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Perylene-3-ylmethanol: Fluorescent Organic Nanoparticles as a Single-Component Photoresponsive Nanocarrier with Real-Time Monitoring of Anticancer Drug Release

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Supporting Information

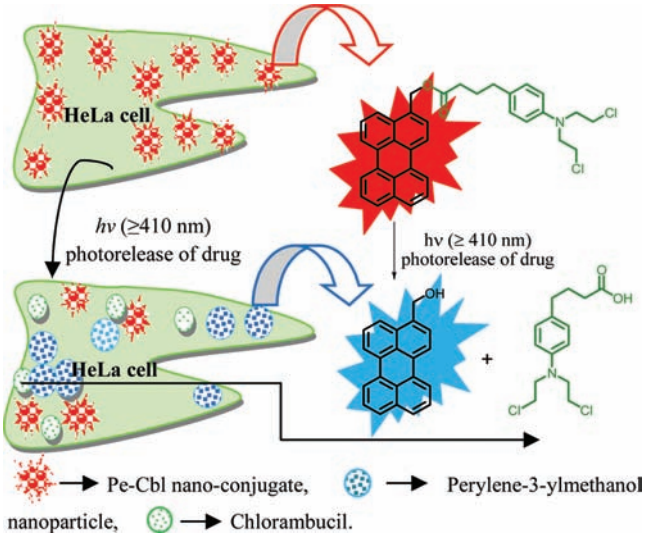
ABSTRACT: We report for the first time the use of perylene-3-ylmethanol fluorescent organic nanoparticles as a drug delivery system. In the present system, perylene-3-ylmethanol nanoparticles performed four important roles: (i) "nanocarriers" for drug delivery; (ii) "phototriggers" for the drug release; (iii) fluorescent chromophores for cell imaging; and (iv) detectors for real time-monitoring of drug release. In vitro biological studies revealed that the newly developed perylene-3-ylmethanol nanoparticles exhibit good biocompatibility and cellular uptake as well as efficient photoregulated anticancer drug release ability. Such fluorescent organic nanoparticles may open up new perspectives for designing a new class of promising photoresponsive nanocarriers for drug delivery.

Photoresponsive nanoparticles have recently gained considerable attention in drug delivery research because of their ability to control the release of bioactive molecules spatially and temporally by external regulated light stimuli.[1] Generally, photoresponsive nanoparticles for use as a drug delivery system (DDS) are composed of two main ingredients: a biocompatible nanocarrier and a "phototrigger". The phototrigger is a small organic molecule that performs two important functions: (i) providing precise control over the drug release and (ii) serving as a linker between the nanocarrier and the drug.

With the use of both of these ingredients, several photoresponsive nanoparticles have been developed for use as DDSs.[2−8] The crucial steps involved in the preparation of photoresponsive nanoparticles are (i) the synthesis of biocompatible nanoparticles, (ii) surface functionalization of the nanoparticles, and (iii) attachment of the phototrigger to the nanoparticles. By any means, if we can synthesize nanoparticle-sized phototriggers, then the phototrigger by itself can act as a nanocarrier for the drug delivery, thereby eliminating the above crucial steps. Hence, we decided to synthesize organic nanoparticles that can act as both the drug nanocarrier and the phototrigger.

Unlike inorganic nanoparticles, the progress on organic nanoparticles has been slow, even though they allow wider variability and flexibility in materials synthesis and nanoparticle preparation.[9,10] This is mainly due to the difficulties associated with their synthesis and isolation.[11,12] However, the introduction of the effective "reprecipitation technique"[13] for the synthesis of organic nanoparticles have sparked interest in them in fields ranging from optoelectronics[14,15] and sensors to medicine.[16−21] In 2006, Latterini et al.[22] synthesized different sizes of perylene nanoparticles by the reprecipitation method and showed that their spectral properties are markedly different from those of perylene molecules. Latter, Baba et al.[23] applied perylene nanocrystals for in vitro fluorescence confocal imaging of living cells. Our group recently demonstrated perylene-3-ylmethyl[24] as an efficient fluorescent phototrigger for carboxylic acids and alcohols in aqueous media under visible-light irradiation. On the basis of the above facts, we report for the first time the use of fluorescent organic nanoparticles of perylene-3-ylmethanol as a single-component photoresponsive nanocarrier for in vitro release of the anticancer drug chlorambucil (Scheme 1).

Scheme 1. Schematic Representation of Photoinduced in Vitro Anticancer Drug Release



First, we synthesized a photocaged perylene−chlorambucil (Pe−Cbl) conjugate using the procedure reported by us.[24] Next, photocaged Pe−Cbl nanoconjugates for use as a DDS were prepared by a reprecipitation technique involving slow

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addition of 10 μL of a 3 mM dimethyl sulfoxide solution of Pe−Cbl caged conjugate into water (20 mL) at room temperature with controlled stirring.[23] The shape and size of the resulting Pe−Cbl nanoconjugates were determined by transmission electron microscopy (TEM), which showed the particles of Pe−Cbl nanoconjugate to be globular in shape with an average particle size of 30 nm (Figure 1a). Interestingly, the TEM

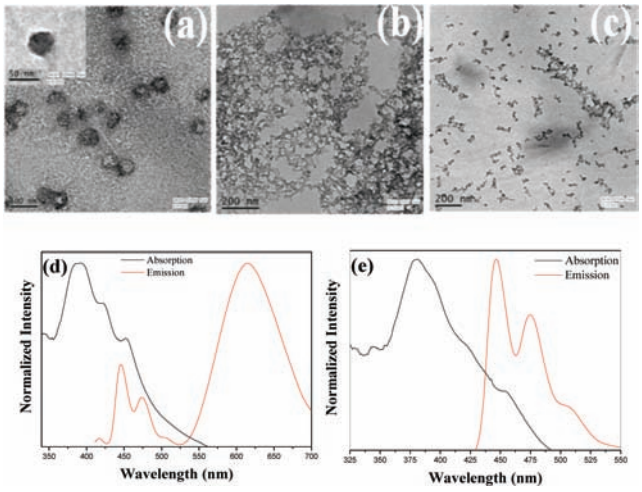

Figure 1. (a−c) TEM images of (a) Pe−Cbl nanoconjugates before photolysis, (b) Pe−Cbl nanoconjugates after photolysis, and (c) perylene-3-ylmethanol nanoparticles. (d, e) Normalized absorption and emission spectra of (d) Pe−Cbl nanoconjugates and (e) perylene-3-ylmethanol nanoparticles.

images of perylene-3-ylmethanol phototrigger nanoparticles synthesized using a similar reprecipitation technique showed the particles to have a reduced size of 20 nm. The absorption and emission spectra of Pe−Cbl nanoconjugates were found to be quite different from those of perylene-3-ylmethanol nanoparticles (Figure 1d,e). A distinct red shift can be seen in the emission spectra of Pe−Cbl nanoconjugates. The broad absorbance of the Pe−Cbl nanoconjugates from 350 to 550 nm and strong emission at 625 nm indicate that our nanoconjugates can be used for both cell imaging and the release of the anticancer drug under visible-light irradiation.

To check the stability of the photocaged Pe−Cbl nanoconjugates in the culture medium, we dispersed the nanoconjugates with 10% fetal bovine serum and incubated them at 37 °C in the dark for 48 h. We observed insignificant (2−3%) release of the drug, which proves that the conjugates are quite stable in the dark.

A suspension of photocaged Pe−Cbl nanoconjugates in water (1×10^{-4} M) was irradiated under visible light (≥410 nm) with a 125 W medium-pressure Hg lamp using a suitable filter (1 M $NaNO_2$ solution), and the time course of the photorelease of chlorambucil was followed by reversed-phase HPLC using acetonitrile as the mobile phase, keeping the flow rate at 1 mL/min. From the HPLC profile (Figure 2), depletion of the peak at a retention time of 6.17 min indicates the decomposition of the Pe−Cbl nanoconjugate, and at the same time, the appearance of two new peaks at retention times of 5.16 and 2.69 min indicates the formation of the photoproduct perylene-3-ylmethanol and the anticancer drug chlorambucil, respectively.

We also demonstrated precise control of the photolytic release of the anticancer drug by monitoring the release of

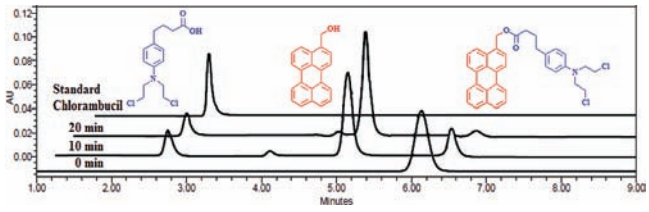

Figure 2. HPLC profile of Pe−Cbl nanoconjugates at regular time intervals of photolysis under visible-light irradiation (≥410 nm). The vertical axes were offset by 15 mAU units and the horizontal axes by 15 s to facilitate better visualization.

chlorambucil after periods of exposure to light and dark conditions, as shown in the Figure 3 inset. The figure clearly shows that the drug release proceeded only under illumination.

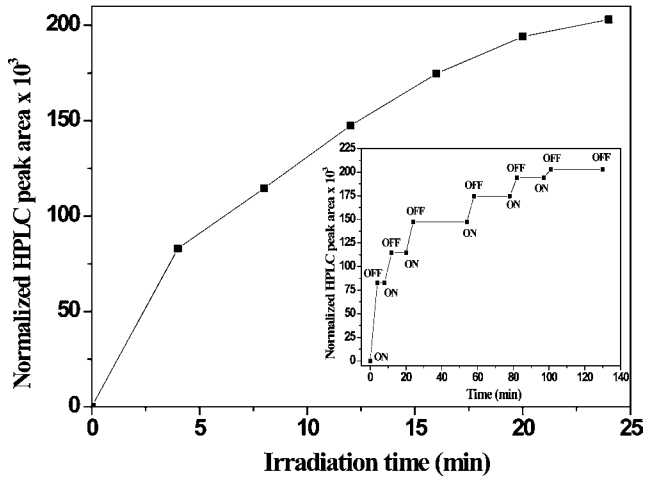

Figure 3. Progress of the release of chlorambucil from Pe−Cbl nanoconjugates under visible-light irradiation (≥410 nm). The inset shows the progress of chlorambucil release under light and dark conditions ("ON" and "OFF" indicate the beginning and end of light irradiation, respectively).

To establish that the photocaged Pe−Cbl nanoconjugates can be used as a versatile DDS, initial cell imaging studies were carried out using a HeLa cell line obtained from the National Centre for Cell Sciences (NCCS; Pune, India), which was maintained in minimum essential medium containing 10% fetal bovine serum at 37 °C and 5% CO_2. To study the cellular uptake of Pe−Cbl nanoconjugates, briefly HeLa cells (5×10^4 cells/well) were plated on 12-well plates and allowed to adhere for 4−8 h. The cells were then incubated with 2×10^{-5} M photocaged Pe−Cbl nanoconjugate in cell culture medium for 4 h at 37 °C and 5% CO_2. Thereafter, the cells were fixed in paraformaldehyde for 15 min and washed two times with phosphate-buffered saline. Imaging was done using an Olympus FV1000 confocal microscope with the appropriate filter. A cellular uptake study after 4 h of incubation revealed that the Pe−Cbl nanoconjugates were internalized by the cell membrane, leading to a uniform distribution of the sample inside the cell (Figure 4).

The cytotoxicity in vitro was measured using the MTT assay[25] [MTT = 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, a yellow tetrazole] on the HeLa cell line. The cytotoxic effect of each treatment was expressed as percentage of cell viability relative to the untreated control cells. The percentage of cell viability was plotted versus the concentration

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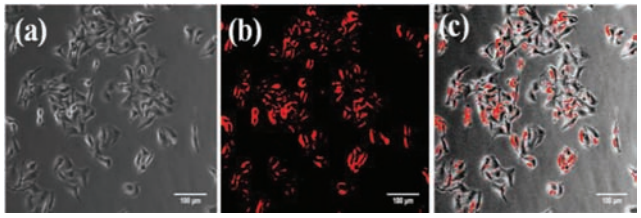

Figure 4. Confocal bright-field and fluorescence images of HeLa cells incubated with 2×10^{-5} M Pe−Cbl nanoconjugate for 4 h: (a) bright-field image; (b) fluorescence image (λ_{ex} = 625 nm); (c) overlay of (a) and (b).

of photocaged Pe−Cbl nanoconjugate at different time intervals (Figure 5). For cells incubated with photocaged Pe−Cbl

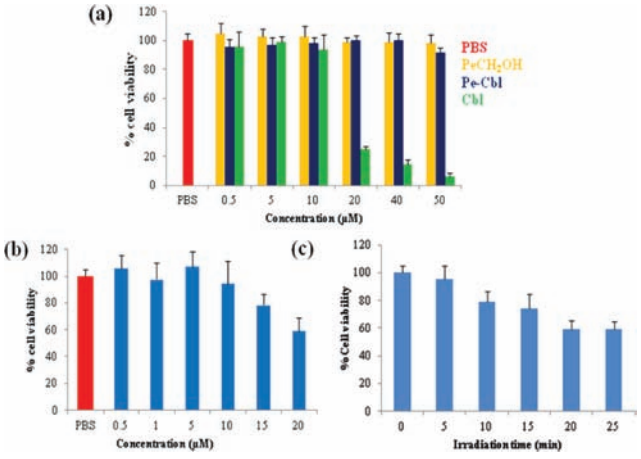

Figure 5. Cell viability tests of Pe−Cbl nanoconjugates, perylene-3-ylmethanol nanoparticles, and chlorambucil on the HeLa cell line: (a) before irradiation; (b) after irradiation in the presence of different concentrations of Pe−Cbl nanoconjugates; (c) after regular time intervals of irradiation in the presence of 2×10^{-5} M Pe−Cbl nanoconjugates. Values are presented as means ± standard deviations.

nanoconjugates, irradiation for 20 min under visible light (≥410 nm) resulted in the release of the anticancer drug chlorambucil, thereby causing cytotoxicity in the cancerous HeLa cell lines, as validated by the MTT toxicity data given in Figure 5. There was no significant cell death observed when the cells without Pe−Cbl nanoconjugates were irradiated. The cytotoxicity was likely caused by the released chlorambucil upon light irradiation. In comparison with chlorambucil at the same concentration as the Pe−Cbl nanoconjugates (Figure 5a), the Pe−Cbl nanoconjugates showed much lower cytotoxicity. However, upon irradiation, the Pe−Cbl nanoconjugates showed an increasing cytotoxicity to cancer cells (Figure 5b) because of photorelease of chlorambucil inside the cells. Thus, the photocaged Pe−Cbl nanoconjugates can serve as both the drug carrier and a versatile photocaged compound for the release of the anticancer drug.

Furthermore, to validate the ability of photocaged Pe−Cbl nanoconjugates to be used for externally regulated drug release, the HeLa cells incubated with 2×10^{-5} M Pe−Cbl nanoconjugate were exposed to visible light (≥410 nm) for different time intervals. We found that the cytotoxicity toward HeLa cells at the irradiation time of 20 min showed the highest level of toxicity (about 40% in comparison to the control), indicating that most of the drugs were released from the nanoconjugates.

Finally, to demonstrate that perylene-3-ylmethanol nanoparticles can serve as photoresponsive nanocarriers with real-time monitoring of the drug release, we investigated the luminescence properties of perylene-3-ylmethanol nanoparticles before and after drug loading. As depicted in Figure 6a,

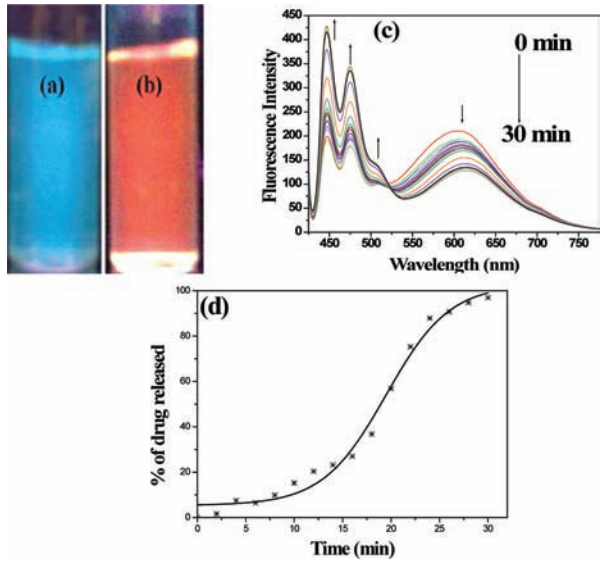

Figure 6. Real-time monitoring of the drug release. (a, b) Perylene-3-ylmethyl nanoparticles (a) before and (b) after drug loading. (c) Change in fluorescence spectral profile with increasing irradiation time. (d) Percentage of drug released as a function of fluorescence intensity change.

the perylene-3-ylmethyl nanoparticles showed blue fluorescence. However, after loading of chlorambucil, the nanoparticles exhibited strong red fluorescence (Figure 6b). In vitro drug release experiments were then carried out by dialysis. Figure 6c shows the fluorescence changes of Pe−Cbl nanoconjugates with different irradiation times. We noticed a gradual decrease in the red emission band at 625 nm for photocaged Pe−Cbl nanoconjugates and a concomitant increase in the blue emission band at 445 nm with increasing irradiation time. We also calculated the percentage of drug release as a function of fluorescence intensity change (Figure 6d). The above change in fluorescence color was also employed to monitor the drug release in the cell in real time using the fluorescence imaging technique (Figure 7a−c).

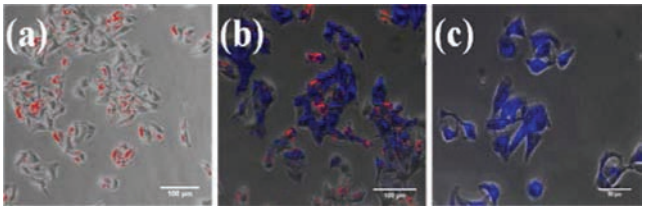

Figure 7. Real-time drug release studies using confocal microscopy. Shown are overlays of the bright-field image and the fluorescence images in the 445 and 625 nm emission channels (a) before photolysis, (b) after 10 min of photolysis, and (c) after 20 min of photolysis.

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In summary, we have developed single-component photoresponsive nanocarrier based on fluorescent organic nanoparticles consisting of perylene-3-ylmethanol. The nanoparticles acted as both the nanocarrier for the drug and the phototrigger for the drug release. Interestingly, the perylene-3-ylmethanol nanoparticles showed good biocompatibility and cellular uptake as well as precise drug release to kill the cancer cells upon irradiation. Furthermore, a change in the fluorescence color of the perylene-3-ylmethanol nanoparticles indicated loading and unloading of the drug. In the future, we are looking forward to the development of fluorescent organic nanoparticles as photoresponsive nanocarriers for targeted drug delivery.

■ ASSOCIATED CONTENT

Supporting Information

Synthesis details, characterization data, and other experimental details. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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