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Anticancer Drug Release from a Mesoporous Silica Based Nanophotocage Regulated by Either a One- or Two-Photon Process

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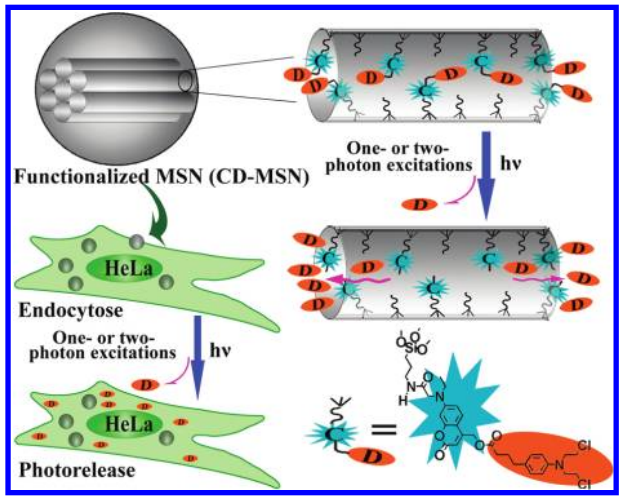
Abstract: An excellent mesoporous silica nanoparticle (MSN) based drug deliver system (DDS) was reported for regulated anticancer drug release upon the irradiation of either one- or two-photon excitation. In this system, the coumarin grafted on MSN acted as both the "phototrigger" for the drug release and fluorescence group for cell luminescence imaging. External light manipulations such as changing irradiation wavelength, intensity, and time can regulate the release of the anticancer drug precisely. Biological studies *in vitro* suggest that the drug carrier can effectively deliver the anticancer drug into intracellular environs and, hence, promote the drug action to kill the cancer cells upon irradiation. We envision that the good biocompatibility, cellar uptake property, and efficient photoregulated drug release will be of great benefit to future controlled release *in vivo* biomedical applications.

Recently, photoresponsive nanocarriers have attracted much attention for their applications in the area of drug and gene delivery, because light can function as a highly orthogonal external stimulus. Release from photoresponsive nanocarriers can be controlled at a specific time and location for phototherapy.[1−3] Ultimately, an excellent photoresponsive drug delivery system (DDS) should process the properties of zero-premature release, near-infrared (NIR) light excitation, clean photolysis without side products, and external precise manipulations. The aim of NIR irradiation is to avoid the cytotoxic effects of UV light whose intensity attenuates quickly in tissue.[4] Especially, the use of two-photon irradiation is capable of noninvasively localizing the photochemistry to any given spot in three dimensions. Clean photolysis without side products is also very important since the presence of side products might cause unpredictable toxicities. External manipulation can control drug release precisely by changing the irradiation parameters such as wavelength, intensity, and time. Thus far, despite the many methods that have been developed for the photoresponsive DDS,[5−7] most of them have limited successes and suffer from a number of intrinsic drawbacks, such as using cytotoxic UV light or generating side products during the photolysis process, rendering them nonfunctional under physiological conditions. Mesoporous silica nanoparticles (MSN) are excellent carriers for drug delivery due to superior properties of mesoporous structure and good biocompatibility.[8,9] Herein, we report a novel MSN-based nanophotocaged DDS whose drug can be effectively uncaged by external precise manipulations under two-photon NIR excitation at 800 nm, as depicted in Scheme 1. To achieve this NIR sensitivity, we incorporated a 7-amino-coumarin chromophore, which has a sufficiently high two-photon absorption cross section (see Supporting Information (SI)),[10] into the grafted molecule as a phototrigger to uncage anticancer drug chlorambucil. Successfully completing such a light-regulated system represents a significant step toward the photoregulated DDS.

Scheme 1. Schematic Representation of Photolysis for Drug Release Using CD-MSN Based DDS under One- Or Two-Photon Excitations



First, we synthesized an aminopropyl-functionalized MCM-41-type MSN material (AP-MSN) using a method reported previously.[6a] TEM, powder X-ray diffraction (XRD), and the nitrogen sorption isotherms analysis confirmed that the 130 nm particles had a honeycomb-like structure with a 2.1 nm average pore diameter and a surface area of 752 m^2/g (SI, Figures S2−S4). Next, 7-amino-coumarin derivative (CD) modified with anticancer drug chlorambucil or pure coumarin derivative (C, for the comparison) was grafted onto the surface of the AP-MSN to obtain the final functionalized MSN materials (CD-MSN, C-MSN). The loading of CD or C on the surface of AP-MSN was validated by FT-IR and UV−vis spectroscopy. The broad absorbance from 300 to 430 nm and strong emission at 463 nm indicated that our DDS could be excited and imaged using visible light (SI, Figures S5−S6). The amount of surface anchored CD or C was determined to be approximately 103 and 239 μmol/g, respectively, by TGA analysis (SI, Figure S7). Additionally, the zeta potential (ζ-potential) change further confirmed the successful preparation of CD-MSN and C-MSN (SI, Table S2).

To check the stability of this CD-MSN based drug release system, we examined the remaining rate under dark conditions by dispersing CD-MSN in PBS solution and the culture media with 10% fetal bovine serum which was kept for 36 h at 37 °C with appropriate

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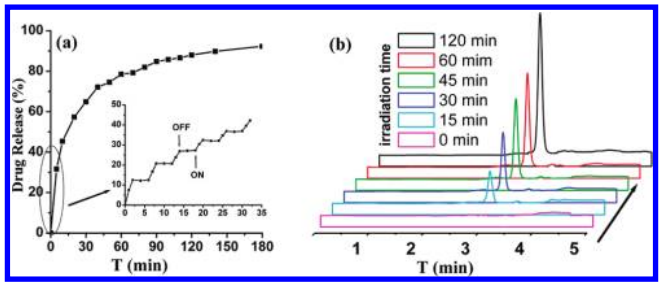

Figure 1. (a) HPLC analysis was used to monitor the drug release process from chlorambucil-grafted CD-MSN material under 420 nm light irradiation (10 mW/cm^2). Inset is the partial progress for the release of chlorambucil from CD-MSN material under bright and dark conditions. "ON" indicates the beginning of light irradiation; "OFF" indicates the ending of light irradiation. (b) As time elapsed, HPLC determined the profiles of released chlorambucil under two-photon NIR excitation at 800 nm.

pH (SI, Table S4). The high remaining rate (94.5%) in culture media suggested that this DDS was quite stable and suitable for the following biological study *in vitro*. The time courses of the drug release under photolysis at both 420 nm visible light (a xenon lamp) (Figure 1a) and two-photon 800 nm NIR light (Figure 1b) were monitored by HPLC. Both photolytic processes progressed effectively; the release controlled by two-photon NIR irradiation reached 40% after 2 h with a ~1 mm diameter beam spot (SI, Figure S9). The maximum release in Figure 1a had reached 97% after 3 h of irradiation (10 mW/cm^2), and the enhancement of the light intensity by using visible light ($\lambda > 400$ nm, 120 mW/cm^2) could complete the release process within 15 min, suggesting that external light intensity could regulate the drug release. In addition, there were no detectable side products in the photolysis for the CD-MSN based DDS. This was an outstanding result compared to the small molecular prodrug system[11] and MSN-based systems with pore-blocking caged caps,[1c,6] because the generation of side products might have a potential negative effect for the therapy. Precise control of the photolytic release was demonstrated by monitoring the progress of chlorambucil release after periods of exposure to light and dark conditions, as shown in the inset of Figure 1a. The distinctive "stepped" profile revealed that the drug release only proceeded under light conditions, thus realizing "light-regulated precise release". All the results indicated that the CD-MSN based DDS could precisely control drug release by manipulating external light intensity, irradiation wavelength, and time. These results hint that it is possible to *precisely* release anticancer drugs containing −COOH, −NH$_2$, −OH, −SH groups et al.[10] both *in vitro* and *in vivo*.

Surface-functionalized MSNs can be efficiently endocytosed by mammalian cells.[9b,12] For the CD-MSN materials, the effective endocytosis was confirmed by both confocal images (Figure 2a−c) and two-photon laser scanning microscopy (SI, Figure S10). ICP-OES determined that the internalized silicon concentration was 3−13 pg/cell after the cells were cultured with 20 μg/mL MSN materials for 6 h (SI, Table S5). Cell viability was quantified by an MTT assay using both HeLa (Figure 2d) and MCF-7 cells (SI, Figure S11). An IC$_{50}$ value of 20 μg/mL (chlorambucil payload 2 μM) was observed upon irradiation of CD-MSN, which was much lower than that for CD-MSN treated with light first and then incubated with cells in the dark (IC$_{50}$ > 160 μg/mL). And as shown in Figure 2e, there was no significant cell death observed when the cells were treated with light in the case of AP-MSN and C-MSN, respectively. The cytotoxicity was likely caused by the released drug, chlorambucil, upon irradiation. Upon comparison with the same amount of chlorambucil as shown in Figure 2d, the CD-MSN upon irradiation showed a higher cytotoxicity to cancer cells, which

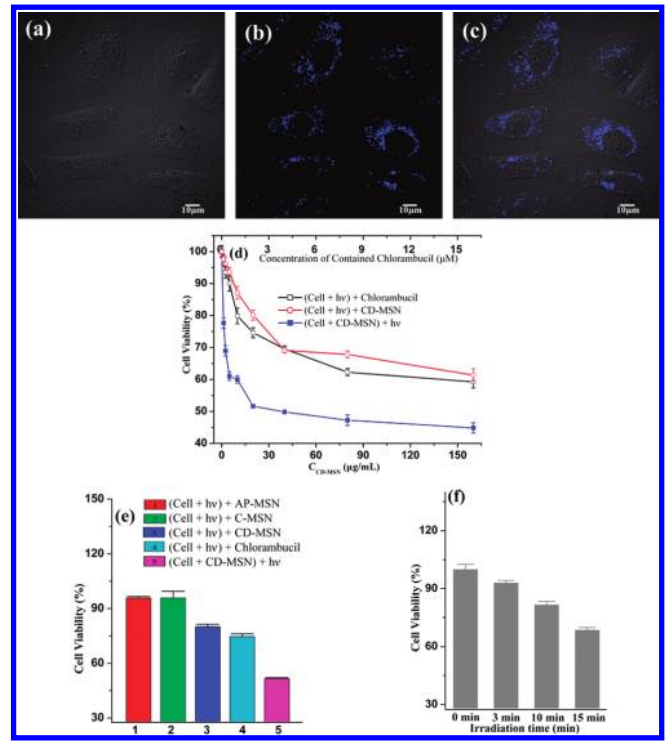

Figure 2. Confocal fluorescence and brightfield images of HeLa cells incubated with 20 μg/mL CD-MSN for 3 h: (a) brightfield; (b) fluorescence (λ_{ex} = 405 nm); (c) the overlay image of a and b. (d) Cytotoxicity to HeLa cells is plotted against the concentrations of chlorambucil and CD-MSN under control conditions and 15 min light exposure. (e) Effect of different conditions on the viability of HeLa cells: the concentration of MSN materials is 20 μg/mL and the corresponding concentration of free chlorambucil is 2 μM, and the light exposure time is 15 min. (f) Cell viability with different durations of light exposure: the concentration of CD-MSN used is 5 μg/mL. Visible light ($\lambda > 400$ nm) intensity used in cell cytotoxicity tests was 120 mW/cm^2.

could be explained by the effective cell endocytosis of CD-MSN resulting in a higher accumulation of chlorambucil inside the cells.[13] Here, CD-MSN serves as both a drug carrier and a photocage for the release of the anticancer drug. Furthermore, to validate our ability to externally regulate drug release, the HeLa cells incubated with CD-MSN were exposed to visible light for 0, 3, 10, and 15 min. These results show that the drug release can be regulated by the duration of the applied light (Figure 2f).

In summary, we have successfully prepared an excellent MSN-based DDS for regulated release under the irradiation of two-photon NIR excitation. In this system, CD-MSN serves as both a carrier and photocage for the drug whose release can be regulated precisely by controlling the irradiation wavelength, intensity, and time of the external light. We envision that good biocompatibility, cellar uptake property, and efficient photoregulated drug release will be of great benefit to future controlled release for *in vivo* biomedical applications.

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Supporting Information Available: Preparation, photolysis mechanism, cellular studies, and other experimental details. This material is available free of charge via the Internet at http://pubs.acs.org.

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