Remotely Triggered Liposome Release by Near-Infrared Light Absorption via Hollow Gold Nanoshells

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A major challenge for drug delivery is to control drug release both spatially and temporally. Liposomes have been evaluated as drug nanocarriers for decades,1–5 but their clinical applications are often limited by slow release or poor availability of the encapsulated drug.6 Here we show that near-complete liposome release can be initiated within seconds (“burst” kinetics) by irradiating hollow gold nanoshells (HGNs) with a near-infrared (NIR) pulsed laser. NIR light penetrates into the tissue up to 10 cm,7 allowing these HGN/ liposome complexes to be addressed noninvasively within a significant fraction of the human body. Our findings on the underlying release mechanism reveal that this approach is conceptually analogous to using optically triggered nano-“sonicators” deep inside the body for drug delivery.

It has proven difficult to create liposomes that are simultaneously resistant to drug leakage in the circulation8,9 and able to rapidly release their contents at the site of interest. Many of the current strategies to enhance temporal or spatial control of drug release focus on incorporating components into the liposome membranes to achieve thermal, pH, photochemical, or enzymatically triggered release.10 Unfortunately, destabilizing agents often promote release in the circulation as well as the site of interest. Active targeting requires specific ligands with high affinities to receptors overexpressed on diseased cells, which can lead to “binding-site barriers” where the tightly bound nanocarriers prevent drug penetration into the tissue.4 In addition, targeting a different site requires the synthesis and characterization of a new ligand.

A new strategy is to delegate the task of controlled drug release to an externally triggered agent, while optimizing liposome composition and structure to enhance circulation time and drug retention. Recently, 2–3 nm gold particles incorporated into thermally sensitized liposome membranes were shown to enhance contents release over 10–20 min during continuous irradiation by UV-light (which limits application to the body surface).11 A NIR light-based approach has been shown to work on polymer carriers,12–15 which unfortunately are still in the research stage for drug delivery.16 Besides, it would be difficult to continuously irradiate a given nanoparticle for 10–20 min before it convects or diffuses out of the irradiation zone. Liposomes were the first type of nanocarriers in clinical use; however, little work on controlled release using NIR light has been reported. Several challenges are: (1) to develop easily synthesized, biocompatible triggering agents with a strong NIR absorption small enough (<50 nm) to load into liposomes;17 (2) to couple the nanoparticle triggers to liposomes without interfering with lipid membrane integrity or the drug contents to avoid premature release or chemical degradation; (3) to require only short bursts of irradiation so that the nanoparticles remain localized during triggering. We have addressed these challenges by synthesizing small HGNs which are either encapsulated within liposomes (by an interdigitization-fusion method8), or tethered to the liposome membrane with an Au-SH-PEG-lipid linker. Gold nanostructures exhibiting plasmon-resonance, for example, nanoshells18–22 and nanorods,23,24 are especially effective at converting NIR light into heat, and have been used successfully to noninvasively heat and eradicate diseased cells and tissues in vivo and in vitro.18,20,21,23,24 HGNs were selected due to their ease of synthesis and small dimensions,19,22 although other NIR-absorbing nanostructures could be used. Electron cryomicroscopy (Cryo-EM)25 images verified that the HGNs were encapsulated, tethered, or free in solution with liposomes (Figure 1 a–c). These HGN/ liposome complexes are as impermeable to dye release as uncomplexed liposomes (data not shown). An important difference between this and previous work is that femtosecond pulses of NIR light induce liposome contents release within seconds. Disruption of the liposome membrane is caused by the formation and collapse of transient vapor bubbles in the solution surrounding laser-heated

Figure 1. Characterization of HGN/liposome complexes: (a–c) Cryo-EM images showing HGNs (red arrows) (a) encapsulated inside, (b) tethered to, (c) suspended freely outside liposomes (blue arrows); (d) absorption spectrum of HGNs showing surface plasmon peak at 820 nm.

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HGNs, similar to the cavitation effects induced by ultrasound. That means release is not due to simple heating as ... (right) of the liposomes (blue arrows).

**Figure 2.** Effect of pulsed-laser power: (a) Kinetics of in situ fluorescence intensity shows the rate of liposome release induced by encapsulated HGNs at various laser powers. The solid lines are single exponential fits, \( F = F_0 + A e^{-\alpha E} \) to the data. (b) Liposome release as a function of laser power induced by HGNs encapsulated inside and suspended freely outside after 9 min of irradiation. The solid curves are sigmoidal fits to the data: \( y = y_{\text{max}}(1 + e^{\left(-E(x)\right)} + y_{\text{min}} \). The maximum release is different for the two coupling methods, but the threshold power density for release is the same (2.2 W/cm²). (c) Typical photoacoustic signal of pressure fluctuations associated with cavitation recorded by a hydrophone from a 0.142 mM HGN solution after a single laser pulse (16.1 W/cm²). The inset is an enlarged view of the first 100 µs. (d) Acoustic signal amplitude as a function of pulsed-laser power. 2.3 W/cm² is necessary to induce the cavitation signal, which is similar to the threshold needed to trigger liposome contents release (Figure 2b).

HGNs, similar to the cavitation effects induced by ultrasound. That means release is not due to simple heating as reported in previous work.¹⁵

As proof of concept, a fluorescent dye, 6-carboxyfluorescein (CF), was encapsulated inside liposomes and used as a soluble model drug. HGNs with a maximum absorption at 820 nm (Figure 1d) were synthesized via galvanic replacement chemistry¹⁹,²² (Supporting Information). HGNs were then coated with 750 Da polyethylene glycol-thiol (PEG) to enhance particle stability and were concentrated by ultracentrifugation. The diameter of the HGNs was 33 ± 13 nm with shell thickness of 3.4 ± 0.9 nm. HGNs were encapsulated within dipalmitoylphosphatidylcholine (DPPC) liposomes together with CF at a sufficient concentration that CF’s fluorescence was self-quenched (Figure 1a and Supporting Information). The unencapsulated HGNs and CF were removed by size-exclusion chromatography and centrifugation. On release from the liposomes, CF is diluted to micromolar concentrations so that the CF fluorescence intensity is proportional to its concentration. The release of CF from the liposomes was quantified by the increase in fluorescence intensity above the background relative to the fluorochrome intensity after all liposomes were lysed.⁸

Disruption of liposomes was triggered by irradiation with NIR pulses from a Ti:Sapphire laser (\( \lambda_0 = 800 \) nm, 130-fs duration, 1 kHz frequency, energy up to 670 µJ/pulse, corresponding to a mean power density of 16.1 W/cm²). We monitored the in situ CF release by recording the evolution of two-photon luminescence over time to determine the release kinetics (Figure 2a). Irradiation with the pulsed-NIR laser at a power exceeding 2.2 W/cm² triggered a near instantaneous increase of fluorescence intensity in the solution of liposomes encapsulating HGNs and CF. NIR laser pulses had no effect on the CF fluorescence intensity in control solutions of DPPC liposomes with CF but no HGNs, unencapsulated CF, or a mixture of HGNs and CF (Supporting Information).

To reveal the mechanism of contents release, we varied the laser power density and compared the fractional CF release. Figure 2b shows a threshold power density is needed to trigger release: no fluorescence increase was detected for a power density less than ∼1.5 W/cm²; while for power densities greater than 4.3 W/cm², the maximum fractional release remained constant at about 71% and 27%, for liposomes encapsulating HGNs (Figure 1a) or mixed with free HGNs (Figure 1c), respectively. The growth rate and magnitude of fluorescence intensity during NIR irradiation increased with the laser power density above the threshold (Figure 2a). With laser power density at 1.3 W/cm² (below the threshold) the fluorescence intensity was constant. At the maximum power level, release is complete within seconds. A similar power threshold (1.5 W/cm²) was reported necessary to damage cancer cells treated with NIR irradiation of gold nanocages.²¹

We investigated the changes of HGN/liposome complexes induced by pulsed laser irradiation. Cryo-EM shows that only minor changes in liposomal morphology are visible after irradiation (Figure 3); the membranes are less circular and appear to be under less tension than before irradiation (Figure 1), which is consistent with the decrease in the osmotic pressure caused by CF release. This lack of change in liposome morphology suggests that irradiation of the HGNs leads to transient defects in the lipid membrane that enable fast contents release, after which the membrane integrity is restored. Meanwhile, there was no observable change in the total CF fluorescence induced by the laser-heated nanoshell indicating that there was little, if any, chemical degradation of the dye. Cryo-EM also shows the change in morphology of the HGNs; the hollow core collapses on itself to form solid gold nanoparticles (red arrow in Figure 3). The HGN changes were confirmed by UV–vis spectroscopy (Supporting Information); the 820 nm absorption peak of HGNs gradually disappears with irradiation,²² along with the growth of a peak at ∼530 nm, which is typical for solid gold nanoparticles. The collapse of HGNs indicates they reach sufficiently high temperatures after absorbing NIR pulses to melt and anneal into more stable shapes. Even though the gold nanoshells are heated above their melting point, the temperature increase of the bulk solution was less than 1 °C above ambient. Hence, the rapid CF release was not due to the increased permeability of DPPC membranes known to occur at the phase transition temperature of 41 °C.³

The laser power threshold and the lack of permanent damage to the liposomes suggest that the triggered release occurs through perforation of lipid bilayers by microbubble formation and collapse, referred to as transient cavitation.²⁰,²⁷,²⁸ When an HGN is irradiated, its temperature rises substantially; heat dissipation to the surrounding water is slower than the electron dynamics in plasmon-mediated
Substantial temperature gradients around the HGNs can then cause the formation of unstable vapor microbubbles, which may grow rapidly and then collapse violently producing the mechanical and thermal effects associated with transient cavitation similar to those induced by ultrasound.  

Continuous-wave (cw) laser ($\lambda_0 = 820$ nm) irradiation for 4 h produced no release of CF from HGN/liposome complexes even at an increased power density (89 W/cm$^2$). Under cw irradiation, HGNs are always near thermal equilibrium with their surroundings; the lack of temperature gradients prevents microbubble formation and liposome disruption.  

The characteristic acoustic signals of pressure fluctuations in HGN solutions associated with cavitation were detected using a hydrophone (Figure 2c). These acoustic signals were absent in CF or buffer solutions which contained no HGNs under the same irradiation. Figure 2d shows the acoustic signal amplitude in the HGN solution as a function of laser power density. The acoustic signal amplitudes were at background up to the laser power density of $\sim$2.3 W/cm$^2$ which coincides with the power threshold for liposome contents release (Figures 2b). Above the threshold, there was a sharp increase in the acoustic signal amplitudes (Figure 2d). The increased laser power leads to higher HGN temperatures, which are then transmitted into larger pressure fluctuations in solution while this energy is dissipated. These results are consistent with reports on laser-induced cavitation.  

Membrane permeabilization by microbubble cavitation is expected to be induced by NIR-absorbing HGNs as long as HGNs are within an optimal distance from the lipid membrane. To test this hypothesis, we mixed DPPC liposomes containing CF with free HGNs (Figure 1c) at various concentrations. Upon pulsed laser irradiation, CF was released and the fractional release increased linearly with external gold concentration up to 0.0315 mM resulting in a maximum release of 35%. To minimize and control the distance between HGNs and the lipid membrane, HGNs were tethered to the liposomes via a thiol-PEG-lipid linker (Figure 1b, Supporting Information). Tethering HGNs directly to the outer surface of the liposomes increased the release fraction to 93%. Therefore, the efficacy of phototriggered contents release is strongly affected by the proximity of the HGN to the lipid membrane which is consistent with the hypothesis that mechanical disruption by microbubbles is responsible for the transient membrane rupture.  

In conclusion, pulsed NIR light absorbed by HGNs triggers the near instantaneous release of liposome contents providing precise spatial and temporal control. The laser-heated HGNs act as optically triggered nano-“sonicators” to temporally disrupt the lipid membrane. HGNs tethered to, encapsulated within, or suspended freely outside liposomes all induce liposome disruption; however tethering achieves the highest release efficacy due to the HGNs’ proximity to the lipid membrane. With this new NIR-activated release, disease cells can be synergistically targeted by combining drug carrying particles (liposomes) and energy absorbing particles (HGNs); continued irradiation of the HGNs can induce localized hyperthermia or permeabilize cell membranes, both of which can facilitate the cellular uptake of large macromolecules including proteins and DNA. This general approach will allow for better control of drug delivery to selected disease sites while minimizing systemic toxicity; no targeting ligands are needed to address different receptors and no “binding-site barriers” would limit drug penetration. In future work, in vivo testing and the long-term HGN safety need to be examined thoroughly, although preliminary studies suggest that gold nanoparticles are nontoxic.