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Photoinduced Intracellular Controlled Release Drug Delivery in Human Cells by Gold-Capped Mesoporous Silica Nanosphere

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Recent developments in designing surface functionalized mesoporous silica nanospheres (MSNs) have revealed the promising potential of utilizing these structurally ordered materials for drug/gene delivery.[1−4] For many practical drug delivery applications, such as chemotherapy, "zero-premature release" and "stimuli-responsive controlled release" of the precious and often toxic pharmaceutical cargo are two important prerequisites that would impact the therapeutic efficacy and cytotoxicity of drug delivery. Unfortunately, constructing drug delivery carriers with control over the location and timing of drug release under physiological conditions remains a challenge.

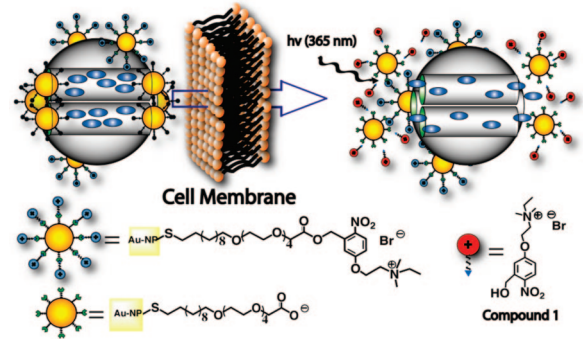
Recently, several MSN-based controlled-release systems with the "zero-premature release" property have been synthesized by using different kinds of pore-blocking caps, such as nanoparticles (NPs),[5−7] organic molecules,[8,9] and supramolecular assemblies.[10,11] Different stimuli-responsive strategies, such as chemical,[5] pH,[9,12,13] electrostatic interaction,[14] enzymatic,[15] redox,[16] and photoirradiation,[17−21] have been applied as "triggers" for uncapping the pores and releasing the guest molecules from MSNs. Despite these burgeoning developments, many of the MSN-based controlled release systems possess only one of the two features, but not both, and are unable to function under physiological conditions. For efficient controlled release of toxic drugs *in vitro* and/or *in vivo*, it would be advantageous to design a capped MSN material that would respond to a noninvasive and externally controllable trigger, such as photoirradiation, under physiological conditions. Herein, we report on the synthesis of a gold nanoparticle-capped MSN material for the photoinduced intracellular controlled release of an anticancer drug, paclitaxel, inside of human fibroblast and liver cells as depicted in Scheme 1.

We first functionalized the surface of the gold nanoparticle with a photoresponsive linker (thioundecyl-tetraethyleneglycolester-*o*-nitrobenzylethyldimethyl ammonium bromide, TUNA) by following a procedure reported by Rotello and co-workers[22] with modifications as detailed in the Supporting Information (SI). The organically derivatized gold nanoparticles (PR-AuNPs) are positively charged (ζ-potential = +4.2 ± 1.4 mV) in PBS (pH 7.4) with an average particle diameter of 5 nm as determined by the transmission electron microscopy (Figure S1 of SI).

We then synthesized an MSN material according to our previously reported method.[11] The honeycomb-like MCM-41 type of mesoporous structure was confirmed by TEM (Figure 1a) and powder X-Ray diffractometry (Figure S2 of SI). As shown in Figure 1a, the MSN particle is spherical in shape with an average diameter of 100 nm. The N_2 sorption analysis of MSN further revealed a type IV BET isotherm with a total surface area of 1083 m^2/g. Also, a narrow BJH pore size distribution was observed with an average pore diameter of 3.0 nm (Figure S3 of SI).

The capping mechanism of this PR-AuNPs-MSN system is based on the electrostatic interaction between the positively charged PR-AuNPs and the negatively charged MSN material (ζ-potential = −23.8 ± 1.8 mV) in water. As illustrated in Scheme 1, upon photoirradiation, the photolabile linker covalently attached to the surface of PR-AuNPs would be cleaved, resulting in the formation of a cationic compound (**1**) as well as the negatively charged, thioundecyltetraethyleneglycolcarboxylate (TUEC)-functionalized AuNPs (NC-AuNPs).[22] The charge repulsion between the NC-AuNPs and MSN would then uncap the mesopores and allowed the release of guest molecules.

Scheme 1. Schematic Illustration of the Photoinduced Intracellular Controlled Release of PR-AuNPs-MSN[a]



[a] Upon UV irradiation, the photolabile linker on the PR-AuNPs was cleaved, changing the surface charge property (ζ-potential) of these gold nanoparticles from positive to negative. The charge repulsion between the AuNPs and MSN would then uncap the mesopores and allowed the release of guest molecules.

TEM microscopy was used to confirm the interaction between MSN and PR-AuNPs. Figure 1 shows the TEM micrographs of MSN before and after capping with PR-AuNPs. In the case of the uncapped MSN (Figure 1a), the hexagonally packed mesoporous channels could be clearly visualized. In contrast, the TEM micrograph of PR-AuNPs-capped MSN (Figure 1b) shows dark spots on the outside edges of the mesopores, representing the aggregation of PR-AuNPs on the exterior surface of MSN. The presence of AuNPs was also confirmed by energy dispersive X-ray (EDX) analysis and powder XRD (Figure S4 of SI).

To investigate the photoinduced controlled release property of the PR-AuNPs-MSN system in aqueous solution, fluorescein was used as a model guest molecule. The fluorescein-loaded PR-AuNPs-MSN sample was prepared, and the loading of fluorescein was determined to be 1.51 μmol/g of MSN as described in the SI. To examine the capping efficiency, the fluorescein-loaded PR-AuNPs-MSN sample was first stirred for 21 h before irradiation. Less than 0.003 μmol/g of fluorescein was leached into the aqueous solution. This amount of leaching is similar to that of the control MSN sample without capping (Figure 2a). The result indicated that the capping strategy was successful with good efficiency. The photoinduced controlled release of fluorescein was investigated at 365 nm under low-power (0.49 mW/cm^2) UV irradiation for 10 min.

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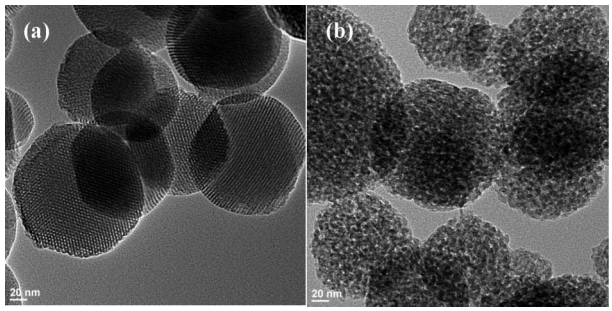

Figure 1. (a) TEM micrograph of MSN; the MCM-41 type mesoporous structure is clearly visualized with the light-colored parallel stripes and the hexagonally packed spots. (b) TEM micrograph of the PR-AuNPs-capped MSN shows the aggregation (dark spots) of the PR-AuNPs on the exterior surface of MSN.

The release reached 100% after stirring in water for 35 h as depicted in Figure 2a. The amount of fluorescein released from MSN was 0.11 μmol/g. A control experiment without UV irradiation was also carried out. No release of fluorescein was observed even after 80 h (Figure 2a). Furthermore, we examined the controlled release property at a wavelength (530 nm) that is known to induce local heating of AuNPs.[23] As detailed in Figure S7 of the SI, less than 10% release of fluorescein was observed under much higher irradiation intensity (3 mW/cm^2) than that of the aforementioned condition at 365 nm (0.49 mW/cm^2). The result indicated that the release of fluorescein from PR-AuNPs-MSN was not due to the photothermal effect of AuNPs.

To validate the feasibility of using the PR-AuNPs-MSN system for intracellular drug delivery in live human cells, a hydrophobic anticancer drug (paclitaxel) was chosen as the guest molecule for the controlled release study in human liver and fibroblast cells. We found that the paclitaxel-loaded PR-AuNPs-MSN material was endocytosed rapidly by these two cell types as determined by flow cytometry as detailed in the SI. After UV irradiation for 10 min, significant decreases in the cell viability, 44.2% and 43.5%, were observed for liver and fibroblast cells containing paclitaxel-loaded PR-AuNPs-MSN, respectively (Figures 2b,c and 3). This result indicated that PR-AuNPs-MSN could indeed transport and release paclitaxel inside these live human cells under the control of photoirradiation. Interestingly, without paclitaxel, the PR-AuNPs-MSN material alone, before and after the UV irradiation, was not toxic to cells as indicated in Figure 3. Also, by encapsulating the paclitaxel inside of MSN with the PR-AuNPs cap, the cytotoxicity of paclitaxel to liver and fibroblast cells was significantly lowered (Figure 3).

In conclusion, we have demonstrated that pacliatxel molecules could be encapsulated inside of PR-AuNPs-MSN without the undesired drug leaching inside of live human fibroblast and liver cells. This "zero premature release" characteristic is of importance for delivery of toxic drugs in chemotherapy. Furthermore, we proved that the cargo-release property of our PR-AuNPs-MSN system could be easily controlled by low-power photoirradiation under biocompatible and physiological condition. We envision that our results would lead to a new generation of carrier materials for intracellular delivery of a variety of hydrophobic toxic drugs.

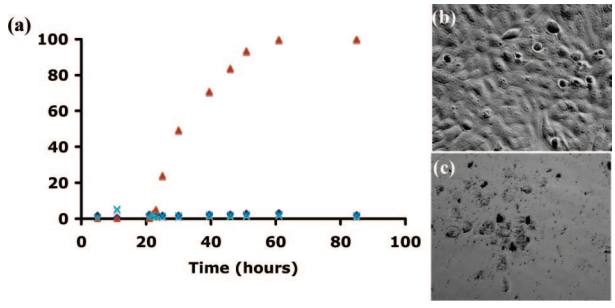

Figure 2. (a) Controlled release profile of fluorescein-loaded PR-AuNPs-MSN after UV irradiation (▲), in the dark (×), and the control sample, fluorescein-loaded MSN without the AuNPs cap (◆). Micrographs of human fibroblast cells containing paclitaxel-loaded PR-AuNPs-MSN before (b) and after (c) UV irradiation.

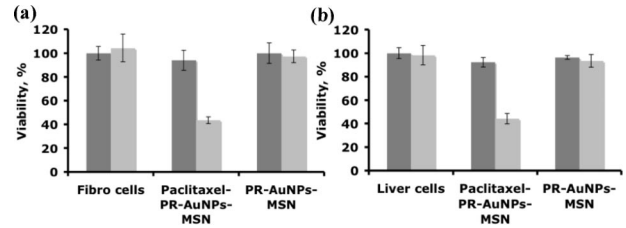

Figure 3. Cell viability study of the paclitaxel-loaded PR-AuNPs-MSN material with Human fibroblast (a) and liver (b) cells. All samples were UV-irradiated (365 nm) for 10 min. The concentration of material used was 10 μg/mL. Dark-gray and light-gray bars represent samples without and with irradiation, respectively.

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Supporting Information Available: Experimental details for the synthesis and characterization of MSN, PR-AuNPs, zeta potential measurement, and cytotoxicity. This material is available free of charge via the Internet at http://pubs.acs.org.

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