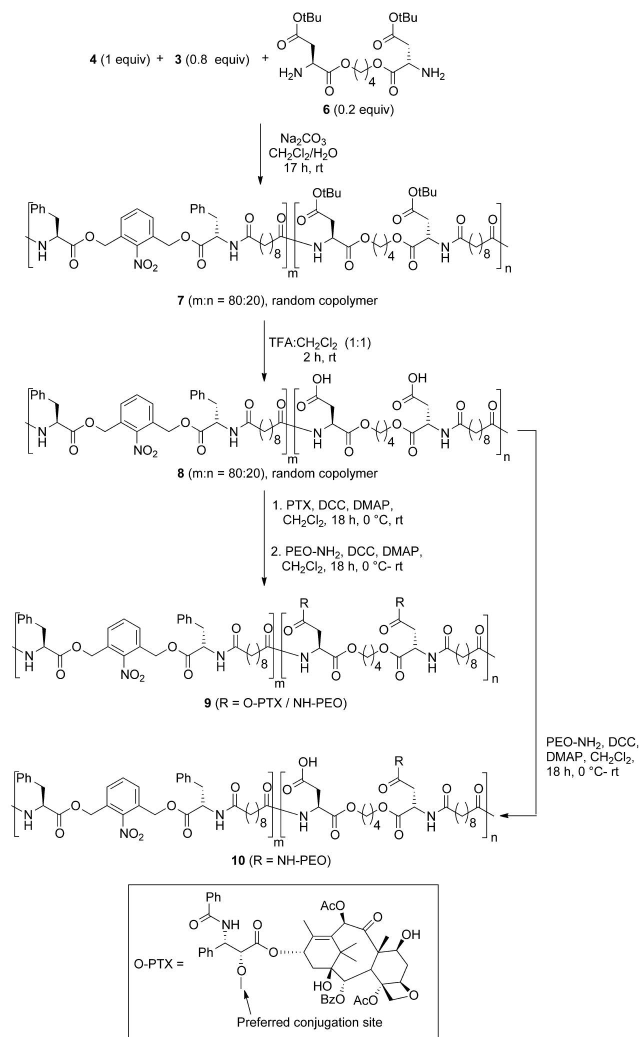


Fig. 2. Photodegradation of polymer **5**: a) UV-vis spectra of polymer **5** in dioxane following different UV irradiation times; b) ^{1}H NMR spectra of polymer **5** in DMSO-d_6 following different UV irradiation times.

Synthesis of a photodegradable amphiphilic PEA-PTX conjugate

Having demonstrated the ability of *o*-nitrobenzyl esters to cleanly impart photodegradability to the PEA backbone, the next step was to incorporate sites for the conjugation of PTX and for the grafting of hydrophilic chains to induce micellization. This was accomplished through the incorporation of L-aspartic acid units throughout the backbone. As shown in Scheme 3, an 80:20 ratio of monomer **3** to the L-aspartic acid-based diester **6**[53] was polymerized interfacially with sebacoyl chloride **4** to provide the random copolymer **7**. The 80:20 ratio was selected to obtain a sufficient number of functional handles throughout the polymer backbone, while still maintaining the maximum number of photodegradable sites. NMR spectroscopy confirmed the expected 80:20 ratio of monomers **3**:**6** in the product (ESI†). The resulting polymer had an M_w of 45600 g/mol and a $Đ$ of 2.9 as measured by SEC.

Using the strategy previously developed by our group for the synthesis of PEAs with pendant functional groups,[53] the *t*-butyl protecting groups on the pendant carboxylic acids of polymer **7** were removed through treatment with 1:1 TFA:CH_2Cl_2 to provide polymer **8** (Scheme 3). The photodegradation of polymer **8** was studied by ^{1}H NMR spectroscopy and the same changes observed for polymer **5** were also observed for this polymer, suggesting that the incorporation of monomer **6** does not interfere with the photodegradation process (ESI†).



Scheme 3. Synthesis of a photodegradable amphiphilic PEA-PTX conjugate.

The next step was the conjugation of PTX as well as the grafting of hydrophilic chains to induce micellization. As shown in Scheme 3, this was accomplished by first the reaction of **8** with 0.5 equiv. of PTX per pendant carboxylic acid moiety in the presence of *N*,*N*′-dicyclohexylcarbodiimide (DCC) and 4-(dimethylamino)pyridine (DMAP). This results in a hydrolytically cleavable ester linkage between the pendant carboxylic acid moieties of the polymer and the 2′-hydroxyl of PTX, which is generally more nucleophilic than the 7-hydroxyl because it experiences less steric hindrance.[49,54,55] PEO was selected as the hydrophilic graft, because of its well known biocompatibility in various therapeutics, as well as its stealthy characteristics, allowing it to prolong the blood circulation time

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of drug delivery systems.[56-58] The intermediate PTX conjugate was then reacted with 0.5 equiv. (per pendant carboxylic acid moiety) of amine terminated PEO (PEO-NH_2, 5000 g/mol), under the same conditions used for the conjugation of PTX to provide the final amphiphilic PTX conjugate **9**. It was noted that the order of PTX and PEO couplings is very important. If PEO conjugation was carried out first, the subsequent PTX coupling failed. This can likely be attributed to the steric influence of PEO, which may block access to unreacted carboxylates. Polymer **9** was purified by dialysis against a 50000 g/mol molecular weight cut-off membrane to remove ungrafted PEO. Based on ^{1}H NMR spectroscopy of polymer **9**, ~30% of the pendant carboxylic acid moieties were coupled to PTX and ~50% were coupled to PEO. The conjugate therefore contains 60 wt% PEO and 6 wt% PTX. The resulting polymer had an M_w of 56000 g/mol and a $Đ$ of 2.3 as measured by SEC, indicating that the dialysis procedure had resulted in some molecular weight fractionation. Control polymer **10** without PTX was prepared by the coupling of PEO-NH_2 directly to polymer **8**.

Preparation and characterization of micelles.

A nanoprecipitation method was used to prepare micelles from polymer **9**. The graft copolymer **9** was dissolved in THF, a good solvent for this polymer. Water was then added to induce the aggregation of the PEA to form the micelle core. Finally, the THF was removed by dialysis. The resulting micelle size was measured by dynamic light scattering (DLS). The z-average diameter and polydispersity index were 95 ± 5 nm and 0.16 respectively, and a representative DLS trace is shown in Fig. 3a. Through the encapsulation of the fluorescent probe nile red, the critical aggregation concentration (CAC) for the polymer was measured to be ~170 mg/L. TEM was also performed to verify the morphology and the size measured by DLS. As shown in Fig. 3c, solid spherical assemblies were indeed observed. The diameter of these micelles was found to be 60 ± 11 nm. The smaller diameter measured by TEM relative to that obtained by DLS can likely be attributed to their dehydrated state.

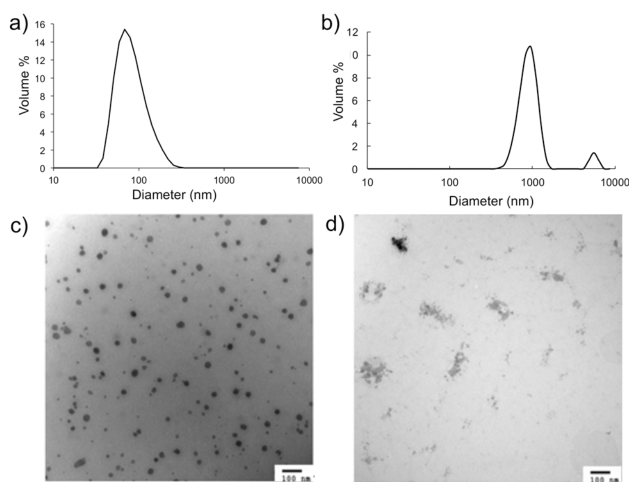

Fig. 3. a) DLS trace for micelles formed from copolymer **9** before UV irradiation; b) DLS trace for copolymer **9** micelles after 20 min of UV irradiation; c) TEM image of micelles formed from copolymer **9** before UV irradiation; c) TEM image of copolymer **9** micelles following 20 min of UV irradiation.

Photoinduced degradation of the micelles

After the preparation of micelles, their photodegradation behavior was studied. In this micellar system, photoirradiation should result in the disintegration of the micellar core due to main-chain degradation of the hydrophobic PEA backbone. Fast photodegradation of the micelles was expected as multiple photo-cleavable moieties were inserted as repeating units into the main chain of the polymer.[32] The fast disintegration of micelles from copolymer **9** was demonstrated by TEM (Fig. 3d). As described above, prior to irradiation, the micelles had a relatively uniform diameter of 60 ± 11 nm, while after 20 min of UV irradiation the micelle structures appeared to disappear and instead just some loose aggregates were observed. These aggregates are likely fragments of the hydrophobic backbone without PEO, which arise from the backbone degradation and are insoluble in water. DLS experiments confirmed the presence of aggregates with diameters of ~ 900 nm (Fig. 3b).

Photodegradation of the system was also studied by UV-vis spectroscopy. When performing this study in water, the results were complicated by the formation of these aggregates, which resulted in some turbidity. However, photodegradation of **9** in dioxane provided the expected changes in the UV-vis spectra (ESI†).

Photoinduced PTX release

The *in vitro* release profile of PTX from micelles formed from polymer **9** was evaluated in pH 7.4 phosphate buffer both with and without UV irradiation. As shown in Fig. 4, in the absence of UV irradiation, the release of PTX from the micelles was slow, with no burst release, and approximately 40% of the drug released over a period of 2 weeks. The slow release can be attributed to the requirement for ester bond hydrolysis to occur between PTX and the PEA backbone at the core of the micelle, where the environment is relatively hydrophobic. It should be noted that although ester hydrolysis would be expected to exhibit a first-order kinetic profile, the release kinetics in Fig. 4 actually appear to be zero-order. This can be attributed to the requirement for not only ester hydrolysis to occur, but also diffusion out of the micelle and subsequently across the dialysis membrane in order for it to be detected as "released". It is not possible to kinetically separate these different processes in this experiment.

Prior to performing the PTX release experiment with photoirradiation, it was important to confirm the stability of PTX to UV irradiation. An aqueous solution of PTX was irradiated, and the purity of the drug was evaluated by HPLC. This experiment indicated that there were no changes in PTX purity following up to 10 minutes of exposure (ESI†). Therefore, a 10 min irradiation time was chosen for the PTX release study. It was confirmed by UV-vis spectroscopy that even at the increased concentrations of micelles used in the release study this 10 min irradiation period was sufficient to obtain significant changes in the UV-vis spectrum of polymer **9** micelles, consistent with partial photodegradation of the material (ESI†). As shown in Fig. 4, after a 10 min UV irradiation of the micelles, there was an initial burst release of 20% of the PTX over the first 24 h. This can likely be attributed to the partial cleavage of the photolabile *o*-nitrobenzyl esters during the 10 min irradiation, which results in some degree of micelle breakdown and increased exposure of

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the ester linkages to water, resulting in accelerated hydrolysis. Thus, the release of PTX can be selectively achieved in the presence of light as a trigger. Following this initial photoinduced burst effect, the release profile followed that of the non-irradiated sample, which likely corresponds to the release of PTX from some intact micelles or from aggregates of polymer fragments that contain PTX.

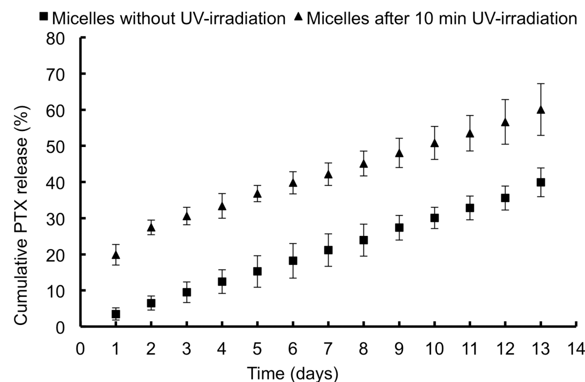

Fig. 4. *In vitro* release of PTX from UV-irradiated and non-irradiated micelles of polymer **9** incubated at 37 °C in phosphate buffer (pH 7.4). Data represent the mean and standard deviation of three independent experiments.

In vitro toxicity studies

The *in vitro* cytotoxic activities of the photodegradable PTX-micelles (polymer **9**) with and without UV irradiation were evaluated and compared with PTX in its CrEL/ethanol formulation in HeLa cells using an MTT assay after 72 h of incubation. Micelles formed from control polymer **10**, without PTX, as well as the CrEL/ethanol vehicle alone were also evaluated. As shown in Fig. 5a, PTX in CrEL/ethanol was toxic (as defined by a cell viability of <70% that of the control[59]) at concentrations of 2.9 nM and higher. The concentration of CrEL/ethanol vehicle required to formulate the higher concentrations of PTX was also toxic, suggesting that some toxicity at the higher PTX concentrations can likely be attributed to CrEL/ethanol.

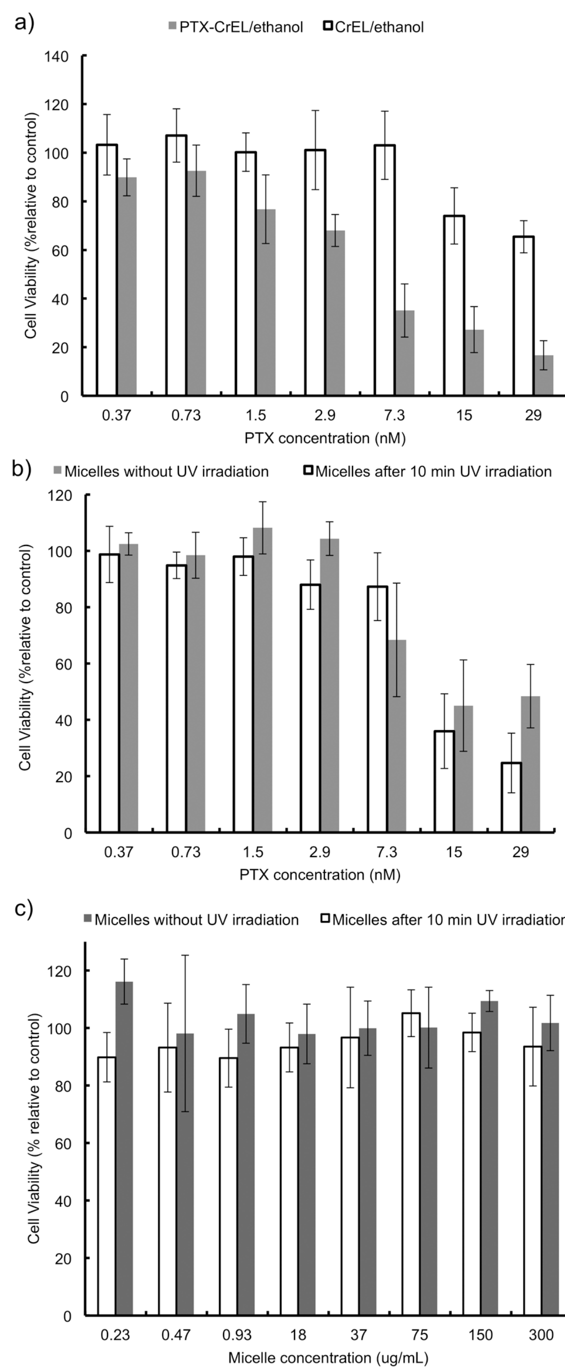

Fig. 5. *In vitro* cytotoxicity, as measured by MTT assays in HeLa cells following a 72 h incubation of a) PTX-CrEL/ethanol and the equivalent dose of CrEL without PTX; b) PTX-micelles from copolymer **9** with and without UV irradiation; c) Micelles from control polymer **10** with and without UV irradiation.

The PTX-micelles (polymer **9**) both with and without UV irradiation were less toxic than the CrEL/ethanol formulation, with toxicity observed at PTX concentrations higher than 7.3 nM (Fig. 5b). This can likely be attributed to the gradual release of drug from these systems. Statistically, the UV-irradiated and non-irradiated PTX-micelles exhibited similar toxicities at all of the investigated concentrations. Although the irradiated micelles may be expected to exhibit higher toxicity, this assay was not sensitive enough to detect this, perhaps because the differences in free drug

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concentration between the two systems (~ 3-fold) over the 72 h time frame of the assay may not be sufficient. It is also conceivable that the release kinetics of PTX measured in phosphate buffer are not the same as those within the cellular environment, where the presence of enzymes and pH gradients may also play a role in mediating ester hydrolysis. Nevertheless, these results do confirm that the photodegradable PTX-micelles are capable of releasing toxic concentrations of PTX *in vitro*.

Micelles formed from control polymer **10** without PTX, both with and without UV irradiation exhibited no significant toxicity at concentrations up to 300 μg/mL, much higher than the concentrations required to deliver toxic concentrations of PTX (Fig. 5c). This demonstrates that the toxicities of the PTX-micelles indeed arise from the PTX, and not from the delivery vehicle or its photodegradation products.

Conclusions

In conclusion, a backbone photodegradable PEA was prepared and was demonstrated to completely degrade upon UV irradiation. This chemistry was extended to the development of photodegradable PEA micelles with PTX conjugated via an ester linkage, based on the hypothesis that breakdown of the micelles induced by UV light would enable enhanced ester bond cleavage through increased exposure to water. The multifunctional graft copolymer was successfully synthesized and was used to prepare sub-100 nm micellar assemblies. Short UV irradiation times led to disruption of the assemblies, as demonstrated by TEM, DLS and UV-vis spectroscopy. It also resulted in a burst release of 20% of the PTX over the first 24 h, in comparison with the non-irradiated control, which exhibited only 3 % release over the same time period. This showed that it is indeed possible to trigger the release of PTX from this system by light. *In vitro* toxicity studies demonstrated that the drug-free micelles were non-toxic up to 300 μg/mL, while the PTX-micelles were highly toxic, with the irradiated and non-irradiated micelles having similar toxicities. The use of longer wavelength light through the incorporation of upconverting nanoparticles,[60] or the use of more photochemically stable drugs can potentially enable enhanced release of drug through the use of longer irradiation times or multiple irradiation periods. Overall, this work provides a new photochemically degradable PEA backbone that can serve as a platform for various applications. It also demonstrates the promise of stimulus-mediated micelle disruption as a means to alter the rate of cleavage of linkages between drugs and polymers, even when the linkages themselves are not directly responsive to the stimulus.

Experimental

General materials and procedures

Compound **2**[61], monomer **6**[53] and PEO-NH_2[62] with a molar mass of 5000 g/mol were synthesized as previously reported. Solvents were purchased from Caledon Labs (Georgetown, ON). PTX (>99%, P-9600) was purchased from LC Laboratories. All other chemicals were purchased from Sigma Aldrich (Milwaukee, WI). Unless noted otherwise, all chemicals were used as received. Anhydrous CH_2Cl_2 was obtained by distillation over CaH_2. Water was purified using an ultra pure water system (Barnstead EASYpure ® II). Dialysis was performed using Spectra/Por 6 regenerated cellulose membranes from Spectrum Laboratories (Rancho Dominguez, CA, USA). ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR spectra were obtained on a Varian Inova 400 spectrometer (Varian Canada Inc., Mississauga, ON). Chemical shifts are reported in parts per million (ppm) and are calibrated against residual solvent signals of DMSO-d_6 (δ 2.50, 40.25 ppm). All coupling constants (*J*) are reported in Hertz (Hz). Fourier transform infrared (FTIR) spectra were obtained using a Bruker Tensor 27 (Bruker Corporation, Milton, ON) using KBr pellets or thin films from CH_2Cl_2 on NaCl plates. High-resolution mass spectrometry (HRMS) was performed using a Finnigan MAT 8400 electron impact mass spectrometer. SEC data were obtained using a Waters 2695 Separations Module equipped with a Waters 2414 Refractive Index Detector (Waters Limited, Mississauga, ON) and two PLgel 5 μm mixed-D (300 mm × 7.5 mm) columns connected in series (Varian Canada Inc., Mississauga, ON). Samples (5 mg/mL) dissolved in the eluent, which was composed of 10 mM LiBr and 1 % (vol/vol) NEt_3 in DMF at 85 °C were injected (100 μL) at a flow rate of 1 mL/min. The calibration was performed using polystyrene standards. DLS was performed on a ZetaSizer Nano instrument from Malvern. UV-vis spectroscopy was performed on a Varian Cary 300 Bio UV–visible spectrophotometer. Photochemical irradiation was performed using a medium pressure mercury lamp (Hanovia S9 PC 451050 /805221), which was contained in a quartz water jacket, approximately 10 cm from the solution. PTX was quantified by isocratic reverse-phase HPLC using a Waters 2695 separations module (Waters, Milford, USA), a Waters 2998 Photodiiode Array Detector and a Jupiter C18 300A column (5 μm, 250 x 4.6 mm, Phenomenex, Torrance, USA). The mobile phase consisted of water/acetonitrile (60:40 vol/vol) with 0.1 vol% TFA. The flow rate was 1.0 mL/min and the detection wavelength was 228 nm. 100 μL of the analyte solution were injected. A calibration curve was prepared for PTX dissolved in water/acetonitrile (60:40 vol/vol).

Synthesis of monomer 3

A suspension of L-phenylalanine (**1**) (5.9 g, 35 mmol, 2.2 equiv.) and *p*-toluenesulfonic acid·H_2O (6.7 g, 39 mmol, 2.4 equiv.) in toluene (100 mL) was heated at reflux in a flask equipped with a Dean-Stark trap for 2 h to remove the residual water. To this suspension, 2-nitro-1,3-benzenedimethanol (**2**)[61] (3.0 g, 16 mmol, 1.0 equiv.) was added and the reaction mixture was heated at reflux for 48 h. The reaction mixture was then cooled and filtered to isolate the crude product, which was recrystallized from water (100 mL) to provide monomer **3** (6.6 g, 50%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.43 (br s, 6H), 7.62-7.66 (m, 2H), 7.48 (d, *J* = 8.2, 5H), 7.22-7.30 (m, 6H), 7.16-7.18 (m, 4H), 7.11 (d, *J* = 7.8, 4H), 5.23-5.31 (m, 4H), 4.39 (t, *J* = 8.0, 2H), 3.06-3.09 (m, 4H), 2.29 (s, 6H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 169.1, 148.3, 145.8, 138.2, 134.7, 132.4, 131.2, 129.7, 129.0, 128.5, 127.7, 125.9, 110.8, 63.3, 53.6, 36.3, 21.2. FTIR (KBr pellet, cm^{-1}): 3313, 2930, 2855, 1735, 1654. HRMS (m/z): calcd for $C_{26}H_{28}N_3O_6$, 478.1978; found, 478.1974 $[M+H]^+$.

Synthesis of polymer 5

Monomer **3** (1.0 g, 1.2 mmol, 1.0 equiv.) and sodium carbonate (0.25 g, 2.4 mmol, 2.0 equiv.) were dissolved in distilled water

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(30 mL). Sebacoyl chloride (**4**) (0.21 mL, 1.2 mmol, 1.0 equiv.) diluted in anhydrous CH_2Cl_2 (15 mL), was added dropwise over 30 min to the biphasic solution and was allowed to react for 17 h. Upon completion of the reaction, solvent was removed *in vacuo.* The resulting polymer was redissolved in DMF permitting filtration of the insoluble salts. The filtrate was then dialysed against DMF with MWCO 12000-14000 g/mol for 24 h, with changing of the dialysate every 12 h. The solvent was removed *in vacuo* to provide polymer **5** (0.41 g, 52%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.26 (d, J = 7.6, 2H), 7.55-7.64 (m, 3H), 7.21-7.23 (m, 10H), 5.16-5.24 (m, 4H), 4.45-4.51 (m, 2H), 2.96-2.99 (m, 2H), 2.82-2.88 (m, 2H), 2.00-2.04 (m, 4H), 1.33-1.36 (m, 4H), 1.07-1.45 (m, 8H). FTIR (KBr pellet, cm^{-1}): 3292, 2928, 2853, 1751, 1654, 1538. SEC: M_w = 32500 Da, $Đ$ = 3.8.

Synthesis of polymer 7

Monomer **3** (0.50 g, 0.61 mmol, 0.80 equiv.) and sodium carbonate (0.13 g, 1.6 mmol, 2.0 equiv.) were dissolved in distilled water (15 mL). Monomer **6** (0.065 g, 0.15 mmol, 0.20 equiv.) was dissolved in CH_2Cl_2 (5 mL) and added to the aqueous phase. After stirring for 30 min, sebacoyl chloride (**4**) (0.15 mL, 0.63 mmol, 1.0 equiv.) diluted in anhydrous CH_2Cl_2 (10 mL), was added dropwise over 30 min to the biphasic solution and was allowed to react for 24 h. This polymer was purified as described above for polymer **5.** (0.24 g, 62%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.26-8.30 (d, J = 8.1, 1.5H), 8.21-8.23 (d, J = 7.5, 0.5H), 7.55-7.64 (m, 2.4H), 7.15-7.25 (m, 8H), 5.16-5.24 (m, 3.2H), 4.50-4.52 (m, 0.5H), 4.45-4.51 (m, 1.5H), 4.03-4.07 (m, 0.8H), 2.98-3.01 (m, 1.6H), 2.84-2.88 (m, 1.6H), 2.50-2.70 (m, 0.9H), 2.00-2.08 (m, 4H), 1.36-1.59 (m, 10H), 1.03-1.07 (m, 10H). FTIR (thin film, cm^{-1}): 3292, 3061, 2928, 2853, 1751, 1653, 1533. SEC: M_w = 45600 g/mol, $Đ$ = 2.9.

Synthesis of polymer 8

Polymer **7** (0.23 g, 0.36 mmol, 1.0 equiv) was dissolved in CH_2Cl_2 (1 mL). TFA (1 mL) was added and the reaction mixture was stirred for 2 h. Toluene (5 mL) was then added and the solvent was removed *in vacuo* to provide the unprotected polymer in quantitative yield. The crude polymer was then washed with cold ethyl acetate (3 mL) three times to provide polymer **8** (0.21 g, 98%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.17-8.23 (m, 2H), 7.50-7.59 (m, 2.4H), 7.13-7.18 (m, 8H), 5.11-5.19 (m, 3.2H), 4.50-4.52 (m, 0.4H), 4.40-4.46 (m, 1.6H), 3.96-4.00 (m, 0.8H), 2.93-2.98 (m, 1.6H), 2.75-2.83 (m, 1.6H), 2.51-2.64 (m, 1H), 1.96 - 2.15 (m, 4H), 1.03-1.49 (m, 19H). IR (thin film, cm^{-1}): 3292, 2929, 2855, 1747, 1651, 1455.

Synthesis of polymer 9

Polymer **8** (0.20 g, 0.065 mmol, 1.0 equiv) was dissolved in distilled CH_2Cl_2 (4 mL). Then, PTX (0.030 g, 0.027 mmol, 0.50 equiv. relative to pendant COOH), DCC (0.018 g, 0.087 mmol, 1.2) and DMAP (0.0073 g, 0.059 mmol, 0.80 equiv. relative to pendant COOH) were added to the above solution at 0 °C. The reaction was carried out under stirring at 0 °C overnight and at room temperature for 8 h. The byproduct dicyclohexylurea was removed by filtration, and the resulting polymer was purified by dialysis against DMF using a 12000-14000 Da MWCO membrane for 24 h, with changing of the dialysate every 12 h to provide the intermediate PTX-conjugate (0.19 g, 83%). This intermediate (0.060 g, 0.017 mmol, 1.0 equiv) was dissolved in distilled CH_2Cl_2 (4 mL). PEO-NH_2 (5000 Da) (0.086 g, 0.017 mmol, 1.0 equiv. relative to pendant COOH), DCC (7.2 mg, 0.025 mmol, 1.5) and DMAP (2.1 mg, 0.017 mmol, 1.0 equiv. relative to pendant COOH) were added at 0 °C. The reaction was carried out under stirring at 0 °C overnight and then at room temperature for 8 h. The byproduct dicyclohexylurea was removed by filtration, and the resulting polymer was purified by dialysis against water using a 50000 Da MWCO membrane for 24 h, changing of the dialysate every 12 h, to provide polymer **9** (0.11g, 74%). ^{1}H NMR (400 MHz, DMSO-d_6): 9.16-9.21 (m, 0.08H), 8.28 (d, J = 7.0 Hz, 1.7H), 7.79 (d, J = 6.6 Hz, 0.26H), 7.78-7.86 (m, 0.43H), 7.41-7.65 (m, 3.4H), 7.13-7.27 (m, 8.6H), 6.29 (br s, 0.11H), 5.81 (d, J = 6.6 Hz, 0.11H), 5.52-5.58 (m, 0.10H), 5.39-5.43 (m, 0.27H), 5.16-5.24 (m, 3.2H), 4.88-4.93 (m, 0.20H), 4.62 (br s, 0.21H), 4.45-4.50 (m, 1.7H), 4.01-4.10 (m, 0.7H), 3.51(br s, 125H), 2.99-3.01 (m, 1.5H), 2.83-2.89 (m, 1.7H), 2.23-2.36 (m, 0.42H), 2.00-2.08 (m, 4.4H), 1.74-1.78 (m, 0.42H), 1.29-1.63 (m, 4.4H), 0.99-1.25 (m, 11H). IR (thin film, cm^{-1}): 3295, 3063, 3030, 2887, 1747, 1651, 1535. SEC: M_w = 56900 g/mol, $Đ$ = 2.3.

Synthesis of polymer 10

To polymer **8** (40 mg, 14 μmol of the pendant carboxylic acid groups, 1.0 equiv) in CH_2Cl_2 (4 mL) at 0 °C, PEO-NH_2 (5000 Da) (30 g, 6.0 μmol, 0.5 equiv), DCC (3.0 mg, 16 μmol, 1.2 equiv) and DMAP (1.5 mg, 10 μmol, 0.80 equiv) were added. After 12 h, the reaction was warmed to room temperature and stirred for another 8 h. The reaction was then filtered to remove the dicyclohexylurea byproduct and the filtrate was concentrated under vacuum to give the crude product. The polymer was then purified by dialysis against DMF using a 50000 g/mol MWCO membrane for 24 h, changing of the dialysate every 8 h, to provide polymer **10** (70 mg, 85%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.26-8.28 (d, J = 7.8, 1.5H), 8.17-8.19 (m, 0.4H), 7.55-7.64 (m, 3H), 7.18-7.24 (m, 8H), 5.16-5.24 (m, 3.2H), 4.47-4.57 (m, 2H), 3.92-4.08 (m, 0.9H), 3.30-3.70 (m, 44H), 2.98 - 3.01 (m, 1.7H), 2.82-2.88 (m, 1.6H), 2.50-2.70 (m, 1.3H), 1.98-2.18 (m, 4H), 1.35-1.58 (m, 6H), 1.03-1.49 (m, 10H). IR (thin film, cm^{-1}): 3293, 3030, 2855, 1747, 1651.

General procedure for monitoring photodegradation by UV-vis spectroscopy

The polymer solution was prepared at a concentration of 4 µg/mL in spectroscopic grade dioxane. In a quartz cuvette, 3 mL of solution was irradiated for various time intervals. UV-vis spectra were obtained at each time point. For micelles, the photodegradation was also studied by preparing micelles by the protocol described below and then diluting them in purified water to 4 µg/mL. They were also prepared and studied at a higher micelle concentration of 1.5 mg/mL. In this case, at each irradiation interval 100 µL of micelle solution was diluted to 3 mL with dioxane and the UV-vis spectrum was obtained.

General procedure for monitoring photodegradation by ^{1}H NMR spectroscopy

The polymer solution was prepared at the concentration of 7.0 mg/mL in DMSO-d_6. The solution was transferred to a quartz NMR tube and irradiated with UV light for various time intervals.

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^{1}H NMR spectra were obtained at selected time intervals.

Micelle formation

Polymer **9** (3.0 mg) was dissolved in THF (0.3 mL). The solution was stirred while distilled water was rapidly added to provide a final volume of 3 mL. THF was then removed by dialysis against distilled water using a 12000–14000 g/mol MWCO membrane. The micelle solution was then filtered with a microfilter (pore size: 0.45 µm, Tuffryn® syringe filter, PALL) to eliminate dust and aggregates prior to characterization.

TEM

The micelle suspension (prepared as described above, 20 µL of 0.1 mg/mL) was placed on a Formvar®/carbon grid and was left to stand for 5 min. The excess solution was then blotted off using a piece of filter paper. The resulting sample was dried under air overnight before imaging. Imaging was performed using a Phillips CM10 microscope operating at 80 kV with a 40 µm aperture.

Procedure for monitoring PTX photodegradation by HPLC

Five samples containing PTX in water at a concentration of 0.3 µg/mL were prepared. Each solution was transferred to a glass cuvette and irradiated. The concentration of PTX was measured as described above over 25 min in 5 min intervals.

***In vitro* release of PTX from PTX-micelles**

The *in vitro* release of PTX from the micelles of copolymer **9** was evaluated by a dialysis method. 3 mL of micelle suspension (PTX concentration: 0.60 µg/mL) were placed into a pre-swollen 3500 g/mol MWCO dialysis bag and immersed into 500 mL of 10 mM pH 7.4 phosphate buffer at 37 °C. Dialysis was performed with gentle stirring and the amount of PTX released into media was measured every 24 h. At each time point an aliquot of release media (15 mL) was withdrawn, and the complete volume of dialysate was removed and replaced with fresh media to ensure sink conditions. For analysis of the PTX concentration, the 15 mL aliquot was dried via lyophilization and the solid was redissolved in 1 mL of 60/40 water/acetonitrile. The solution was filtered with a 0.2 µm pore size (Tuffryn® syringe filter, PALL) filter into a vial for detection of the PTX concentration by HPLC as described above. The cumulative PTX release was calculated. The release experiments were conducted in triplicate, and the results presented are the mean ± standard deviation.

***In vitro* release of PTX from photoirradiated PTX-micelles**

The same procedure described above for the non-irradiated micelles was used except that the micelle solution was exposed to UV light for 10 min prior to beginning the dialysis.

***In vitro* cytotoxicity assay**

HeLa cells were cultured at 37 °C and 5% CO_2 in Dulbecco's Modified Eagle Medium (DMEM)(Invitrogen) supplemented with 10% (vol/vol) fetal bovine serum (Invitrogen) and antibiotics (penicillin and streptomycin, 100 units/mL each). The cells were seeded into a Nunclon® 96-well U bottom transparent polystrol plate at a density of 2500 cells per well in a final volume of 100 µL of DMEM. Cells were allowed to adhere for 24 h at 37 °C in a humidified incubator with 5% CO_2. Next, the growth medium was aspirated and was replaced with either the positive control - sodium dodecyl sulfate (SDS) in the cell culture medium at concentrations of 0.2, 0.15, 0.10, or 0.05 mg/mL, just the medium or the testing materials. Several materials were also evaluated. Micelles formed from polymer **9** (prepared as described above) with and without 10 min of UV irradiation at PTX concentrations ranging from 0.37 - 29 nM were evaluated. Micelles formed from control polymer **10** (prepared as for polymer **9**) were also evaluated at concentrations from 0.23 to 300 µg/mL both with and without 10 min of UV irradiation. For comparison, PTX solubilized in CrEL/ethanol was also prepared according to Lee *et al*.[63] In short, 6 mg of PTX was dissolved in 0.5 mL dehydrated ethanol and to this solution 0.5 mL of CrEL was added. This mixture was sonicated for 30 min. This sample also diluted to provide concentrations of PTX the same as those for the micelle systems. A control formulation without PTX was prepared using 1:1 CrEL/ethanol. This solution was diluted similar to PTX formulated with CrEL/ethanol. 8 replicates per concentration of each system were performed. After 72 h, the media was aspirated and replaced with 110 µL of fresh medium containing 0.5 mg/mL (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) (MTT) reagent. After 4 h, the media was carefully aspirated and the purple crystals were dissolved by addition of 50 µL of spectroscopic grade DMSO. After shaking (1 second, 2 mm amp, 654 rpm), the absorbance of the wells at 540 nm was read using an M1000-Pro plate reader (Tecan). The absorbance of wells not containing cells but treated by all of the above steps was subtracted as a background and the cell viability was calculated relative to wells containing cells that were exposed to just culture medium. No (0%) cell viability was detected for the cells exposed to the highest concentrations of the positive control SDS, confirming the sensitivity of the assay.

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1 N. Kamaly, Z. Xiao, P. M. Valencia, A. F. Radovic-Moreno and O. C. Farokhzad, *Chem. Soc. Rev.*, 2012, **41**, 2971.
2 S. Liu, R. Maheshwari and K. L. Kiick, *Macromolecules*, 2009, **42**, 3.
3 A. Rösler, G. W. M. Vandermeulen and H.-A. Klok, *Adv. Drug. Delivery Rev.*, 2012, **64 supp**, 270.
4 T. M. Allen and P. R. Cullis, *Adv. Drug. Delivery Rev.*, 2013, **65**, 36.
5 R. Langer, *Nature*, 1998, **392**, 5.
6 Z. Ge and S. Liu, *Chem. Soc. Rev.*, 2013, **42**, 7289.

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Polymer Chemistry Accepted Manuscript

7 E. G. Kelley, J. N. L. Albert, M. O. Sullivan and T. H. Epps III, *Chem. Soc. Rev.*, 2013, **42**, 7057.
8 M. Cohen Stuart, W. T. S. Huck, J. Genzer, M. Muller, C. Ober, M. Stamm, G. B. Sukhorukov, I. Szieifer, V. V. Trukruk, M. Urban, F. Winnik, S. Zauscher, I. Luzinov and S. Minko, *Nat. Mater.*, 2010, **9**, 101.
9 E. Fleige, M. A. Quadir and R. Haag, *Adv. Drug. Delivery Rev.*, 2012, **64**, 866.
10 H. Wei, R.-X. Zhou and X.-Z. Zhang, *Prog. Polym. Sci.*, 2013, **38**, 503.
11 Y. Lu and K. Park, *Int. J. Pharm.*, 2013, **453**, 198.
12 K. Miyata, R. J. Christie and K. Kataoka, *Reactive Funct. Polym.*, 2011, **71**, 227.
13 H. Maeda, H. Nakamura and J. Fang, *Adv. Drug. Delivery Rev.*, 2013, **65**, 71.
14 W. Gao, J. M. Chan and O. C. Farokhzad, *Mol. Pharm.*, 2010, **7**, 1913.
15 Y. Bae and K. Kataoka, *Adv. Drug. Delivery Rev.*, 2009, **61**, 768.
16 X. Guo, C. Shi, J. Wang, S. Di and S. Zhou, *Biomaterials*, 2013, **34**, 4544.
17 O. Soga, C. F. van Nostrum, M. Fens, C. J. F. Rijcken, R. M. Schiffelers, G. Storm and W. E. Hennink, *J. Controlled Release*, 2005, **103**, 341.
18 S. Q. Liu, Y. W. Tong and Y.-Y. Yang, *Biomaterials*, 2005, **26**, 5064.
19 P. Liu, B. Shi, C. Yue, G. Gao, P. Li, H. Yi, M. Li, B. Wang, Y. Ma and L. Cai, *Polym. Chem.*, 2013, **4**, 5793.
20 A. Marin, H. Sun, G. A. Husseini, W. G. Pitt, D. A. Christensen and N. Y. Rapoport, *J. Controlled Release*, 2002, **84**, 39.
21 A. Marin, M. Muniruzzaman and N. Rapoport, *J. Controlled Release*, 2001, **75**.
22 Z.-G. Gao, H. D. Fain and N. Rapoport, *J. Controlled Release*, 2005, **102**, 203.
23 A. P. Goodwin, J. L. Mynar, Y. Ma, G. R. Fleming and J. M. J. Fréchet, *J. Am. Chem. Soc.*, 2005, **127**, 9952.
24 H.-I. Lee, W. Wu, J. K. Oh, L. Mueller, G. Sherwood, L. Peteanu, T. Kowalewski and K. Matyjaszewski, *Angew. Chem. Int. Ed.*, 2007, **46**, 2453.
25 J.-F. Gohy and Y. Zhao, *Chem. Soc. Rev.*, 2013, **42**, 7117.
26 J. S. Katz and J. A. Burdick, *Macromol. Biosci.*, 2010, **10**, 339.
27 J. Jiang, X. Tong, D. Morris and Y. Zhao, *Macromolecules*, 2006, **39**, 4633.
28 J. Jiang, X. Tong and Y. Zhao, *J. Am. Chem. Soc.*, 2005, **127**, 8290.
29 O. Bertrand, J.-F. Gohy and C.-A. Fustin, *Polym. Chem.*, 2011, **2**, 2284.
30 G. Liu and C.-M. Dong, *Biomacromolecules*, 2012, **13**, 1573.
31 J. Babin, M. Pelletier, M. Lepage, J.-F. Allard, D. Morris and Y. Zhao, *Angew. Chem. Int. Ed.*, 2009, **48**, 3329.
32 D. Han, X. Tong and Y. Zhao, *Macromolecules*, 2011, **44**, 437.
33 D. Han, X. Tong and Y. Zhao, *Langmuir*, 2012, **28**, 2327.
34 D. Yamanouchi, J. Wu, A. N. Lazar, K. Craig Kent, C.-C. Chu and B. Liu, *Biomaterials*, 2008, **29**, 3269.
35 D. K. Knight, E. R. Gillies and K. Mequanint, *Biomacromolecules*, 2011, **12**, 2475.
36 S. H. Lee, I. Szinai, K. Carpenter, R. Katsarava, G. Jokhadze, C. C. Chu, Y. Huang, E. Verbeken, O. Bramwell, I. De Scheerder and M. K. Hong, *Coron. Artery Dis.*, 2002, **13**, 237.
37 Y. Huang, L. Wang, S. Li, X. Liu, K. Lee, E. Verbeken, F. van de Werf and I. de Scheerder, *Acute Card. Care*, 2006, **8**, 210.
38 K. M. Defife, K. Grako, G. Cruz-Aranda, S. Price, R. Chantung, K. MacPherson, R. Khoshabeh, S. Gopalan and W. G. Turnell, *J. Biomater. Sci., Polym. Ed.*, 2009, **20**, 1495.
39 X. Pang and C. C. Chu, *Polymer*, 2010, **51**, 4200.
40 P. Karimi, A. S. Rizkalla and K. Mequanint, *Materials*, 2010, **3**, 2346.
41 A. C. Fonseca, M. H. Gil and P. N. Simoes, *Prog. Polym. Sci.*, 2014, **39**, 1291.
42 J. Wang, K. G. Boutin, O. Abdulhadi, L. D. Personnat, T. Shazly, R. Langer, C. L. Channick and J. T. Borenstein, *Adv. Healthcare Mater.*, 2013, **2**, 1329.
43 G. J. Zilinskas, A. Soleimani and E. R. Gillies, *Int. J. Polym. Sci.*, 2012, **Article ID**, 564348.
44 E. K. Rowinsky and R. C. Donehower, *N. Engl. J. Med.*, 1995, **332**, 1004.
45 D. M. Vyas, H. Wong, A. R. Crosswell, A. M. Casazza, J. O. Knipe, S. W. Mamber and T. W. Doyle, *Bioorg. Med. Chem. Lett.*, 1993, **3**, 1357.
46 P. Ma and R. J. Mumper, *J. Nanomed. Nanotechnol.*, 2013, **4**, 6.
47 N. I. Marupudi, J. E. Han, K. W. Li, V. M. Renard, B. M. Tyler and H. Brem, *Expert Opin. Drug Saf.*, 2007, **6**, 609.
48 S. Singh and A. K. Dash, *Crit. Rev. Ther. Drug Carrier Syst.*, 2009, **26**, 333.
49 R. Tong and J. Cheng, *Angew. Chem. Int. Ed.*, 2008, **120**, 4908.
50 A. Nazemi, T. B. Schon and E. R. Gillies, *Org. Lett.*, 2013, **15**, 1830.
51 V. Yesilyurt, R. Ramireddy and S. Thayumanavan, *Angew. Chem. Int. Ed.*, 2011, **50**, 3038.
52 M. A. Kostiainen, D. K. Smith and O. Okkala, *Angew. Chem. Int. Ed.*, 2007, **46**, 7600.
53 K. M. Atkins, D. Lopez, D. K. Knight, K. Mequanint and E. R. Gillies, *J. Polym. Sci. Part A: Polym. Chem.*, 2009, **47**, 3757.
54 Z. Xie, H. Guan, X. Chen, C. Lu, L. Chen, X. Hu, Q. Shi and X. Jing, *J. Controlled Release*, 2007, **117**, 210.
55 K. Nam, H. Y. Nam, P.-H. Kim and S. W. Kim, *Biomaterials*, 2012, **33**, 8122.
56 J. M. Harris and R. B. Chess, *Nat. Rev. Drug Discov.*, 2003, **2**.
57 R. B. Greenwald, C. D. Conover and Y. H. Choe, *Crit. Rev. Ther. Drug Carrier Syst.*, 2000, **17**.
58 M. L. Nucci, R. Shorr and A. Abuchowski, *Adv. Drug. Delivery Rev.*, 1991, **6**, 133.
59 *International Standard 10993-5 Biological Evaluation of Medical Devices—Tests for In vitro Cytotoxicity*, 2009.
60 B. Yan, J.-C. Boyer, N. R. Branda and Y. Zhao, *J. Am. Chem. Soc.*, 2011, **133**, 19714.
61 M. R. Pavia, W. H. Moos and F. M. Hershenson, *J. Org. Chem.*, 1990, **55**, 560.
62 S. Zalipsky, *Bioconjugate Chem.*, 1995, **6**, 150.
63 S. C. Lee, C. Kim, I. C. Kwon, H. Chung and S. Y. Jeong, *J. Controlled Release*, 2003, **89**, 437.

Polymer Chemistry Accepted Manuscript