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Light-responsive CO₂ bubble-generating polymeric micelles for tumor cell ablation†‡

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We report a novel UV-responsive polymeric micelle system based on coumarin ester for tumor treatment. The micelle itself can generate CO₂ under UV exposure owing to the photolysis of (7-diethylaminocoumarin-4-yl)methyl (DEACM). Without loading any drugs, the micelle causes significant cell death under 8 s UV irradiation *in vitro*. This is the first time to use light to decompose the nano-carrier itself into CO₂ bubbles for tumor cell ablation.

Conventional chemotherapy of cancer has caused severe systemic toxicity and multidrug resistance.¹ Drug resistant tumors necessitate high doses of chemotherapy drugs, which in turn increase the systemic toxicity of chemotherapy. Therefore, to keep the dose at its minimum to reduce toxicity, a specific delivery system of the drug to the tumor site is highly encouraged. Recently, polymeric micelles (PMs) as site-specific drug delivery systems (DDS) are proving their effectiveness pre-clinically and clinically. Polymeric micelles are based on block-copolymers with hydrophilic and hydrophobic units that self-assemble in an aqueous environment into structures composed of a hydrophobic core stabilized by a hydrophilic shell. PMs possess several distinct advantages, such as biodegradability, passive tumor targeting, prolonged circulation and high drug loading.^{2,3} Currently, many drug-loaded polymeric micelles for anticancer therapy are under investigation in preclinical studies to improve drug efficacy.⁴ For example, Genexol-PM is a micellar paclitaxel formulation consisting of PEG and poly(D,L-lactic acid) (PDLLA). Preclinical *in vivo* studies with Genexol-PM demonstrated a 3-fold increase in the maximum tolerated dose (MTD) and a significantly increased anti-tumor efficacy compared with free PTX.³

However, the remaining anticancer agent may harm normal cells and tissues as well after killing the cancer cells.⁵ Moreover, certain drugs may undergo transformation to generate chemically reactive metabolites that can cause significant side effects. For example, it is well documented that doxorubicinol (*i.e.*, the metabolite of doxorubicin) can induce acute and chronic cardiac toxicities.⁶

To avoid the toxicity of chemotherapy drugs to normal tissues, herein we propose a UV-responsive polymeric micelle that produces CO₂ bubbles for direct tumor ablation. To the best of our knowledge, it is the first time to use light to decompose the nano-carrier into CO₂ bubbles for effective tumor cell ablation. The use of light to trigger tumor ablation is one of the most attractive fields currently being investigated owing to its advantages including noninvasiveness and allowing remote, temporal, and spatial control.^{7–11} In our design, the hydrophilic block of our polymer micelle is poly(ethylene glycol) (PEG) and the hydrophobic block is a photo-labile group, coumarin ester. On UV light irradiation (365 nm, 15 mW cm⁻²), the photolysis of the coumarin ester will lead to the cleavage of coumarins and production of CO₂ (Fig. 1). As a proof of concept, a large amount of CO₂ bubbles has been demonstrated

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Fig. 1 (a) A schematic illustration showing the self-assembly and UV-induced gas generation process of the micelle; (b) changes of the copolymer structure before and after UV irradiation.

to burst from this CO₂ releasing system, which instantly explode to induce the necrosis of tumor cells. As a result of the transient formation, growth, and collapse of CO₂ bubbles, a disruptive force will be produced similar to the cavitation effect induced by ultrasound. Compared to chemotherapy drugs, physical treatment using CO₂ bubbles will not leave any toxic agents behind. In this way, only the cells that internalize the micelles will be destructed under UV exposure, while the surrounding cells can still remain alive and unharmed.

(7-Diethylaminocoumarin-4-yl)methyl (DEACM) carbonate was selected as the photo-labile group and conjugated with PEG to form the amphiphile DEACM-PEG derivatives, which was the backbone of the micelle system. The synthesis process of the block polymer is shown in Fig. S1, ESI.† As reported, coumarin-4-ylmethyls have higher photolysis efficiencies than others such as 2-nitrobenzyls, so that the incident light intensity for the uncaging reaction could be lowered, leading to minimization of cell damage while maintaining higher spatial resolution.¹¹ Under UV exposure, DEACM absorbed the energy of light and was released from the micelle through a C–O bond cleavage. This process could be explained by a radical mechanism, which involved electron transfer between the oxygen and coumaryl moieties which formed the intramolecular radical ion pair and caused the subsequent cleavage of the C–O bond.^{12,13} After the cleavage of the C–O bond, the remaining PEG-carbonate part hydrolyzed and generated CO₂ in the water.

According to the transmission electron microscopy (TEM) images, the amphiphile PEGylated DEACM self-assembled in water into ~200 nm spherical micelles (Fig. 2a). Under UV irradiation, the polymeric micelle decomposed into smaller fragments due to the cleavage of the hydrophobic block. Dynamic light scattering (DLS) measurements also showed that the size of micelles before and after 12 s UV exposure was

~193 nm and ~43 nm, respectively (Fig. 2b). The shorter irradiation failed to degrade the micelles, probably because the light intensity was lower than the minimal requirement for photolysis of DEACM (Fig. 2c). These experiments consolidated that DEACM-PEG derivatives could form micelles, and UV exposure of appropriate energy was able to cleave the amphiphile and thereby decompose the micelles. During the decomposition, the UV absorption band of the micelle solution underwent a blue shift from 392 nm to 383 nm with a concomitant change in color from yellow to orange (Fig. 3A and B). The changes of UV absorption and color were credited to the breakage of the amphiphile and subsequent disruption of the micelles.

Then we investigated the production of CO₂ from micelles under UV exposure. We monitored the decrease of the carbonate amount after UV irradiation by proton magnetic resonance (¹H-NMR) imaging in CDCl₃ solution, manifested by the decreased signal at site a (Fig. 3c). The decrease of carbonate indicated the hydrolysis of carbonate in the water and generation of CO₂. The spectra also showed the formation of DEACM-OH as a photolysis product by the appearance of a signal at 4.84 ppm and a weakened signal at site b. As a photo-labile group, the DEACM monomer *per se* remained stable under UV irradiation (Fig. S2, ESI†). Similarly, the Fourier transform IR (FTIR) spectra further consolidated the breakage of the carbonate linkage and removal of DEACM. According to Fig. 3d, the decrease of the C=O, C–O and C=C bonds (1751 cm⁻¹, 1014 cm⁻¹ and 1517 cm⁻¹, respectively) in the spectrum of micelles after UV irradiation indicated the breakage of the carbonate linkage and removal of DEACM from the amphiphile. The strong O–H bond (3426 cm⁻¹) in the spectrum of micelles after UV irradiation suggested the formation of PEG-OH after the cleavage of DEACM. Both of ¹H-NMR and FTIR spectra substantiated the decrease of the carbonate amount under UV exposure. As mentioned above, the breakage of the

Fig. 2 UV exposure (365 nm, 20 W) degrades micelles formed by self-assembly of DEACM-PEG. (a) TEM images of micelles before and after 12 s UV exposure. (b) Size distribution of micelles before and after 12 s UV exposure. (c) Average size of micelles under 0 s, 4 s, 8 s, 12 s, 16 s, 30 s and 60 s UV exposure, respectively.

Fig. 3 (A) Photographs of micelles before and after 12 s UV exposure. (B) Absorption spectra of micelles during 12 s UV exposure. (c) ¹H-NMR spectra of micelles before and after 12 s UV exposure. (d) FTIR spectra of micelles and PEG. The blue line is PEG, the orange line is micelles before UV irradiation, and the red line is micelles after UV irradiation.

Fig. 4 (a) Viability of Renca cells receiving 0 s to 10 s UV exposure. (b) Viability of Renca cells treated with micelles or DEACM, before or after UV irradiation, as evaluated by the CCK8 assay. (c) Fluorescence imaging showing the viability of cells treated with micelles and 8 s UV irradiation. The dead cells were stained red (propidium iodide) (scale bar, 100 µm).

carbonate linkage was accompanied by the hydrolysis of carbonate into CO₂ in the water. As expected, we observed the generation of a large amount of CO₂ bubbles from micelle solutions right after UV irradiation which remained for about 10 s (Movie S1†). In contrast, water as a negative control showed no generation of bubbles (Movie S2†). Therefore, we demonstrated that micelles could generate CO₂ under UV exposure in aqueous solution.

We then investigated the cytotoxicity of micelles under UV exposure using Renca cells. Considering that UV exposure *per se* was cytotoxic, we firstly evaluated the influence of UV exposure on the cell viability. As Fig. 4a indicated, while 10 s-UV exposure was capable of causing obvious cell death, the cytotoxicity of 8 s-irradiation was negligible. Thus, we chose 8 s-UV exposure for further investigation of the influence of CO₂ generated from micelles on the cell viability. Without UV exposure, micelles showed no obvious cytotoxicity in a concentration range of 0 to 0.5 µg ml⁻¹ (Fig. 4b). The results indicated the bio-safety of our micelles *in vitro*. However, under 8 s-UV exposure, micelles caused significant cytotoxicity that killed ~86% cells at a concentration of 0.5 µg ml⁻¹. A negative correlation existed between cell viability and micelle concentration, which indicated that it was the micelles that caused cell death. To verify the results, cells treated with micelles combined with 8 s-UV exposure were stained with propidium iodide (PI) (Fig. 4c). The higher red fluorescence intensity of PI indicates higher cytotoxicity, because PI stains only dead cells. Results showed that the red fluorescence intensity of cells was positively related to the concentration of micelles. We then investigated whether the photolysis products of micelles caused the cytotoxicity. To this end, we irradiated the micelle solution for 8 s before adding it to the cell medium for overnight incubation. The negligible cell death indicated that it was not the photolysis products but the changes of micelles during the UV exposure that were responsible for the concentration dependent cell death. According to the structure of micelles, the only two changes under UV exposure are the photolysis of DEACM and CO₂

production. To exclude the influence of photo-labile DEACM on cell viability, we also evaluated the cytotoxicity of DEACM monomers under UV exposure. In the concentration range of 0 to 0.5 µg ml⁻¹, DEACM monomers showed no cytotoxicity under 8 s-UV exposure. After ruling out the influence of photolysis and release of DEACM, the generation of CO₂ was the only factor that contributed to the cell death of Renca cells.

We have developed a UV-responsive CO₂-generating polymeric micelle system without loading any drugs. UV exposure could break up the backbone polymer of the micelle system, thus causing the degradation of micelles. The degradation was substantiated by TEM imaging and DLS measurements. H¹-MNR and FTIR spectra also indicated the breakage of the C=O bond and production of CO₂. Further, we substantiated that our micelle could kill tumor cells under UV exposure through CO₂ generation.

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