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Ionizing Radiation-Induced Release from Poly(ε-caprolactone-*b*-ethylene glycol) Micelles

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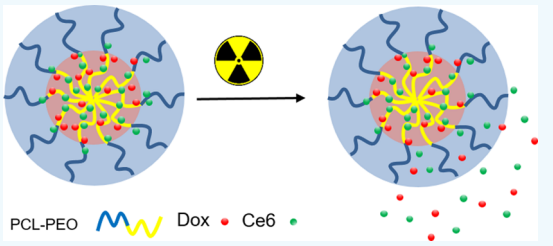
ABSTRACT: Polymeric micelles, due to their easy preparation and versatile properties, have been widely applied as one of the most popular carriers for chemotherapeutic agents. Such micelles primarily prevent the leakage of drugs during transportation and thus protect healthy tissue. Controlled drug release, which releases the drugs at the site of interest using internal or external stimuli as triggers, can further improve the safety of the drug delivery process. In this paper, we investigate whether ionizing radiation can be used to initiate release, focusing on using Cerenkov light as a possible trigger. For this purpose, micelles composed of the degradable polymer poly(ε-caprolactone-*b*-ethylene glycol) (PCL-PEO) were first loaded with the photosensitizer chlorin e6 (Ce6) and subsequently exposed to gamma or X-ray radiation of varying radiation doses. The results reveal that Ce6 was released from the micelles under radiation, regardless of the energy of incident photons, showing that Cerenkov light was not the driving force behind the observed release. SANS measurements showed that the volume fraction of the micelles containing Ce6 was reduced after exposure to radiation. This change in volume fraction suggests that the number of micelles was reduced, which was probably responsible for the release of Ce6. The exact mechanism, however, remains unclear. Subsequently, the PCL-PEO micelles were loaded with Ce6 and one of the following drugs: doxorubicin (Dox), docetaxel (DTX), and paclitaxel (PTX). Under radiation exposure, Dox, which is quite stable in single-loaded micelles, shows an enhanced release profile in the presence of Ce6, while DTX and PTX remained in the micelles, regardless of the presence of Ce6.



KEYWORDS: *PCL-PEO micelles, ionizing radiation, Cherenkov light, Chlorin e6, controlled release*

INTRODUCTION

Cancer is one of the major causes of death in the developed world, and new treatment strategies are continuously being pursued to increase the life expectancy of patients and to improve their life quality. In the case of metastasized tumors, chemotherapy is one of the most often-applied cancer treatments. Chemotherapy is indispensable in the clinic, but it is still facing many challenges.[1] Two of the main problems of chemotherapy are the hydrophobic nature of many anticancer drugs, such as paclitaxel and docetaxel, limits their solubility in blood and therefore their bioavailability and the lack of targeting properties that could lead to various adverse health effects.[2] Polymeric carriers have been proposed as a possible solution to both problems relying on the so-called enhanced permeability and retention (EPR) effect to limit toxicity to healthy tissue and to provide high loading capacity for hydrophobic substances.[3,4] A few polymeric carriers have already been approved by the FDA for clinical application, among which poly (ε-caprolactone)-containing vehicles are considered to be one of the best candidates because of their biodegradability.[5,6] In addition to carriers for chemotherapeutic agents, micelles composed of this polymer have also been used in combined therapies such as photodynamic and photothermal therapy.[7]

Toxicity to tissue can be further reduced if the delivery systems release the drugs primarily at the tumor site.[8] Various stimuli have been used to precisely discharge the active substances at the tumor, for example, pH, hypoxia, and enzymes are commonly implemented as internal triggers,[9−11] while heat and light are often applied as external stimuli.[12,13] Light-responsive drug delivery systems have been widely studied because of the noninvasive nature of light[14,15] but suffer from the low penetration depth in tissue, which is limited to a few millimeters.[16]

In this respect, ionizing radiation such as X-rays offers much better penetration possibilities and has been implemented as a trigger for release, although publications on this topic remain scarce. One example is the work of Deng et al. who have used

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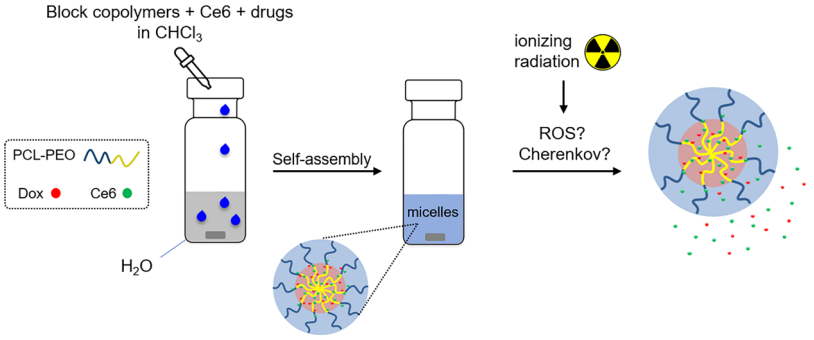
Scheme 1. Formation of Ce6&Dox-Coloaded Micelles and the Drug Release after Being Exposed to Ionizing Radiation



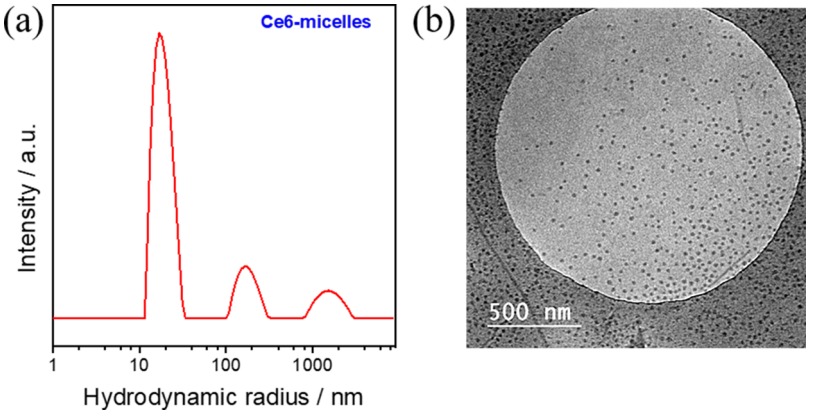


Figure 1. (a) Hydrodynamic radius of PCL-PEO micelles loaded with Ce6 as determined by DLS (the polymer concentration was 0.14 mg/mL); (b) Cryo-TEM images of the same micelles. The polymer concentration was 2.9 mg/mL, and the initial Ce6 concentration was 20 μM.

liposomes and verteporfin to generate singlet oxygen under X-ray exposure, which in consequence destabilized the carriers and released their encapsulated cargo.[17] Other applications of ionizing radiation have been demonstrated by Xu's group who have developed a series of radiation-sensitive polymeric carriers for controlled drug delivery.[18,19] Apart from directly utilizing the energy of radiation, accompanying phenomena, such as the so-called Cerenkov light, can also work as an internal light stimulus but has so far not been implemented in drug release studies.[20,21] Cerenkov light has a broad emission spectrum, and it is emitted when charged particles pass through a medium with a speed greater than the speed of light in this medium.[22] Cerenkov light is typically observed when ionizing radiation interacts with water and tissues and has drawn increasing attention recently as an internal light source in photodynamic therapy.[20,23]

Inspired by these studies, we have designed PCL-PEO micellar systems containing a typical photosensitizer, that is, Chlorin e6 (Ce6), and one of the following drugs: doxorubicin (Dox), paclitaxel (PTX), and docetaxel (DTX), which are commonly applied in chemotherapy. Initially, we had intended to use Cerenkov light induced by radiation as an internal trigger to activate Chlorin e6 and destroy the micelles by simply exploiting the destructive character of reactive oxygen species (ROS), in particular, singlet oxygen (as shown in Scheme 1). To investigate whether such a system can be used for triggered release, we have performed a systematic study by applying both X-rays and gamma rays (γ-ray) and varying the radiation dose. Drug release has been shown to occur, but the mechanism behind these effects appears not to be related to Cerenkov light.

RESULTS AND ANALYSIS

Ce6-Loaded PCL-PEO Micelles. The incorporation of Ce6 into the PCL-PEO (2800−2000) micelles was carried out during the self-assembly process, and high loading efficiency was achieved (69.3 ± 10.6%). The morphology of the Ce6-loaded micelles was determined by dynamic light scattering (DLS) and cryo-TEM. Figure 1a shows the obtained DLS data which reveal that most of the micelles have a hydrodynamic radius of ∼16 nm. Two other peaks corresponding to larger sizes suggest that there are also larger species present. The cryo-TEM image (Figure 1b) shows that the Ce6-loaded micelles are primarily spherical and small but a few worm-like micelles are also visible. The peaks observed in the DLS data at larger diameters possibly correspond to these worm-like micelles. The morphologies of the empty micelles (Figure S1) and the ones loaded with Ce6 appear to be the same.

To determine whether Cerenkov light can lead to release, we exposed the Ce6-loaded micelles to γ-rays (above the Cerenkov light energy threshold) and X-rays (below the Cerenkov light energy threshold) at different radiation doses. The release data shown in Figure 2 are obtained within 20 min after irradiation and indicate that the released amount goes up as the radiation dose increases. γ-Rays lead to slightly higher release (48.5 ± 3.5% at 50 Gy) than X-rays (39.3 ± 2.0% at 50 Gy). Exposure of Ce6 stock solution (10 μM) to γ-radiation resulted in negligible change in the UV signals at 665 nm of the Ce6 molecules (Figure S2), revealing that Ce6 itself is not affected by the radiation exposure under these experimental conditions. Based on the DLS and SANS data (Figures S3 and S4), the morphology including size and shape of the empty PCL-PEO micelles also did not change when exposed to

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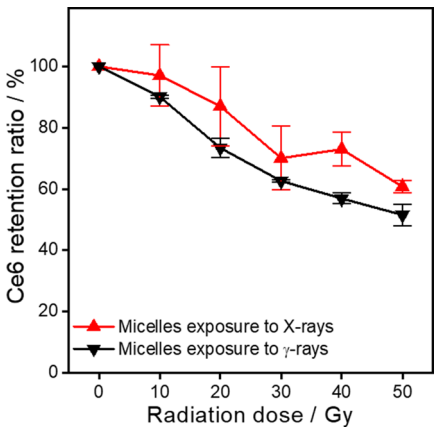

Figure 2. Ce6 retention ratio in the micelles as a function of radiation dose for Ce6-loaded PCL-PEO micelles when exposed to γ-rays delivered by the ^{60}Co source and X-rays of 240 kVp energy.

ionizing radiation. Moreover, according to the DSC result, the thermal properties of the irradiated sample also show no difference when compared with the original sample (Figure S5).

The Co-60 source delivers γ-rays of two main energies (1.17 and 1.33 MeV), while the X-ray source that was used supplied X-rays with a maximum energy of 240 keV. The interaction mechanism of ionizing photons depends on their energy and on the Z number of the materials with which they interact. In this case, the Compton effect in which photons give part of their energy to an electron occurs, the so-called Compton electron. The energy threshold for electrons to produce Cerenkov light is 261 keV in water,[22] which is above the value that the Compton electrons reach for the used X-ray energy. The γ-rays do have sufficient energy to create Compton electrons with energies above 261 keV, and Cerenkov light is expected to be produced.

The fact that release is also observed when using X-rays reveals that release is caused by ionizing radiation rather than Cerenkov light. To determine whether the release might be associated to the change in self-assemblies, we first investigated the morphology of the irradiated Ce6-loaded micelles by DLS and cryo-TEM (Figure 3).

According to the results discussed above, these radiation doses are not sufficient to lead to the destruction of the PEO-PCL polymer, which is in accordance with the literature on similar systems where much higher doses (in the kGy range) have been shown to result in polymer damage.[24] Additionally, empty micelles exposed to a radiation of 50 Gy have identical SANS patterns and intensity as the nonexposed ones (Figure S4). However, the SANS data in Figure 4 do show that the volume fraction of micelles containing Ce6 was reduced after exposure to ionizing radiation, while the morphology of the micelles remained the same. The Supporting Information contains more details on the SANS fitting parameters.

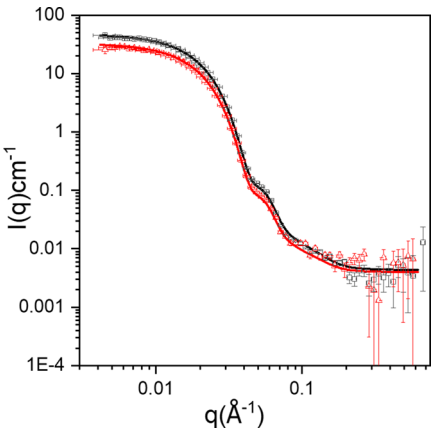

Figure 4. SANS curves obtained for micelles containing Ce6. Black square: Ce6-micelles; black line: the fitting curve for Ce6-micelles using a core−shell model; red triangle: irradiated Ce6-micelles, measured 1.5 h after being irradiated by 50 Gy gamma rays; red line: fitting curve using a core−shell model of the irradiated Ce6-micelles.

Ionizing radiation can interact with matter directly or indirectly through various radicals created by the radiolysis of water. Gamma rays have much less of a chance to directly damage Ce6, because Ce6 is composed of light elements with low stopping power, which suggests that indirect effects may dominate the Ce6 release from micelles. To check this assumption, we irradiated the micelles in a HEPES buffer which is a well-known radical scavenger.[25,26] Figure 5a shows that no Ce6 was released in HEPES, while 48.6 ± 3.2% was released in water when the micelles were exposed to the same dose of radiation, that is, 50 Gy, showing that radicals play the main role in the Ce6 release from the micelles.

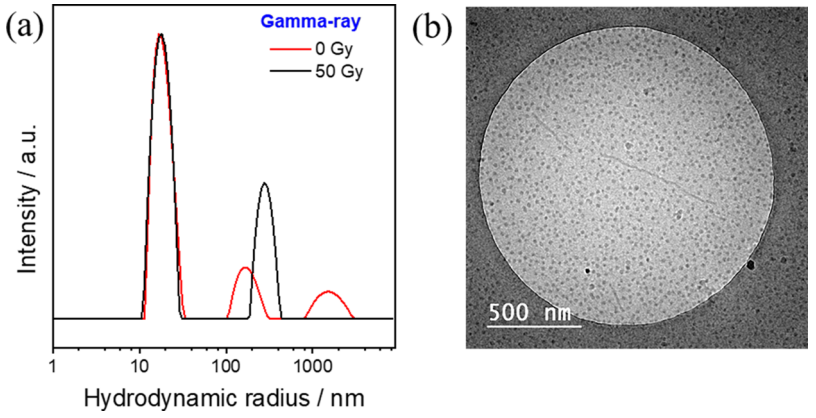

Figure 3. (a) Hydrodynamic radius obtained by DLS of Ce6-loaded PCL-PEO micelles before and after exposure to 50 Gy gamma rays (b) Cryo-TEM images of Ce6-loaded PCL-PEO micelles after exposure to 50 Gy delivered by gamma rays. The polymer concentrations were 0.14 and 2.9 mg/mL for DLS and Cryo-TEM, respectively.

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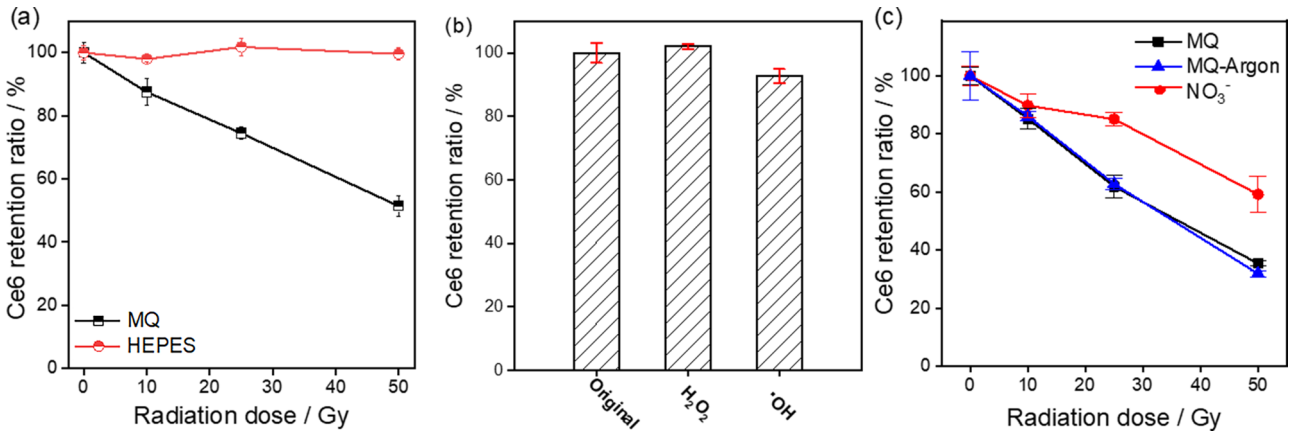

Figure 5. Release profiles of Ce6 from PCL-PEO micelles (a) in MQ and HEPES solution (1 mM) after gamma-ray exposure, (b) after reaction with H_2O_2 and OH for half an hour, and (c) in argon-saturated aqueous solution and NO_3^- aqueous solution (20 mM) after gamma exposure. Error bars represent the experimental uncertainties of at least three samples.

The radiolysis of water is a complex phenomenon, which results in various radicals and chemical species, among which OH radicals and hydrated electrons (e_{aq}^-) are some of the most reactive and H_2O_2 is one of the most abundant and persistent. In order to determine whether OH radicals and H_2O_2 can play a role in the release, we exposed the micelles to these species obtained in a chemical way. The H_2O_2 solution was prepared by diluting commercial H_2O_2 solutions, to a concentration of 400 μM which is much higher than that generated under radiation treatment in our experiments.[27] The OH radicals were generated through a typical Fenton reaction between H_2O_2 and Fe^{2+} ions.[28] As shown in Figure 5b, the presence of H_2O_2 or OH radicals exhibited negligible influence on the release of Ce6. Although there are only very few reports on the presence of singlet oxygen purely because of the radiolysis of water,[29] we tried to eliminate singlet oxygen effects using argon-saturated samples, which decreased the possibility of $^1O_2$ generation. As shown in Figure 5c, the micelles dispersed in argon-saturated aqueous solution showed a similar release behavior compared to those in air-saturated water, that is, around 65% of Ce6 was released from the micelles, indicating that $^1O_2$ was not the main cause for Ce6 release.

Besides the abovementioned reactive oxygen species, hydrated electrons are another main product of the radiolysis of water. To evaluate the influence of hydrated electrons, we studied the release profiles of Ce6-loaded micelles in aqueous solution containing NO_3^- (20 mM), which is a typical scavenger of e_{aq}^-. Under these conditions, only 40.8 ± 6.3% of Ce6 leaked out from the micelles after being exposed to 50 Gy gamma rays, which is significantly less than that for samples dispersed in MQ water with 64.7 ± 0.8% of the Ce6 being released. Note that the percentage of release differs somewhat per micelle batch; hence, the data are shown in one single figure using the same batch of Ce6-loaded micelles.

In previous experiments, we observed that Ce6 could also be loaded into preformed micelles over a 24 h period, which means that the Ce6 can actively enter the micelles. Hence, after the radiation exposure, we immediately separated the released Ce6 molecules to avoid the reloading process. In the experiments shown in Figures 2 and 5, the separation process has been managed within 20 min (sample transportation) after radiation exposure. To check whether ionizing radiation can induce a sustained release of Ce6 from the micelles, we exposed such samples to gamma rays of increasing radiation dose and separated the free Ce6 molecules after certain time intervals. Figure 6 shows that the immediate release after irradiation increases with the radiation dose, which is in agreement with the results in Figure 2. Moreover, the Ce6 retention in the samples remains relatively stable, although it is slightly increased after 2 days compared to that at 0 h. The residual Ce6 in irradiated samples is evidently less than that for non-irradiated samples, suggesting that the released Ce6 molecules are not likely to totally return to micelles under experimental conditions. Thus, irradiation gives a long-term change in the Ce6−micelle interaction.

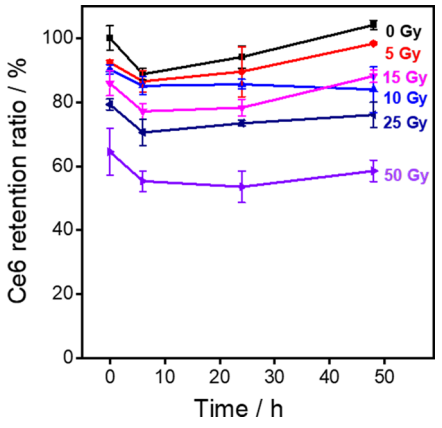

Figure 6. Retention of Ce6 in PCL-2800 micelles as a function of time after exposure to γ-rays at different radiation doses. The polymer concentration was 2.9 mg/mL. The Ce6 retention ratio for samples at 0 h was calculated using the light absorption intensity at 660 nm of the immediately separated samples after irradiation, and the average light absorption intensity of the non-irradiated samples was used as the reference for all samples.

We also carried out control experiments in which we separately irradiated either Ce6 or empty micelles and carried out the synthesis process again, which showed that encapsulation efficiencies were nearly the same (Figure S7). Clearly, the release must be due to a change in interaction between Ce6 and the polymeric micelles occurring during irradiation. The exact mechanism, however, remains unknown.

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Drug-Loaded PCL-PEO Micelles. Although the mechanism of Ce6 release is not yet clear, we were interested in finding out whether such radiation-induced release was also observed for drugs with or without the presence of Ce6. For this purpose, we loaded the same micelles with different anticancer agents, that is, Dox, PTX, and DTX. The loading efficiencies of the drugs with or without the presence of Ce6 are summarized in Table 1, revealing that the more hydrophobic the drug is, the higher loading efficiency (LE) it would achieve, that is, the LE is 23.5 ± 3.5% for Dox, while it is 80.0 ± 14.2% and 95.8 ± 4.5% for DTX and PTX, respectively. Even though the efficiency differed in all cases, the morphology of the micelles appeared not to be affected for all samples (Figure S8). Interestingly, the LE(%) of Ce6 in these coloaded samples shows quite different behavior. The LE of Ce6 significantly decreased to 33.8 ± 6.6% in the presence of Dox, while that for Ce6&DTX-micelles and Ce6&PTX-micelles was 57.6 ± 3.2% and 62.4 ± 8.1%, respectively. When compared to loading of Ce6 alone (69.3 ± 10.6%), the presence of PTX and DTX appeared not to be competing with Ce6, while Dox did.

Table 1. Summary of the Loading Efficiency of Therapeutic Drugs and Ce6 in Micelles

sample	drug loading/%	Ce6 loading/%
Dox-micelles	23.5 ± 3.5[a]	
Ce6&Dox-micelles	18.9 ± 2.5	33.8 ± 6.6
DTX-micelles	80.0 ± 14.2	
Ce6&DTX-micelles	61.7 ± 9.3	57.6 ± 3.2
PTX-micelles	95.8 ± 4.5	
Ce6&PTX-micelles	78.1 ± 4.5	62.4 ± 8.1
Ce6-micelles		69.3 ± 10.6

[a]The error indicates standard deviation based on three measured samples.

Subsequently, we exposed the Ce6&drug coloaded and the Ce6 or drug single-loaded micelles to gamma radiation of different doses. The data summarized in Figure 7 a,b,c illustrate that there were more Ce6 molecules released from the coloaded samples than in the case of the single Ce6-loaded micelles, which means the presence of the drugs somehow influences the interaction between Ce6 and the polymeric micelles. However, the release of drugs showed quite different behaviors. The micelles only loaded with Dox, PTX, and DTX had no evident drug release even when exposed to 50 Gy. In the case of the coloaded micelles, only Dox showed a significant release.

To better understand the interaction between micelles and drugs, we freeze-dried all the samples and used differential scanning calorimetry (DSC) to detect changes in thermal behavior of the PCL-PEO micelles as a result of the presence of drugs and Ce6. As shown in Table 2, the presence of Dox and Ce6 slightly decreases the melting temperature of PCL from 43.0 to 42.3 and 41.2 °C, while DTX and PTX increase

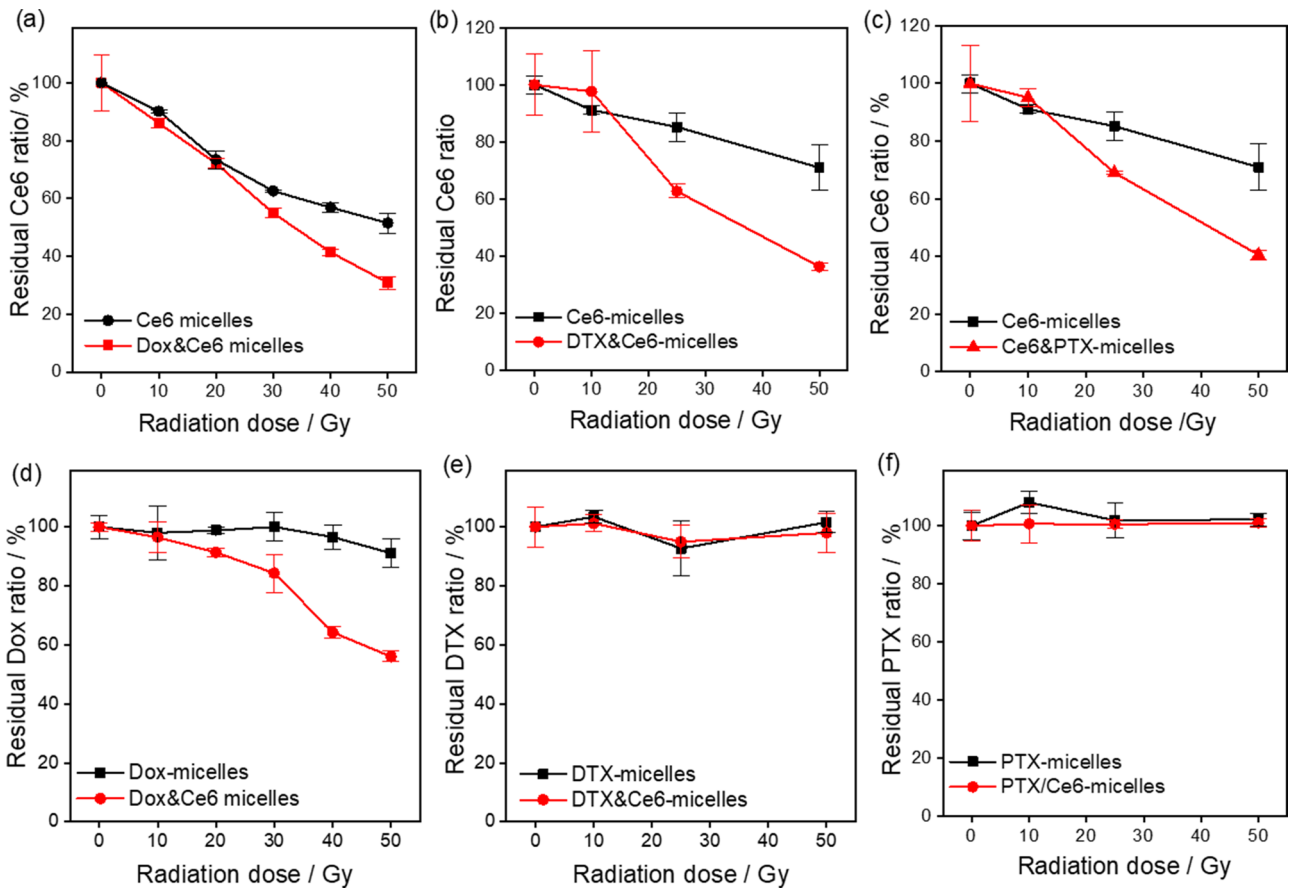

Figure 7. First row: the Ce6 retention of Ce6-micelles and coloaded micelles with Ce6 and (a) Dox, (b) DTX, and (c) PTX; second row: the release of the different drugs for single drug-loaded micelles and micelles coloaded with Ce6 and (d) Dox, (e) DTX, and (f) PTX under various γ-ray doses delivered by the ^{60}Co source.

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Table 2. Summary of Melting Temperature, Crystallization Temperature, and Heat of Fusion of the Samples Containing Ce6, Dox, PTX, and DTX

sample	T_m(PCL) (°C)	T_m(PEO) (°C)	T_c(PCL) (°C)	T_c(PEO) (°C)	$\Delta H_{f,s}$(PCL) (J g^{-1})	$\Delta H_{f,s}$(PEO) (J g^{-1})
PCL-PEO	43.0	52.1	19.5	26.0	67.7	119.5
Dox-micelles	42.3	52.1	19.8	28.3	93.6	144.1
Ce6-micelles	41.2	52.1	18.7	29.6	75.7	123.6
DTX-micelles	44.6	51.7	19.6	37.8	52.0	87.9
PTX-micelles	45.8	51.7	21.6	37.6	53.2	88.8

the melting temperature of PCL to 44.6 °C and 45.8 °C, respectively. In contrast, all drugs had a negligible influence on the melting behavior of the PEO. In terms of the crystallization temperature, the presence of the drugs had a larger influence on the PEO block and the crystallization temperature increased to 28.3 °C (Ce6), 29.6 °C (Dox), 37.8 °C (DTX), and 37.6 °C (PTX), which were substantially higher than that of the empty micelles (26.0 °C). These results indicate that drugs stimulate the nucleation of PEO segments at higher temperature.[30]

The released energy during the crystallization process was calculated based on the DSC curve in Figure S10. As summarized in Table 2, the PCL and PEO segments for empty PCL-PEO micelles showed energy release of 67.7 and 119.5 J/g, respectively, during crystallization, which increased because of the addition of Dox and Ce6, while decreased because of the addition of DTX and PTX. However, Ce6 showed a smaller influence on the energy released during crystallization than the others, indicating less interaction between the Ce6 molecules and the micelles, which might be an explanation for the release of Ce6 under ionizing radiation. Moreover, the evident drop in loading efficiency of Ce6 in the presence of Dox implies that they are somehow synergistically working, leading to a release of Dox along with Ce6.

CONCLUSIONS

In this study, we prepared block copolymer micelles that were loaded with Ce6 or coloaded with Ce6 and drugs and evaluated their radiation-induced release behavior. PCL-PEO micelles with a hydrodynamic radius of ∼16 nm exhibited excellent encapsulation ability of Ce6, DTX, PTX, and Dox without any effect on the morphology of the micelles. Once exposed to ionizing radiation, these micelles exhibited release of Ce6 as a function of dose by both gamma rays and X-rays, which is possibe partly because of the interaction with hydrated electrons generated by water radiolysis. There was no significant difference in release after interacting with ionizing photons above and below the Cerenkov light threshold, indicating that Cerenkov light was not part of the mechanism for the observed phenomenon. Moreover, the radiation used under these experimental conditions did not damage the Ce6 or the micelles but rather induced a long-term change in the interaction between the Ce6 and micelles.

Micelles single-loaded with drugs, that is, Dox, PTX, and DTX, did not show drug release in the presence of radiation. In contrast, Dox exhibited an enhanced release profile upon exposure to radiation in the presence of Ce6, while PTX and DTX remained in the micelles. The DSC data revealed that the addition of Ce6 showed a small influence on the crystallization of PCL and PEO segments of the micelles, indicating a relatively loose interaction between Ce6 and PCL-PEO micelles. The radiation-induced release of Ce6 could be used to further trigger the release of other anticancer substances, that is, Dox, which could be applied for controlled release using radiation as an external stimulus.

However, the full mechanism for radiation-induced drug release from PCL-PEO micelles remains unclear and requires further exploration. Currently, the radiation-induced release of Dox is only significant under high dose, that is, 50 Gy, which could be utilized in some radiotherapy treatments, but more efforts are required to increase the radiation sensitivity of the Ce6−drug micelles system for the clinical applications.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsapm.0c01258.

Materials and methods section; DLS, Cryo-TEM, and SANS of different micelles; SANS fitting parameters; and DSC curves of PCL-PEO micelles (PDF)

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Author Contributions

H.L. performed the experiments and data analysis and drafted the manuscript. A.C.L. helped with all the Cryo-TEM pictures. J.P. helped preparing samples for SANS and fitted the SANS data. S.R.P. performed the SANS experiments together with R.M.D. who has also assisted in setting up the whole SANS measurement setup. Y.M. helped the DSC data analysis and joined all discussions about this manuscript. R.E. and A.G.D. guided the whole experimental process, analyzed the data, and revised the manuscript.

Notes

The authors declare no competing financial interest.

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