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Hybrid Polyester Self-Immolative Polymer Nanoparticles for Controlled Drug Release

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ABSTRACT: Delivery systems have been developed to address problematic properties of drugs, but the specific release of drugs at their targets is still a challenge. Polymers that depolymerize end-to-end in response to the cleavage of stimuli-responsive end-caps from their termini, commonly referred to as self-immolative polymers, offer high sensitivity to stimuli and have potential for the development of new high-performance delivery systems. In this work, we prepared hybrid particles composed of varying ratios of self-immolative poly(ethyl glyoxylate) (PEtG) and slowly degrading poly(D,L-lactic acid) (PLA). These systems were designed to provide a dual release mechanism consisting of a rapid burst release of drug from the PEtG domains and a slower release from the PLA domains. Using end-caps responsive to UV light and reducing thiols, it was found that triggered particles exhibited partial degradation, as indicated by a reduction in their dynamic light-scattering count rate that depended on the PEtG:PLA ratio. The particles were also shown to release the hydrophobic dye Nile red and the drug celecoxib in a manner that depended on triggering and the PEtG:PLA ratio. In vitro toxicity assays showed an effect of the stimuli on the toxicity of the celecoxib-loaded particles but also suggested it would be ideal to replace the sodium cholate surfactant that was used in the particle synthesis procedure in order to reduce the background toxicity of the delivery system. Overall, these hybrid systems show promise for tuning and controlling the release of drugs in response to stimuli.

INTRODUCTION

The controlled release of therapeutics using drug delivery systems has been studied in depth over the last several decades.¹−³ Drug delivery systems can provide improved solubility or dispersibility of hydrophobic drugs, improved bioavailability, and more specific targeting, leading to fewer adverse side effects. Polymers have been widely explored for drug delivery, as they can be engineered to assemble into a variety of nanostructures such as vesicles, micelles, and solid-core particles by tuning both their chemical structures and their processing conditions.⁴,⁵ Polymeric solid-core particles, composed of a hydrophobic polymer core that is stabilized in aqueous solution using a surfactant coating, are among the most widely investigated drug delivery systems due to their ease of preparation by various techniques such as emulsification or nanoprecipitation.⁶−⁸ A hydrophobic cargo molecule can be encapsulated within the hydrophobic cores of the particles during their preparation. Thus far, most polymeric particles have been composed of biodegradable polymers such as poly(lactic acid), poly(glycolic acid), poly(caprolactone), and their copolymers.⁹−¹¹ While these have been effective at improving the delivery of therapeutics, the hydrolytic degradation of polyesters is inherently a random process, and it is challenging to control its rate.¹² In some cases, degradation may be more rapid than desired, while in other cases it may be too slow. To address this limitation, stimuli-responsive particles have been developed and shown to release drug molecules in response to stimuli such as heat, reactive oxygen species, reducing agents, or changes in pH.¹³,¹⁴

Over the past decade, a new class of stimuli-responsive polymers was introduced. Often termed self-immolative polymers (SIPs), these polymers undergo complete end-to-end depolymerization in response to stimuli-mediated cleavage of end-caps from the polymer termini.¹⁵,¹⁶ This offers the potential to amplify responses to stimuli, making SIPs of significant interest for a wide range of applications including sensors,¹⁷−¹⁹ responsive plastics and coatings,²⁰−²² lithography,²³ and drug delivery.¹⁸,²⁴−²⁸ Backbones including polycarbamates,¹⁷,²⁴ polyaldehydes,⁹,²⁰,²³,²⁹−³⁴ polycarbonates,³⁵ polythiocarbamates,³⁶ and poly(benzyl ether)³⁷ have been developed, and end-caps responsive to stimuli including light,³⁵,²⁶,³¹ thiols,²⁶,³⁶ acid,³⁸ fluoride,²⁰,²¹,³⁹ heat,⁴⁰,⁴¹ and H₂O₂¹⁹,⁴² have been studied.

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Only a few studies thus far have explored the potential of SIPS for drug delivery. Our group demonstrated the triggered release of Nile red as a model drug from assemblies composed of poly(ethylene glycol) (PEG) block copolymers with a polycarbamate SIP based on 4-hydroxybenzyl alcohol and N,N-dimethylthelylene diamine. Almutairi and co-workers prepared nanoparticles from the same polymer backbone capped with a UV light-responsive end-cap and studied the release of Nile red from these particles in response to UV and two-photon irradiation. Liu and co-workers reported self-immolative vesicles based on polycarbamates composed of 4-aminoxybenzyl alcohol as the hydrophobic block and demonstrated vesicle disruption and the release of payloads including fluorophores, enzymes, and the drugs camptothecin and doxorubicin (Dox) in response to light or reducing conditions as stimuli. They also prepared amphiphilic hyperbranched polycarbamate−PEG copolymers and demonstrated the release of Dox that was covalently bound to the polymers as well as DNA that was complexed to cationic poly(2-(dimethylamino)ethyl methacrylate) chains conjugated to the periphery of the hyperbranched polymer. Vallet-Regi, Manzano, and co-workers used polycarbamates as blocking agents to control drug release from mesoporous silica nanoparticles. Despite this progress, the release of potentially toxic quinone methides and azainoquinone methides from these systems during their depolymerization is a possible drawback. To address this, we recently reported the study of micelles based on poly(ethyl glyoxylate) (PÉtG)−PEG block copolymers. PÉtG depolymerizes to ethyl glyoxylate, which is subsequently hydrolyzed to generate ethanol and glyoxylic acid (Figure 1a). Glyoxylic acid is a metabolic intermediate that can be processed in the liver and should be nontoxic in low concentrations. Previous work has demonstrated the nontoxicity of PÉtG degradation products in a Caenorhabditis elegans (invertebrate) model. We demonstrated that by simple tuning of the end-cap linker molecule between the PEG and PÉtG blocks it was possible to easily change the stimulus to which the micelles responded, and the triggered release of doxorubin and curcumin was demonstrated.

We describe here an alternative approach for formulating drug delivery systems using PÉtG. Using an emulsion method, we prepared sodium cholate-stabilized PÉtG particles. Furthermore, in order to add an additional level of control over the drug release, we explored particles composed of blends of PÉtG and poly(β,ε,-lactic acid) (PLA). It was envisioned that triggering of PÉtG degradation would result in erosion and rapid drug release from the PÉtG domains, leaving drug-loaded PLA for a sustained drug release. 6-Nitroveratryl carbonate-capped PÉtG was selected for its rapid responsiveness to UV light and served as an ideal model system. Disulfide-capped PÉtG was selected as a thiol-sensitive system, as elevated concentrations of reducing agents such as glutathione (GSH) are associated with the intracellular environment as well as hypoxic tumor tissue. The degradation of these hybrid particles in response to UV light and thios was studied by dynamic light scattering (DLS) and the release of Nile red as a probe. The drug celecoxib was encapsulated in the hybrid particles, and its stimuli-responsive release was explored. Initial in vitro experiments were performed to explore the behavior of the system in cells.

![Figure 1](image)

**Figure 1.** (a) Depolymerization scheme for PÉtG. (b) Chemical structures of the stimuli-responsive and control PÉtGs used in this study.

### RESULTS AND DISCUSSION

Preparation and Characterization of PÉtG and PÉtG−PLA Blends. PÉtGs with different end-caps (Figure 1b) were synthesized as previously reported, and the characterization data for the specific batches used here are included in the Supporting Information. PÉtG with a 6-nitroveratryl carbonate end-cap (PÉtG-NVOC) was selected because the NVOC can be easily and cleanly removed using UV light. Although UV light is not practical for most in vivo applications, this serves as a valuable model. PÉtG with a disulfide end-cap (PÉtG-disulfide) was selected because it can be triggered using reducing thios via an initial reduction of the disulfide, followed by cyclization of the resulting thiol to produce a cyclic thiocarbonate, releasing uncapped PÉtG. Increased concentrations of the reducing agent glutathione are found in intracellular compartments and in hypoxic tumor tissue, potentially allowing for the selective release of drugs in these environments. A control PÉtG had a benzyl carbonate end-cap (PÉtG-BnCO) that was not expected to be specifically responsive to stimuli but contained the same carbonate linkage to the PÉtG terminus as the other polymers.

In addition to the pure polymers, blends of PLA with the PÉtGs were also studied. Ratios of 9:1, 7:3, and 1:1 PÉtG:PLA were prepared. Lower ratios of PÉtG:PLA were not explored because it was anticipated that they would exhibit primarily nonstimuli-responsive behavior. They were prepared by codissolving the polymers in CH2Cl2. After removing the solvent, the blends were characterized by thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC). These experiments utilized PÉtG-NVOC, which behaves very
similarly to the other PEtGs in terms of its thermal properties. TGA showed that in agreement with the literature pure PLA was stable up to ~300 °C (Figure 2a). Pure PEtG with the NVOC end-cap was stable up to 200 °C, also in agreement with the reported value. PEtG is less thermally stable than PLA due to its susceptibility to depolymerize. A two-step thermal degradation was observed for each of the three blends, and the mass fractions lost in each step were consistent with the reported value. PEtG is less thermally stable than PLA due to its susceptibility to depolymerize. A two-step thermal degradation was observed for each of the three blends, and the mass fractions lost in each step were consistent with the reported value.31 PEtG is less thermally stable than PLA due to its susceptibility to depolymerize.

Particle Preparation. Particles were prepared by an emulsion—evaporation procedure. Various parameters including the surfactant identity, surfactant and polymer concentration, and sonication time were investigated with the aim of preparing particles of each polymer blend composition with the reported value.31 PEtG is less thermally stable than PLA due to its susceptibility to depolymerize. A two-step thermal degradation was observed for each of the three blends, and the mass fractions lost in each step were consistent with the reported value.31 PEtG is less thermally stable than PLA due to its susceptibility to depolymerize.

Figure 2. Thermal characterization data for PEtG-NVOC, PLA, and their blends: (a) thermogravimetric analysis and (b) differential scanning calorimetry.

First, DLS was used to study the degradation of the particles in response to stimuli. The DLS count rate is proportional to both the number of scattering particles in suspension and their mass if the attenuator of and laser position are fixed at constant values. A decrease in either the mass or number of particles arising from degradation would lead to a reduction in the count rate. Keeping the attenuator fixed, the count rate was measured initially and then at time points after introduction of the stimuli. For the PEtG—NVOC particles, the stimulus was the application of 2.8 mW/cm² of light radiation for 30 min, as we have previously found this irradiation time to be sufficient to achieve complete end-cap cleavage for other systems at similar concentration. For PEtG—disulfide particles, 7.7 mM dithiothreitol (DTT) was added as a reducing agent, as this concentration was effective in triggering the depolymerization of assemblies based on PEtG block copolymers. Control experiments involved the application of stimuli to non-responsive PEtG—BnCO control particles to account for any nonspecific effects of the stimuli as well as background degradation.

For particles with each ratio of PEtG—NVOC:PLA, there was a rapid decrease in count rate over the first hour following UV irradiation that likely corresponded to degradation (Figure 4a). In addition, there were plateaus in the count rates at percentages corresponding approximately to the percentage of PLA in the particles. Pure PEtG particles approached zero counts at the 24 h time point; 9:1 PEtG—NVOC:PLA particles degraded to ~10% of their original count rate and then remained constant; 7:3 PEtG—NVOC:PLA particles plateaued at ~30% of the initial count rate; and 50 wt % of PLA particles plateaued at ~50% of the initial count rate. Examination of the PEtG T_g from −8 to −1 °C and simultaneous decrease in the PLA T_g from 46 to 40 °C as the PEtG content decreased to 50% suggested that some degree of mixing between the two polymers also occurred.

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As observed in our previous work with PEtG−PEO block copolymer assemblies, the reduction in count rate occurred more slowly for the PEtG−dilsulfide:PLA systems in response to DTT (Figure 4b). This may result from the reduction reaction being slower than the photochemical end-cap cleavage. In addition, DTT is likely only able to react with end-caps at the particle surface, whereas UV light can penetrate the particles to some extent. Again, the particles with higher PEtG content exhibited larger reductions in the count rate over the experiment in general, corresponding to more complete degradation. Both pure PEtG−dilsulfide and 1:1 PEtG−dilsulfide:PLA particles underwent only minor fluctuations in Z-average diameter and PDI, suggesting that even while PEtG was eroded, resulting in a decrease in count rate, there were still particles remaining at 48 h (Tables S7−S8). The count rates from the pure PLA particles and all of the PLA:PEtG-BnCO particles remained near 100% of their original values over 48 h.

The particle degradation was also studied using a fluorescent probe. Nile red is a hydrophobic dye that is highly fluorescent in hydrophobic environments but undergoes aggregation and fluorescence quenching in water. Therefore, Nile red encapsulated within the particles fluoresces, and as it is released into the surrounding aqueous environment, the fluorescence decreases. Nile red was encapsulated using the same particle preparation method described above except that 1 wt % of Nile red was used. Nile red encapsulation was confirmed using a fluorescent microscope. Nile red encapsulated within the particles fluoresces, and as it is released into the surrounding aqueous environment, the fluorescence decreases.

Table 1. Z-Average Diameters and PDI Values Measured by Dynamic Light Scattering for Particles Prepared from PEtG−NVOC and PLA

| Blend        | Z-average diameter (nm) | PDI     |
|--------------|-------------------------|---------|
| pure PEtG    | 144 ± 13                | 0.19 ± 0.07 |
| 9:1 PEtG:PLA | 137 ± 19                | 0.26 ± 0.11 |
| 7:3 PEtG:PLA | 128 ± 7                 | 0.11 ± 0.03 |
| 1:1 PEtG:PLA | 133 ± 8                 | 0.12 ± 0.03 |
| pure PLA     | 132 ± 10                | 0.13 ± 0.04 |

Figure 4. Count rate as a function of time after application of stimulus for: (a) particles composed of PEtG−NVOC:PLA or controls PEtG−BnCO:PLA, where the stimulus was UV light and (b) particles composed of PEtG−dilsulfide:PLA or controls PEtG−BnCO:PLA, where the stimulus was DTT. The error bars correspond to standard deviations.
red relative to polymer was incorporated into the CH$_2$Cl$_2$ phase. The initial fluorescence intensity was measured for each sample, and then UV light and DTT were applied as stimuli to PEtG−NVOC:PLA, PEtG−disulfide:PLA, and PEtG−BnCO:PLA particles as described above, and the fluorescence was measured over 24 h. PEtG−NVOC:PLA particles exhibited a significant burst release of Nile red within the first hour after being exposed to UV light (Figure 5a). This can be attributed to the PEtG fraction of the particles being degraded. Similar to the DLS study, the fluorescence plateaued at higher percentages of the initial fluorescence as the PLA content of the particles increased. This suggests that it may be possible to selectively release a payload from the PEtG phase of the particles, while retaining the load in the PLA phase. After application of light to PEtG−BnCO:PLA particles, small reductions in the Nile red fluorescence were observed. This could be attributed to some background degradation of these systems, such as hydrolysis of the carbonate linkage between the end-cap and polymer, which would induce depolymerization. However, based on the DLS results described above, it likely corresponds to the passive diffusion of some Nile red from the peripheries of the particles or other factors that would alter the environment of Nile red.

As observed in the DLS study, Nile red release from the PEtG−disulfide:PLA particles following application of DTT was slower than for the light-responsive particles (Figure 5b). Again, this can likely be attributed to slower end-cap cleavage. However, this experiment also suggested that the extent of Nile red release was dependent on the PLA content. The levels of release from the PEtG−BnCO:PLA particles treated with DTT were similar to those observed following UV irradiation of the same particles. Overall, the DLS and Nile red release data show that it is possible to trigger degradation of the particles specifically by matching the stimulus with the end-cap and that it appears possible to trigger different percentages of degradation by tuning the PLA content in the particles.

**Drug Loading and Triggered Release.** Celecoxib was selected as an initial drug for investigating loading and release. It is a widely used nonsteroidal anti-inflammatory and has shown potential in cancer therapy. However, it exhibits low aqueous solubility and adverse gastrointestinal and cardiac effects when administered systemically. It was loaded into PEtG−NVOC:PLA, PEtG−disulfide:PLA, and PEtG−BnCO:PLA particles during their preparation by the addition of 30 wt % of drug relative to polymer in the CH$_2$Cl$_2$ phase of the emulsion. Unloaded drug was removed by filtration and dialysis. The loading efficiencies ranged from 17 to 33% and the drug content from 4.9 to 8.8 wt %. However, there were no statistically significant differences between the loadings of the different PEtG:PLA ratios (Table S9). The particle diameters were very similar to those of the unloaded particles described above (Tables S10–S12, Figures S8–S10).

Celecoxib release in response to UV light and DTT was investigated for the PEtG−NVOC:PLA and PEtG−disulfide:PLA systems, respectively, with the absence of stimuli and the application of stimuli to 100% PEtG−BnCO:PLA particles serving as controls. Initially, measurement of the released celecoxib posed a significant challenge due to the low water solubility of this drug. Upon application of the stimuli, the scattering of the suspension clearly decreased, indicative of degradation, but at the same time, the celecoxib precipitated (Figure S11). Due to this precipitation, use of a protocol involving dialysis into buffer resulted in measurement of the precipitated drug’s dissolution rate rather than its actual release. The addition of Polysorbate 20, which we have used successfully with celecoxib in other studies in our lab, resulted in rapid release from control systems, suggesting that it destabilized the particles. Thus, the released and precipitated drug was instead centrifuged, dissolved in CH$_3$CN, and then quantified by UV−visible spectroscopy based on its absorbance at 253 nm.

Application of UV light as a stimulus to the PEtG−NVOC systems resulted in a rapid release of celecoxib (Figure 6a). The percentage of released drug ranged from 15 to 82% in the first 30 min, depending on the PEtG−NVOC:PLA ratio. Over the first 4 h, 26−89% of celecoxib was released. It is possible that the remaining ~10% of the drug in the case of the pure PEtG−NVOC system can be solubilized by the sodium cholate surfactant in the system. The percentage of drug released depended on the PEtG:PLA ratio, with higher PEtG content generally leading to increased drug release. However, more release was observed for the 1:1 system than for the 7:3 system, and the reason for this is not clear. Less release was observed from pure PLA particles (Figure 6a), controls consisting of nonirradiated PEtG−NVOC:PLA or UV-irradiated particles composed of PEtG−BnCO:PLA (Figure 6b). Interestingly, the irradiated particles generally had higher release than the nonirradiated particles, suggesting the possibility of a non-specific reaction involving UV light. However, this was not observed in the DLS or Nile red experiments.

The addition of DTT to the PEtG−disulfide systems also resulted in the rapid release of celecoxib with similar trends.
observed with respect to the PEtG:PLA ratio as for the PEtG−NVOC systems (Figure 6c). However, consistent with the DLS and Nile red studies, this release was slower than for the UV light-triggered release, with 7−44% of celecoxib released over the first 4 h and 32−80% released over 24 h, again suggesting end-cap cleavage as the rate-limiting step. Again, control PLA particles (Figure 6c), PEtG−disulfide:PLA without DTT added and PEtG−BnCO:PLA (Figure 6d) with DTT added, exhibited slower release than the triggered systems. Nevertheless, the level of release from the control systems appeared to be relatively high, ranging from 18 to 49% over 4 h in the UV light experiment and 21−46% over 24 h in the DTT experiment. For this reason, we assessed the composition of the precipitated and centrifuged material from the 4 and 24 h time points by dissolving it in CD3CN and analyzing it by 1HN M R spectroscopy (Figures S12−S20). By comparison of the spectra with those of celecoxib, PLA, and PEtG, it was found that after application of DTT to the PEtG−disulfide:PLA particles the pellet was primarily composed of celecoxib and not polymer, confirming its release from the particles and effective isolation by centrifugation. On the other hand, the pellets obtained from PEtG−disulfide:PLA particles not exposed to DTT or PEtG−BnCO:PLA contained mostly intact PEtG and PLA, indicating that some drug-loaded particles were destabilized and isolated during the centrifugation procedure, resulting in apparent release percentages for the controls that are likely higher than the true celecoxib release rates. Thus, the true leakage rates of celecoxib from the untriggered particles are likely lower than those reflected in Figures 6b and 6d. Nevertheless, this was still the most effective protocol to measure and demonstrate the triggered release of celecoxib from these systems and the ability to tune the release profile by tuning the PLA content.

**In Vitro Studies.** As noted above, celecoxib has been shown to exhibit anticancer properties, and there is value in releasing anticancer drugs specifically in cancer cells in order to mitigate the side effects that can result from their systemic administration. Therefore, the MDA-MB-231 breast cancer cell line was selected to evaluate the toxicities of different particles in both the absence and presence of stimuli using a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. This study focused on the systems composed of pure PEtG rather than blends with PLA, in order to maximize the effect of the stimuli.

Following a 48 h incubation of the drug with cells, free celecoxib was found to have an IC50 value of ~30 μg/mL in this cell line (Figure 7a). When celecoxib-loaded PEtG−NVOC particles were incubated with the cells for 48 h in the absence of any stimuli, they were found to be somewhat less toxic than the free drug, with an IC50 value near the maximum concentration studied of ~75 μg/mL (concentration of loaded drug). On the other hand, when celecoxib-loaded PEtG−NVOC particles were incubated with the cells for 24 h (to allow uptake into cells), irradiated with 360 nm light (LED flashlights, 90 mW/cm2) for 15 min, and then incubated an additional 24 h, the IC50 was reduced to ~30 μg/mL, similar to that of free celecoxib. This is consistent with the release of drug from the system. It should be noted that cells exposed to 360 nm light,
but no particles, did not exhibit any significant reduction in metabolic activity relative to nonirradiated cells, showing that this short irradiation process did not alone lead to toxicity. The toxicity of PEtG−NVOC particles without drug was also studied. The particles began to exhibit some toxicity at concentrations of 500 μg/mL and above (Figure 7c). Initially this was surprising as the PEtG and its degradation products were expected to be benign. However, when we studied the toxicity of the free sodium cholate, the surfactant used to stabilize the particles, we found that it began to cause toxicity at ∼300 μg/mL (Figure 7d). Based on mass recovery studies, we found that our particles were composed of ∼1.5:1 mass ratio of sodium cholate:PEtG. Therefore, despite cholic acid being one of the major bile acids produced in the liver, in this assay it is likely that most of the toxicity arose from the sodium cholate:PEtG. Therefore, despite cholic acid being one of the major bile acids produced in the liver, in this assay it is likely that most of the toxicity arose from the sodium cholate:PEtG. In addition, based on the drug loading of ∼8 wt % for the PEtG−NVOC system (1000 μg/mL of PEtG−NVOC needed to deliver 80 μg/mL of celecoxib), some of the toxicity arising from the nonirradiated celecoxib-loaded PEtG−NVOC system (Figure 7a) likely resulted from the delivery system itself (due to the cholate). The effect of the UV light would perhaps be larger were this not the case.

The PEtG−disulfide system was studied in a similar manner, except that in this case the reducing agent glutathione (10 mM) was added as the stimulus following 24 h of incubation, and then the system was incubated for an additional 24 h. As for the PEtG−NVOC system, the triggered system and free celecoxib had similar IC_{50} values of ∼30 μg/mL, while the celecoxib-loaded PEtG−disulfide without GSH added had a somewhat higher IC_{50} value of ∼60 μg/mL (Figure 7b). Thus, it appears that the GSH has a modest effect.

■ CONCLUSIONS

Blends of PLA with self-immolative PEtG were studied for the preparation of drug delivery particles. UV light-responsive, thiol-responsive, and nonresponsive control end-caps were used. Thermal studies suggested that PEtG and PLA were mainly phase separated, as T_g values of both blocks were observed using DSC. Using an emulsification−evaporation procedure with sodium cholate as a surfactant, we prepared particles with diameters of 130−150 nm. Upon application of the appropriate stimuli, the PEtG portions of the particles degraded, resulting in partial degradation of the systems as a function of the PEtG:PLA ratio. Using Nile red as a fluorescent probe and celecoxib as a drug, it was demonstrated that the triggered PEtG depolymerization could be used to release a portion of the loaded drug, leaving the remaining drug encapsulated in the PLA phase. The fraction of rapidly released drug could be tuned based on the PEtG:PLA ratio. Celecoxib-loaded PEtG particles with UV light and thiol-responsive end-caps were investigated in MDA-MB-231 breast cancer cells. Triggering of these systems with their corresponding stimuli in the presence of cells led to toxicities similar to that of free celecoxib and somewhat higher than the corresponding untriggered systems. However, differences between the triggered and untriggered systems were reduced by some background toxicity of the systems at high concentrations that likely results from the sodium cholate surfactant. In the future,

Figure 7. Metabolic activities, measured by MTT assays, of MDA-MB-231 cells following 48 h exposure to varying concentrations of (a) free celecoxib compared with celecoxib encapsulated in PEtG−NVOC particles without stimulus or with UV light applied for 15 min at 24 h; (b) free celecoxib compared with celecoxib encapsulated in PEtG−disulfide particles without stimulus or with 10 mM glutathione added at 24 h; (c) particles composed of either PEtG−NVOC or PEtG−disulfide without drug; and (d) sodium cholate. The error bars correspond to standard deviations.
this limitation can be mitigated by changing the surfactant, increasing the drug loadings, or using a more potent drug that requires less drug and vehicle to achieve the desired biological effect.

### METHODS

**General Materials and Procedures.** PEtG–NVOC ($M_n = 70$ kg/mol, $D = 2.4$), PEtG–BnCO ($M_n = 56$ kg/mol, $D = 1.6$), and PEtG–disulfide ($M_n = 24$ kg/mol, $D = 1.4$) were prepared as previously reported.$^{31,38}$ Their molar mass data were obtained from size exclusion chromatography (SEC) in THF at 1 mL/min using a Viscotek GPC Max VE2001 solvent module equipped with a Viscotek VE3580 RI detector operating at 30 °C, two Agilent Polypropylene (300 × 7.5 mm) columns, and a Polypropylene guard column (50 × 7.5 mm). A calibration curve was obtained using polystyrene standards. Sodium cholate hydrate, GSH, DTT, Nile red, and PLA (18–28 kg/mol) were obtained from Sigma-Aldrich.Celecoxib was purchased from Ontario Chemicals, Inc. TGA was performed on a Q50 from TA Instruments at a heating rate of 10 °C/min from 35 to 500 °C under a nitrogen atmosphere. DSC was performed using a Q2000 from TA Instruments (New Castle, DE). The heating/cooling rate was 10 °C/min from −75 to +120 °C. Data were obtained from the second heating cycle. Fluorescence spectra were obtained using a QM-4 SE spectrometer from Photon Technology International equipped with double excitation and emission monochromators. UV–visible spectra were obtained on a Varian UV/vis Cary 300 spectrophotometer. Ultrapure water was obtained from a Barnstead EASYpure II system. Spectra/Por regenerated cellulose membranes were used for dialysis. 0.22 μm Acrodisc Syringe Filters (nonpyrogenic) with 13 mm diameter were used to filter particle suspensions for analysis. DLS was performed using a Zetasizer Nano ZS instrument from Malvern Instruments at 25 °C at a concentration of 0.1 mg/mL of polymer assemblies. The Z-average diameter and PDI index for each series of particles were measured in triplicate. TEM imaging was done using a Phillips CM10 microscope operating at an acceleration voltage of 80 kV with a 40 μm aperture. An amount of 20 μL of particle suspension (1.0 mg/mL) was placed on a copper grid, and the excess liquid was wicked off with a Kimwipe. The resulting sample was air-dried for 24 h before imaging.

**Preparation of PLA–PEtG Blends.** Blends of 9:1, 7:3, and 1:1 PEtG:PLA were prepared by combining 9.0, 7.0, and 5.0 mg of PEtG with 1.0, 3.0, or 5.0 mg of PLA, respectively. These polymer samples were then dissolved in CH$_2$Cl$_2$ (1 mL) and stirred for 30 min to allow for complete mixing of the polymers. The solvent was removed in vacuo for analyses.

**Preparation of Particles.** PEtG, PLA, or PEtG:PLA blend (10 mg) was dissolved in CH$_2$Cl$_2$ (1 mL). A separate solution of sodium cholate (50 mg) was prepared in distilled water (10 mL). The organic phase was added to the aqueous phase in a 20 mL glass vial. The sonication amplitude was set to 10% intensity on a Branson 450 Digital Sonifier. The biphasic mixture was then sonicated for three 30 s intervals with 10 s breaks in between for a total of 90 s of sonication over 120 s. The resulting emulsion, a magnetic stir bar was added, and the solution was vigorously stirred overnight (16 h) to evaporate the organic phase. After evaporation of the organic phase, the particle suspension was dialyzed (3500 g/mol molecular-weight cutoff (MWCO) dialysis membrane, 8 h) against distilled water to remove any excess sodium cholate or trace organic solvent.

**Particle Degradation Studied by DLS.** Particles were prepared as described above, with the exception that the resulting suspensions were dialyzed against 100 mM, pH 7.4 phosphate buffer (1 L, 24 h, water changed once at ∼12 h). The final polymer concentration was diluted to ∼0.1 mg/mL. The count rate was measured by DLS while fixing the attenuator at 7 (t = 0 reading). For PEtG–NVOC and the PEtG–BnCO control, irradiation with UV light was performed in an ACE Glass photochemistry cabinet containing a mercury light source (450 W bulb, 2.8 mW/cm$^2$ of UVA radiation) for 30 min. A t = 30 min count rate measurement was obtained. Then the samples were incubated at 37 °C in the dark, and the DLS count rate was measured at additional time points over 24 h. For PEtG–disulfide and the PEtG–BnCO control, DTT (7.7 mmol) was added, and then the samples were incubated at 37 °C in the dark. The DLS count rate was measured at various time points over 24 h. Control experiments without stimulus were also included in each case. Each experiment was performed in triplicate.

**Loading and Triggered Release of Nile Red.** Nile-red-loaded PEtG, PLA, or PEtG:PLA particles were prepared by the addition of Nile red (0.1 mg) to the CH$_2$Cl$_2$ solution of PEtG or PEtG:PLA. The solution was stirred for several hours to ensure complete mixing, and then the particle preparation was performed as described above. The resulting suspensions were dialyzed against 100 mM, pH 7.4 phosphate buffer. The suspensions were then diluted 10-fold in the same buffer. Using an excitation wavelength of 540 nm, the initial emission intensity of Nile red was measured at 602 nm to obtain fluorescence at T0 (starting time). At this point, the appropriate stimuli (UV light for PEtG–NVOC and DTT for PEtG–disulfide) were applied as described above for the DLS study, and the suspensions were placed in a 37 °C oven. Fluorescence measurements were taken at time points over a 24 h period. By comparing the fluorescence at each time point with its initial fluorescence, the percent initial fluorescence was calculated. These measurements were taken in triplicate.

**Preparation of Celecoxib-Loaded Particles.** Particles were prepared as described above with the addition of celecoxib (30 wt %, 3.0 mg, 7.9 μmol) dissolved in the CH$_2$Cl$_2$ phase with the polymer mixtures before sonication. After evaporation of the organic phase, the particle suspension was filtered through a pipet containing a cotton filter to remove any unloaded drug crystals. The resulting suspension was further dialyzed (3500 g/mol molecular-weight cutoff (MWCO) dialysis membrane, 16 h) against distilled water (1 × 1 L) to remove excess sodium cholate and any soluble free drug. Afterward, 0.1 mL of the particle suspension was diluted with 1.4 mL of acetonitrile. The absorbance of the resulting solution was measured by UV–visible spectroscopy at 253 nm. The concentration was calculated based on a celecoxib calibration curve ($ε = 16 400$ L/mol-cm, acetonitrile, 253 nm), and the loading efficiency and drug content of the particles were calculated as follows.

\[
\text{Loading Efficiency} = \frac{\text{Drug Encapsulated}}{\text{Drug Added}} \times 100%
\]

\[
\text{Drug wt %} = \frac{\text{Encapsulated Drug Mass}}{\text{Mass of Polymer and Drug}} \times 100%
\]
Release of Celecoxib from Particles. Celecoxib-loaded particles in pH 7.4 buffer were prepared as described above. An initial absorbance measurement was taken by removing 200 μL of each sample and dissolving it in 1 mL of acetonitrile. Insoluble buffer salts were removed by filtration, and the absorbance was measured at 253 nm in order to quantify the initial amount of drug in the system. Subsequently, the appropriate stimuli were applied as described above for the DLS degradation study. The samples were then each placed in an individual 20 mL vial equipped with a small bar magnet, stirred at low rpm, and kept in a 37 °C oven for the duration of the study. At specified time points, each sample was centrifuged for 3 min at 2800g to separate the precipitated drug from the suspended particles. The suspension was decanted back into the initial vial and placed back into the oven, while the pellet was dissolved in 1 mL of acetonitrile and the absorbance at 253 nm measured to quantify the amount of drug that had been released and consequently precipitated (appropriate dilutions performed to ensure an absorbance less than 1.0). This amount was compared to the initial amount of encapsulated drug in order to determine the percentage of released drug. 1H NMR spectra of the pellets were obtained at 400 MHz in CD3CN at 4 °C. The metabolic activities of the cells. Absorbance at 540 nm was measured to quantify the relative absorbance

In Vitro Cell Toxicity Study. Dulbecco’s Modified Eagle Medium (DMEM) with 4.5 g/L of d-glucose and 110 mg/L of glutamine added in an atmosphere of 5% CO2 at 37 °C, to each well to solubilize the purple crystals. The plate was then removed, and the MTT reagent solution was placed in a plate reader (Tecan Infinite M1000 Pro), and the absorbance was measured to quantify the amount of drug that had been released and consequently precipitated (appropriate dilutions performed to ensure an absorbance less than 1.0). This amount was compared to the initial amount of encapsulated drug in order to determine the percentage of released drug. 1H NMR spectra of the pellets were obtained at 400 MHz in CD3CN at 4 °C, to confirm the identity of the precipitated material.

In Vitro Cell Toxicity Study. Dulbecco’s Modified Eagle Medium (DMEM) with 4.5 g/L of d-glucose and 110 mg/L of sodium pyruvate was obtained from Gibco. Penicillin-streptomycin (Pen Strep, 10000 U/mL), l-glutamine, fetal bovine serum (FBS), and trypsin-EDTA (0.25%) were obtained from Gibco. The MDA-MB-231 cells were cultured in DMEM with 10 vol % FBS, 100 units/mL of Pen Strep, and 2 mM l-glutamine added in an atmosphere of 5% CO2 at 37 °C. The cells were seeded in a 96-well plate (Corning Flat Bottom Plate) at a density of 10 000 cells/well and allowed to adhere for 24 h. The medium was then aspirated and replaced with varying concentrations of particles, just medium as a negative control, or sodium lauryl sulfate as a positive control. The medium was then aspirated and replaced with varying concentrations of particles, just medium as a negative control, or sodium lauryl sulfate as a positive control. Controls did not involve stimuli. The medium was then aspirated and replaced with 100 μL of fresh medium containing 0.5 mg/mL of MTT reagent and allowed to react for 4 h in the incubator. After 4 h the plate was removed, and the MTT reagent solution was aspirated. An amount of 50 μL of dimethyl sulfoxide was added to each well to solubilize the purple crystals. The plate was then placed in a plate reader (Tecan Infinite M1000 Pro), and the absorbance at 540 nm was measured to quantify the relative metabolic activities of the cells.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsomega.8b00534.

NMR spectra and SEC data for the polymers, Tg values for polymer blends, additional DLS data, particle stability in mouse serum, drug content and loading efficiencies, photo showing particle degradation and drug precipitation, and NMR data for the centrifuged pellets from the drug release study (PDF)

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Notes

The authors declare no competing financial interest.

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