J|A|C|S COMMUNICATIONS

Published on Web 04/07/2009

Photoregulated Release of Caged Anticancer Drugs from Gold Nanoparticles

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A key attribute of drug delivery systems (DDSs) is their ability to regulate drug release, minimizing side effects and improving therapeutic efficacy of conventional pharmaceuticals.[1] Two approaches can be used to regulate the release of the therapeutic payload from the carrier: endogenous and exogenous activation. Endogenous activation strategies[2] exploit specific physiochemical characteristics of the pathological microenvironment, providing biologically controlled release. Exogenous activation[3] provides a complementary approach, employing orthogonal external stimuli to effect drug release.

Light provides a highly orthogonal external stimulus, allowing spatiotemporal control of payload release. In recent applications of this strategy, drug encapsulated carriers of 100−500 nm size (i.e., mesoporous silica, self-assembled molecular aggregates) containing a photoswitch for cargo release have been developed.[4] In an alternative approach, caged drugs have been developed where the activity of the drug is suppressed by attaching it to a blocking element through a photoremovable protecting group.[5]

Monolayer protected gold nanoparticles (AuNPs) provide an appealing synthetic scaffold for the creation of DDSs due to their functional versatility, better biocompatibility, and low toxicity.[6] Moreover, through the appropriate choice of particle size (2−10 nm), enhanced biodistribution (i.e., passive targeting) can be obtained through the enhanced permeation and retention (EPR) effect.[7] The EPR effect arises from the increased permeability of the tumor tissue vasculature, which allows nanocarriers to extravasate into the interstitial space,[1,7] resulting in an enrichment of the carriers within the tumor tissue. We describe here the use of AuNPs for photocontrolled release of a caged anticancer drug (5-fluorouracil, 5-FU) by conjugating the drug to the particle surface through a photoresponsive *o*-nitrobenzyl (ONB) linkage. In this approach, the particle serves to both cage and transport the therapeutic.

The fluorouracil conjugated gold nanoparticles (**Au_PCFU**) synthesized for this study possess a gold core diameter of ∼2 nm and feature a surface functionality comprising a mixed self-assembled monolayer of photocleavable and zwitterionic thiol ligands. The two ligands feature a common basic structure, where an alkyl segment is used to confer stability on the particle, while the tetra(ethylene glycol) component provides water solubility and superior biocompatibility.[8,6d] The zwitterionic ligand serves to enhance solubility and prevent cellular uptake,[9] while the photocleavable ligand integrates fluorouracil (5-FU) moieties to the nanoparticle surface through a terminally anchored *ortho*-nitrobenzyl (ONB) group. The ONB group has long-term stability under ambient light in biological environments. However, it undergoes photolytic cleavage at 365 nm when exposed to UV radiation, thus allowing controlled uncaging of the covalently attached 5-FU moieties.[5b,10]

The time course of the photolytic release was first monitored by means of UV−vis spectroscopy. We characterized the uncaging behavior of a free photocleavable thiol ligand and **Au_PCFU** by irradiating solutions using 365 nm UV-A radiation. As the photolytic reaction proceeds, a decrease of absorbance was observed at 200, 230, and 280 nm, along with a noticeable increase at 215, 250, and 375 nm (see Supporting Information Figure S8). These results confirm that the photolytic reaction of **Au_PCFU** proceeds in an analogous fashion to that of the free photocleavable thiol ligand.

The identity and amount of the photoreleased product (5-FU) of **Au_PCFU** were verified by irradiation followed by spin filteration through a 50 000 MW cutoff filter to remove the nanoparticle from solution. As shown in Figure 1b the absorption spectra of solution

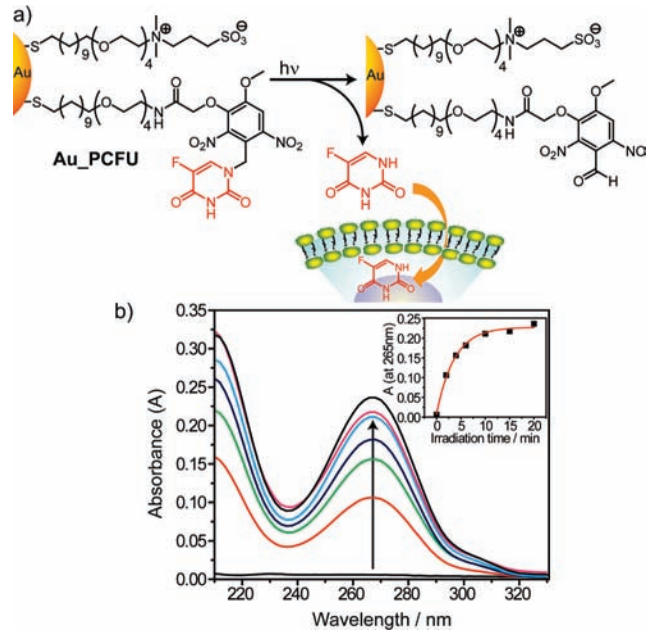

Figure 1. (a) Photochemical reaction of **Au_PCFU** and delivery of payload to cell. (b) Overlaid UV−vis spectral changes showing light dose dependent increase of 5-FU concentration. Inset: The plot of absorbance at 265 nm against irradiation time.

clearly indicate the presence of free 5-FU. This release is dependent on irradiation time, with maximum release observed at ∼10 min (inset, Figure 1b). Based on the absorption value at 265 nm, the maximum number of 5-FU molecules released from a single nanoparticle surface was estimated to be 14, 82% of the 17 photocleavable ligands on **Au_PCFU** as determined by UV−vis spectroscopy.[11] The release of 5-FU was also monitored after exposing the nanoparticle solution to alternating periods of light and dark (see Supporting Information Figure S9). The step profile of the product formation reveals no release is observed in the dark and that uncaging is spatiotemporally restricted to the illuminated region.

We next evaluated the use of **Au_PCFU** as a photocontrolled DDS through MCF-7 cell culture studies. **Au_PCFU** dispersed in cell culture media was added to the cells, which were then irradiated

10.1021/ja900591t CCC: $40.75

at 365 nm for 20 min. After 96 h further incubation, optical micrographs were taken to visualize the change in cell morphology, and the live cells were stained with calcein AM (Figure 2a−d).

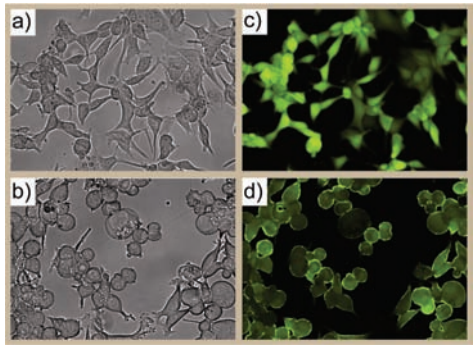

Figure 2. Bright-field and fluorescence-microscopy images of the cells irradiating before treated with **Au_PCFU** (a and c) and irradiating after treated with **Au_PCFU** (b and d).

Cell viability was quantified by Alamar blue assay. An IC_{50} value of 0.7 μM was observed upon irradiation for **Au_PCFU** on a per particle basis, corresponding to 11.9 μM on a per drug basis. In contrast, when the cells were first exposed to light and afterward incubated with the particle, there were no signs of toxicity (Figure 3a). Likewise, no significant cell death was observed in cells treated

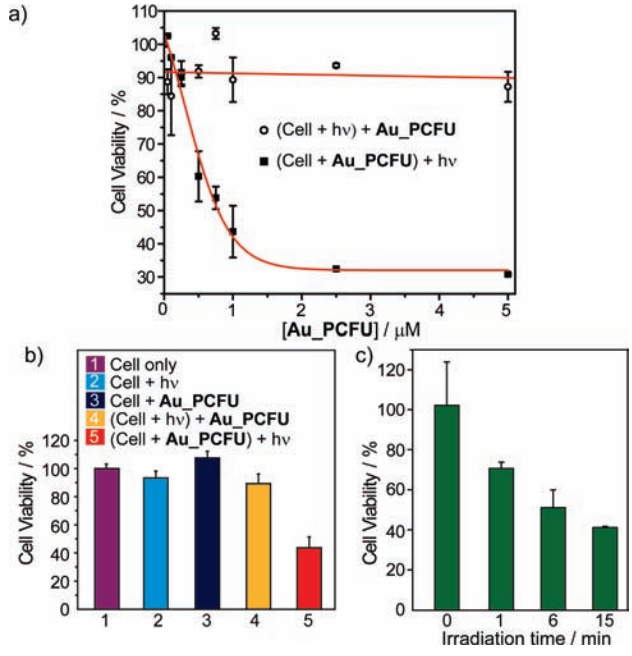

Figure 3. (a) Cytotoxicity of different concentrations of **Au_PCFU** under uncaging and control conditions. The IC_{50} value was 0.7 μM per particle, 11.9 μM per drug. (b) Effect of different conditions on the cell viability of MCF-7 cell line. The concentration of **Au_PCFU** used is 1 μM, and the light exposure time is 20 min. (c) Cell viability with different durations of light exposure.

with only light or only **Au_PCFU** (Figure 3b), demonstrating the lack of toxicity from either light or particle. Taken together, these observations demonstrate that **Au_PCFU** serves as a drug carrier as well as a caging group for 5-FU function.[12]

An important benefit of exogenous control is the ability to externally regulate drug dosing. To demonstrate this capability MCF-7 cells were first treated with a 1 μM solution of **Au_PCFU** and then exposed to 365 nm UV light for 0, 1, 6, and 15 min. The cytotoxicity studies after 96 h of incubation show that the cell viability decreases with increasing duration of the applied light (Figure 3c). The light dependent change in cell viability thus effectively correlates the dose of the liberated drug with duration of the exposure to light.

In summary, we have demonstrated the light-controlled release of a therapeutic from a nanoscale gold nanoparticle carrier. In this system, the particle served as both cage and carrier for the drug, providing a nontoxic conjugate that effectively released the payload upon long wavelength UV irradiation. The small size (hydrodynamic diameter ~10 nm, as measured by DLS) of the carrier coupled with the engineered monolayer of this system should provide a long circulation time and preferential accumulation into the tumor tissues via the EPR effect.[7] We envision that using photolabile linkers responsive to two-photon excitation will enhance tissue penetration and reduce phototoxicity for *in vivo* application, which is an area under investigation.

Acknowledgment. This research is supported by the NIH (GM077173).

Supporting Information Available: Synthesis of ligands, preparation of the nanoparticle, protocols for *in vitro* and cellular studies, and other experimental procedures. This information is available free of charge via the Internet at http://pubs.acs.org.

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(11) See Supporting Information for detail analysis and characterization.
(12) Cytotoxicity experiment with free 5-FU under similar conditions showed an IC_{50} value of 4.5 μM (see Supporting Information Figure S10), roughly 50% of the per drug IC_{50} of **Au_PCFU** (9.7 μM) after factoring in loading and release efficiency.

JA900591T