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Remotely triggered liposomal release by near-infrared light absorption via hollow gold nanoshells

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Abstract

An elusive goal for systemic drug delivery is to provide both spatial and temporal control of drug release. Liposomes have been evaluated as drug delivery vehicles for decades [1–5], but their clinical significance has been limited by slow release or poor availability of the encapsulated drug [6]. Here we show that near-complete liposomal release can be initiated within seconds by irradiating hollow gold nanoshells (HGNs) with a near-infrared (NIR) pulsed laser. Our findings reveal that different coupling methods, such as having the HGNs tethered to, encapsulated within, or suspended freely outside the liposomes, all triggered liposomal release but with different levels of efficiency. For the underlying content release mechanism, our experiments suggest that microbubble formation and collapse due to the rapid temperature increase of the HGN is responsible for liposome disruption, as evidenced by the formation of solid gold particles after NIR irradiation and the coincidence of a laser power threshold for both triggered release and pressure fluctuations in the solution associating with cavitations. These effects are similar to those induced by ultrasound and our approach is conceptually analogous to use optically triggered nano-"sonicators" deep inside the body for drug delivery. We expect HGNs can be coupled with any nanocarriers to promote spatially and temporally controlled drug release. In addition, the capability of external HGNs to permeabilize lipid membranes can facilitate the cellular uptake of macromolecules, including proteins and DNA and allow for promising applications in gene therapy.

One major challenge for current drug delivery is to control the drug release both spatially and temporally. Liposomes have been evaluated as drug delivery vehicles for decades [1–5], but their clinical significance has been limited by slow release or poor availability of the encapsulated drug [6]. Here we show that near-complete liposomal release can be initiated within seconds ("burst" kinetics) by irradiating hollow gold nanoshells (HGNs) with a near-infrared (NIR) pulsed laser. Tissues are relatively transparent to NIR light which penetrates into body up to 10 cm [7]. This allows these HGN/liposome complexes to be addressed non-invasively 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.

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Liposomes optimized to be highly stable and resistant to drug leakage in the circulation [8,9] are hampered by suboptimal drug release to serve as drug carriers. Current endogenous strategies in drug release have focused on incorporating components into liposomes to achieve either thermal, pH, enzymatically triggered or receptor-targeted liposomes [3–5], however, none of them has led to marketed drugs [10]. It is difficult to include a destabilizing agent into the liposomes to promote release without compromising their stability. Besides, active targeting generally requires specific ligands with strong affinities to receptors over-expressed on diseased cells, which can also lead to "binding-site barriers" where the tightly bound nanocarriers prevent drug penetration into the tissue [4].

Approaches to separate the tasks of controlled release and drug retention were attempted by incorporating externally triggerable agents, however, there are issues limiting their applications in medical practice. For example, 2–3 nm gold particles absorbing UV-light were used to induce liposomal release [11] but this method was limited to only surface accessible areas such as the skin. NIR light-based approach has been shown to work on polymer carriers [12,13], which unfortunately are still in research stage for drug delivery [14]. For liposomes, the first type of nanoparticles currently in clinical use, however, no work has been reported on its efficient content release using NIR light. This may be due to several challenges: first, it is difficult to develop biocompatible triggering agents small enough (<50 nm) to load into liposomes while retaining a strong NIR absorption and with easy synthesis [15]; second, care has to be taken to couple triggering colloidal particles to liposomes without interfering lipid membrane integrity to avoid undesirable release. We have successfully addressed these issues by synthesizing small HGNs and then have them either encapsulated inside (by interdigitation-fusion vesicle method that has ~60% encapsulation efficiency for colloidal particles [8]), or tethered to liposomes (through a Au-SH-PEG lipid linkage) and efficient liposomal release has been observed. Recently, silica core-gold nanoshells of diameter larger than 100 nm were encapsulated by liposomes [16] but no characterization of liposome/nanoshell complex was made. We unambiguously confirm the HGN-liposome coupling by electron cryo-microscopy (Cryo-EM) imaging and verify that coupled liposomes have comparable content retention as those free of HGNs (data not shown). More importantly, beyond simple heating in the case of polymer capsules [13], our study reveals that laser-heated HGNs induce the formation of transient vapor bubbles which cause temporal rupture of lipid membranes and contents release upon collapse, similar to the cavitation effects induced by ultrasound.

As a proof of concept, a fluorescent dye, 6-carboxyfluorescein (CF), was encapsulated inside liposomes and used as a soluble model drug. Gold nanostructures exhibiting plasmon-resonance, i.e., nanoshells [17–21] and nanorods [22,23] are especially effective at converting NIR light into heat, and have been used successfully to non-invasively heat and eradicate diseased cells and tissues *in vivo* and *in vitro* [17,19,20,22,23]. We selected HGNs due to their ease of synthesis and small dimensions [18,21], although other absorbing nanostructures could be used.

HGNs with a maximum absorption at 820 nm (Fig. 1d) were synthesized via galvanic replacement chemistry [18,21] (Supporting Information). HGNs were then coated with 750-Da polyethylene glycol-thiol to enhance particle stability, and concentrated by ultracentrifugation. The dimensions of HGNs were 33 ± 13 nm in diameter and 3.4 ± 0.9 nm in shell thickness, analyzed using transmission electron microscopy. HGNs were encapsulated within dipalmitoylphosphatidylcholine (DPPC) liposomes (Supporting Information) together with CF at a sufficient concentration that CF's fluorescence was self-quenched. Cryo-EM [24–26] results confirm the encapsulation (Fig. 1a and Supporting Information). In Fig. 1, the liposome membranes are almost perfectly circular in the images, consistent with observations of membranes under tension caused by the higher osmotic pressure inside the liposome due to the internal CF. The unencapsulated HGNs and CF were removed by size exclusion column

and centrifugation. After release, the CF is diluted to micromolar concentrations so that CF fluorescence intensity is proportional to its concentration. The release of CF from the liposomes was detected by an increase in fluorescence intensity above the background [8].

Disruption of liposomes was triggered by irradiation with NIR pulses from a Ti:Sapphire laser (λ_0 = 800 nm, 130-fs duration, 1 kHz repetition rate, energy up to 670 μJ/pulse, corresponding to the mean power density of 16 W/cm^2). We monitored the *in situ* release of CF by recording the evolution of two-photon photoluminescence with time to determine the release kinetics. As shown in Fig. 2a, irradiation with the pulsed-NIR laser at a power exceeding 2.2 W/cm^2 triggered a near instantaneous increase of fluorescence in the solution of liposomes encapsulating HGNs and CF, but had no effect on the control solutions tested with either DPPC liposomes with CF but no HGNs, or with un-encapsulated CF, or a mixture of HGNs and CF (data not shown).

To reveal the mechanism of content release, we varied the power of the pulsed-laser when comparing the fractional release. Fig. 2b shows a distinct power threshold to trigger the release: almost no fluorescence increase was detected for a power lower than ~ 1.5 W/cm^2; while for powers greater than 4.3 W/cm^2, the maximum fractional release remained constant at about 71% and 27%, for liposomes encapsulating HGNs or mixing with free HGNs, respectively (Fig. 2b). The rate of fluorescence growth during NIR irradiation increased with the laser power (Fig. 2a). The *in situ* fluorescence intensity was constant at 1.3 W/cm^2, which is below the threshold. Above the threshold, the fluorescence intensity grows exponentially, with a time constant increasing with the decrease of the laser power. At maximum power level, release completes within seconds. A similar power threshold (1.5 W/cm^2) was reported for the damage of cancer cells treated with gold nanocages [20].

We further investigated the changes of HGN/liposome complexes induced by pulsed laser. Cryo-EM shows that only minor changes in liposomal morphology are visible after irradiation; the membranes are less circular and appear to be under less tension, consistent with the decrease in the positive osmotic pressure caused by CF release (Fig. 3). The minor changes in the liposome shapes or sizes suggest that the irradiation of the HGNs leads to transient defects in the lipid membrane that enable drug release, after which membrane's integrity is restored. Meanwhile, there was no observable change of CF fluorescence induced by the laser-heated nanoshells, indicating that there was little chemical degradation of the dye after the treatment. Importantly, cryo-EM shows the collapse of the HGNs at the hollow center (Fig. 3). The HGN changes were also revealed by absorption spectroscopy (Supporting Information); the 820 nm absorption peak of HGNs gradually disappears with irradiation [21], 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 the 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 occurring upon the phase transition near 41 °C [3].

The laser power threshold suggests that the mechanism of triggered release is through perforation of lipid bilayers mediated by microbubble formation and collapse, referred to as transient cavitation [19,28,29]. When an HGN is irradiated, its temperature rises substantially in one NIR pulse; dissipation of heat to the surrounding water is slower than the electron dynamics involved in plasmon-mediated heating [21,30]. Substantial gradients around the HGNs can 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 [29,31]. Control experiments with continuous-wave (cw) laser (λ_0 = 820 nm) produced no release of CF from HGN/liposome complexes,

even at an increased power (89 W/cm^2) after 4 hours. Under cw irradiation, HGNs are always in a state of thermal equilibrium with its surroundings, hence much smaller temperature gradients are insufficient to give rise to microbubble formation [21,30].

Indeed, we detected acoustic signals of pressure fluctuations associated with cavitation using a hydrophone after a laser pulse in HGN solutions (Fig. 2c). These acoustic signals were absent in CF or buffer solutions which contain no HGNs under the same irradiation. Fig. 2d shows the acoustic signal amplitude in the HGN solution as a function of laser power. The acoustic signal amplitudes were at background up to the laser power of ~ 2.3 W/cm^2, which coincides with the power threshold for liposomal content release (Figs. 2b). Above the threshold, there was a sharp increase in the acoustic signal amplitudes (Fig. 2d). The increased laser power leads to higher HGN temperatures [21], which are then translated into larger pressure fluctuations in solution while this energy is dissipated. These results agree well with report on laser-induced cavitations [32].

Membrane permeabilization with micro-bubble cavitation is expected to be induced by NIR-absorbing HGNs in the solution, 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 (Fig. 1c) at various concentrations. Upon pulsed laser irradiation, CF was released and the fractional release increases linearly with external gold concentration up to 0.0315 mM and reached the 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 (Fig. 1b) [33,34] (Supporting Information). Tethering HGNs directly to the outer surface of the liposomes increased the release fraction to 93%. Therefore, the efficacy of photo-triggered contents release is strongly affected by the proximity of HGN to the lipid membrane, consistent with the hypothesis that mechanical disruption by microbubbles is responsible for the temporal membrane rupture [19,22,35].

In conclusion, pulsed NIR light absorbed by HGNs trigger the near instantaneous release of the liposomal content, where laser-heated HGNs act as optically triggered nano-“sonicators” to temporally disrupt the lipid membrane. Among three coupling methods: HGNs tethered to, encapsulated within, or suspended freely outside liposomes, tethering achieves the highest release efficacy due to HGNs’ proximity to the lipid membrane. With this new NIR-activated release, disease-cell 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 on cells and no “binding-site barriers” would limit drug penetration [4].

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

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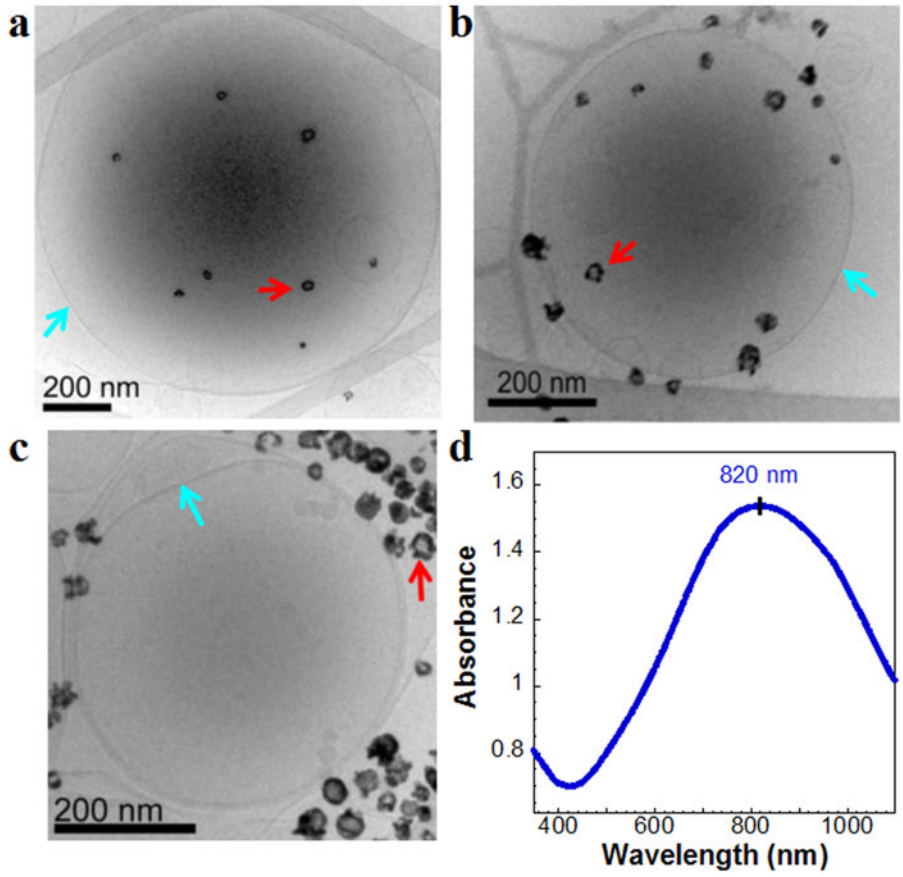

Figure 1. Characterization of HGN/liposome complexes

(a-c) Cryo-EM images showing HGNs (red arrows) **(a)** encapsulated inside, **(b)** tethered to, and **(c)** suspended freely outside liposomes (blue arrows) [8,27]. **(d)** Absorption spectrum of HGNs.

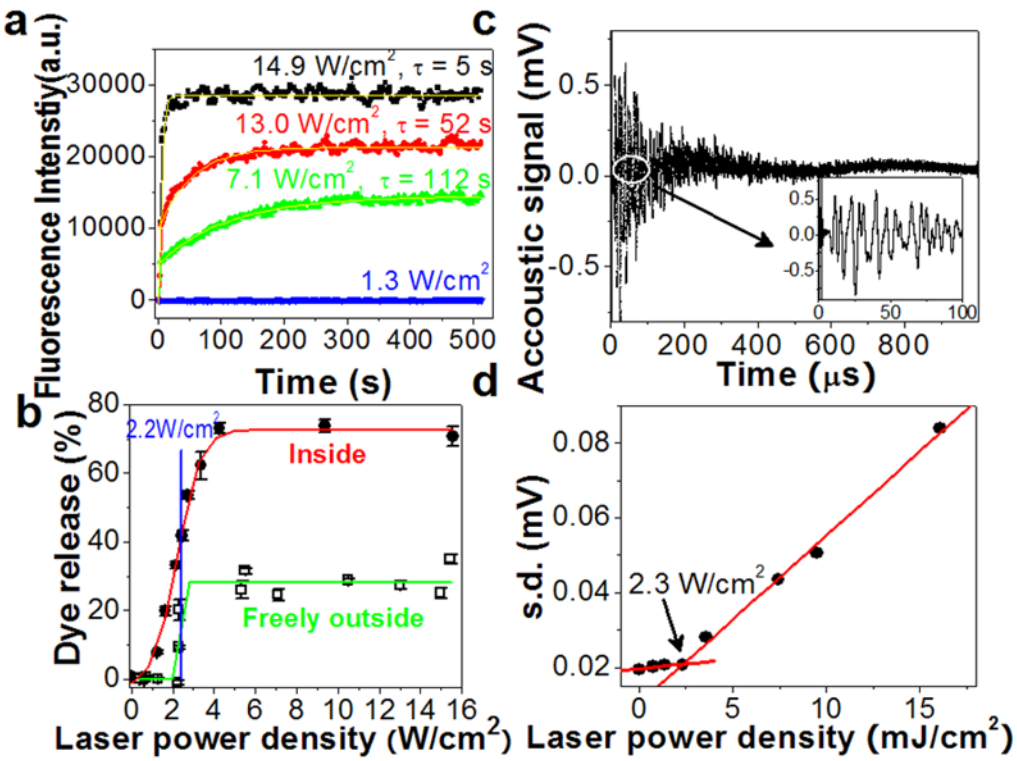

Figure 2. Effect of pulsed-laser power

(a) Kinetics of *in situ* fluorescence shows the rate of liposomal release induced by encapsulated HGNs at various laser powers. The solid lines are single exponential fits, $F = Fo + Ae^{-/\tau}$to the data. **(b)** Liposomal release as a function of laser power induced by HGNs encapsulated inside and suspended freely outside after 9 minutes irradiation. The solid curves are sigmoidal fit to the data: $y = (y_{max}-y_{min})/(1 + e^{(E-Eo)/\Delta E})+y_{min}$ The maximum release is different for the two coupling methods, but the threshold power for release is the same (2.2 W/cm^2). **(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 (average power 16.1 W/cm^2). The inset is an enlarged view. **(d)** Acoustic signal amplitude as a function of pulsed-laser power. A threshold of laser power at 2.3 W/cm^2 to induce the cavitation is similar to the threshold to trigger the liposomal contents release (Fig. 2b).

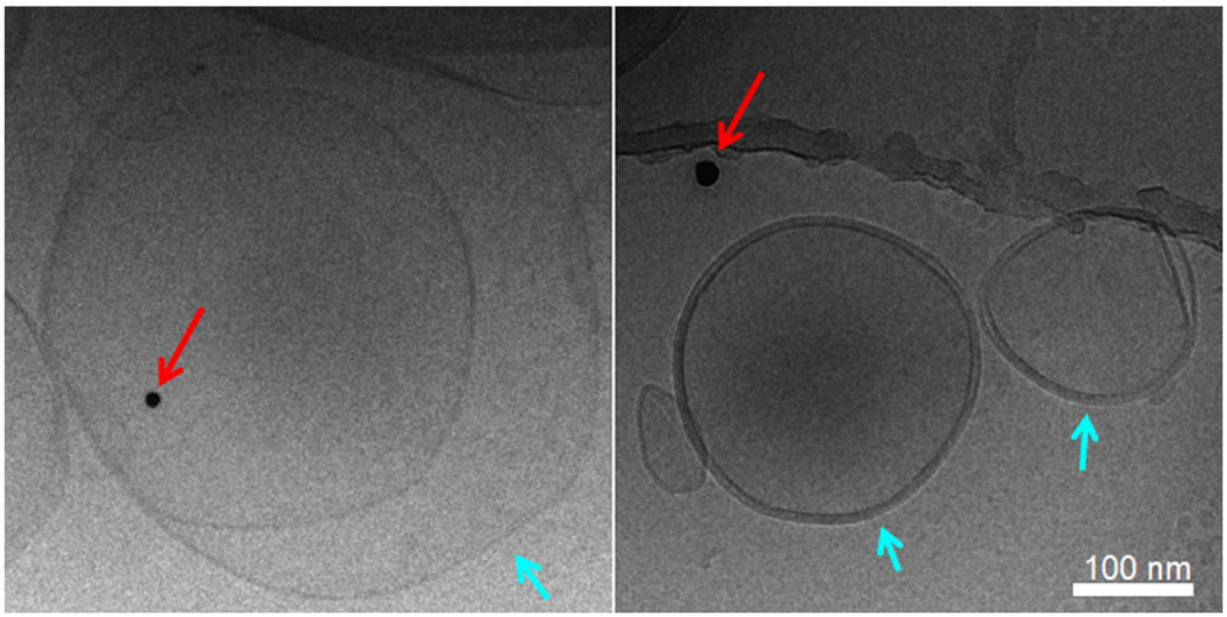

Figure 3. Morphology of HGN/liposome complex after laser irradiation

Cryo-EM images showing that HGNs become solid-core nanoparticle (red arrows) after NIR pulsed-laser irradiation (16.1 W/cm^2), both inside (left) and outside (right) of the liposomes (blue arrows).