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Wavelength-Selective Light-Induced Release from Plasmon Resonant Liposomes

Sarah J. Leung, Xenia M. Kachur, Michael C. Bobnick, and Dr. Marek Romanowski*

Department of Biomedical Engineering, University of Arizona, 1657 E. Helen Street, Tucson, AZ 85721, USA

Abstract

Biodegradable, spectrally tunable plasmon resonant nanocapsules are created via the deposition of gold onto the surface of 100 nm diameter thermosensitive liposomes. These nanocapsules demonstrate selective release of encapsulated contents upon illumination with light of a wavelength matching their distinct resonance bands, which correspond to 760 and 1210 nm in this study. Spectrally selective release is accomplished through the use of multiple, low intensity laser pulses delivered over a period of less than four minutes, ensuring that illumination affects only the gold-coated liposomes and avoids heating the surrounding media. The result of this illumination scheme for selective release using multiple wavelengths of light is a biologically safe mechanism for realizing drug delivery, microfluidic, and sensor applications.

Keywords

Nanostructures; Plasmon Resonance; Self-Assembly; Liposomes; Drug Delivery

1. Introduction

Widespread use of nanotechnology in biological and human health applications will require extensive efforts to combine therapeutic functions with biologically relevant mechanisms for the degradation or elimination of structures synthesized on the scale of nanometers. Indeed, the lingering concerns of poor elimination and potential toxicity of nanoparticles remain major impediments in the development of clinical applications of nanoscale materials.^[1,2] In the spirit of the longstanding interest in man-made materials inspired by biological self-assembly,^[3–8] we recently designed a composite structure, gold-coated liposomes, that combines the optical properties of plasmon resonant gold nanoshells with the biodegradability of liposomes.^[9,10] Here we report spectrally controlled release from these gold-coated liposomes. For the first time, we demonstrate selective content release, with nanocapsules of a particular color individually activated by a pulsed laser of a matching wavelength.

The encapsulation, targeting, and controlled release of therapeutic content remain the preferred means for delivering agents that are either unstable following administration or produce adverse effects when interacting with normal cells and tissues.^[11,12] Among the many systems capable of encapsulation and targeting, long-circulating liposomes are particularly successful and enable circulatory lifetimes in excess of 24 hrs^[12,13] and targeting to molecular markers of cancer,^[14] with a proven track of clinical and commercial development.^[15] Controlled release is frequently accomplished by designing lipid

*Fax: 520-626-4824, marekrom@email.arizona.edu.

compositions that are responsive to changes in temperature,^[16] pH,^[17] enzymatic action,^[18] or ultrasound.^[19] In comparison, release triggered by light offers unparalleled spatial and temporal control of the process. However, despite the long history of research efforts to produce spatially and temporally controlled release by light,^[20–29] there is no clinically viable liposome system capable of on-demand content release.

The recently demonstrated efficient photothermal conversion in nanoparticles of gold spurred interest in content release from composites combining gold nanostructures with thermosensitive materials. Laser-initiated photothermal conversion with subsequent thermal release provides the desired temporal and spatial control of the delivery process.^[30–36] Some delivery technologies rely on shock waves produced by extremely short laser pulses^[37] or employ continuous illumination, which has the potential to thermally ablate tissue.^[38–42] However, the size of gold nanoparticles required for these processes is in the range of 30–200 nm,^[38–41] which is not compatible with renal clearance requirements. The gold coated liposomes we introduced recently combine a thermosensitive lipid composition^[43] that releases content at temperatures corresponding to the lipid pre-transition or main phase transition with a shell-like assembly of gold clusters having a diameter below the limit of renal clearance.^[10] When fully assembled on the surface of a 100 nm diameter liposome, these gold clusters produce optical properties resembling those of a solid gold shell of this diameter, yielding a new class of biodegradable plasmon resonant capsules as reported by us earlier.^[9]

The present work elucidates two aspects of these gold-coated liposomes. First, their unique preparation method yields liposomes of the same diameter, and yet tuned to respond to different light wavelengths. In this first demonstration of spectrally controlled release, two samples of the gold-coated liposomes, each exhibiting different resonances, discharge their content when illuminated with light of a matching wavelength, and exhibit minimal release upon off-resonant illumination. Second, this content release relies on the cumulative action of relatively low energy pulsed illumination, with spectral selectivity controlled by the pulse width. The use of pulsed illumination ensures that resulting photothermal changes affect only the gold-coated liposomes, with no shock wave and minimal thermal impact on surrounding medium.

2. Results and Discussion

2.1. Particle Stability

Deposition of gold on the surface of liposomes (see Experimental Methods) follows a method of lipid vesicle metallization by the formation of zero-valent metal-lipid complexes.^[44] Stable gold deposition has previously been confirmed using a combination of scanning electron microscopy (SEM) and energy-dispersive X-ray spectroscopy (EDS) imaging of samples, which displayed co-localization of liposomes and gold.^[9] The gold deposition process does not appear to compromise liposomal stability or ability to encapsulate content. The results of particle sizing of intact and degraded gold-coated liposomes are presented in Figure 1. As determined by quasi-elastic light scattering, the average liposome diameter was approximately 98nm, number weighted (Malvern Zeta Sizer). To examine the stability of the nanoparticles over time, uncoated and plasmon resonant liposomes were sized and photographed over a two week period of incubation at 4 °C (Figure 2). While uncoated and gold-coated liposomes resonant at 760 nm showed stable diameter over the two week period, gold-coated liposomes resonant at 1210 nm demonstrated an almost two-fold increase in average diameter at one week, suggesting that an increased density of gold clusters at the surface of liposomes reduces their colloidal stability (Figure 2b). Sample photographs acquired over this period correlate with this finding. Suspensions of uncoated and 760 nm liposomes exhibit rather consistent and

uniform color over two weeks, while 1210 nm liposomes exhibit gradual color changes in their appearance over the observation period. These different stabilities of various gold-coated liposomes can be attributed to the different amount of gold required to produce these resonances. It requires 473 μg of gold per 14.7 mg of lipids to produce resonance at 760 nm, and 749 μg of gold per the same amount of lipids to produce resonance at 1210 nm. The added mass in these long-wavelength resonant liposomes likely accelerates their sedimentation, which is possibly followed by aggregation within the sediment. Lastly, the measured zeta potential of all samples was rather minimal, with its absolute value at or below 1 mV, suggesting that the observed colloidal stability was controlled by the polyethylene glycol coating present in all tested preparations, rather than electrostatic repulsion.

To evaluate the influence of the gold-coating process on the stability of liposomes, we compared the amount of fluorescein retained in various liposome preparations one day after sample fabrication. We measured fluorescence intensity changes accompanying the 100% release from uncoated and gold-coated liposomes resonant at 760 nm and 1210 nm; fluorescence measurements are provided in Figure 3. The release was accomplished by adding 1% of Triton X-100 (by volume) to the sample. Fluorescence intensity following complete release is representative of the total amount of fluorescent dye encapsulated in the liposome. As indicated by the minimal decrease in fluorescence between uncoated and gold-coated liposomes, the gold reduction process appears to have little influence on the ability of liposomes to retain their content.

2.2. TEM Imaging

Negative stain TEM images demonstrate that uncoated liposomes, gold-coated liposomes, and gold-coated liposomes exposed to laser radiation maintain stable morphology (Figure 4). The negative staining method allows for determining the morphology and size distribution of specimen that are generally unstable under the high energy beam of the electron microscope, such as the liposomes. Based on the lack of discernable variation between the three images of liposomal samples obtained at different stages of experimentation, it can be concluded that neither the gold coating procedure nor the on-resonant irradiation of the sample causes observable changes in liposome morphology or size distribution. This conclusion lends further support to the liposome sizing data obtained by quasi-elastic light scattering, described previously.

There are no identifiable gold clusters present in electron micrographs of gold-coated liposomes (Figure 4b and c). Spatial resolution in TEM is one-tenth the thickness of the specimen, as explained by Cosslett.^[45] We estimate that the carbon substrate is approximately 20 nm and the liposome with molybdenum stain contributes approximately 100 nm to the thickness of the specimen; therefore, the limit of spatial resolution in the negative stain representation of liposomes is approximately 12 nm. While gold coating is evident in combination of scanning electron microscopy (SEM) and energy-dispersive X-ray spectroscopy (EDS) imaging of samples,^[9] these gold clusters are evidently below the limit of spatial resolution of negative stain TEM.

2.3. Spectral Modeling

Because these gold liposomes retain their ability to release content, we hypothesize that the coating does not form a continuous layer of gold, but rather discrete aggregates, or clusters, of gold distributed on the surface of the liposome.^[9] The gold coating is therefore approximated as an imaginary shell surrounding the liposome and partially filled with gold (Figure 5a). The Maxwell Garnet effective medium theory allows for treating this shell as a composite material with an effective dielectric constant, ϵ_{eff} , derived from the dielectric

constants of gold and the surrounding medium, ϵ_{Au} and ϵ_m , and the fill factor f , i.e., the ratio of the volume of the gold clusters to the volume of the shell [Eq. (1)].

$$\epsilon_{eff} = \epsilon_m \frac{\epsilon_{Au}(1+2f) + \epsilon_m(1-f)}{\epsilon_{Au}(1-f) + \epsilon_m(2+f)} \quad (1)$$

To apply the Maxwell Garnet model, we assume that distribution of gold nanoparticles within the volume of imaginary shell remains isotropic. The overall volume of the gold clusters, hence the fill factor, is controlled by varying the amount of tetrachloroaurate ions added to the suspension of liposomes; therefore, ϵ_{eff} can be controlled experimentally. In the manner explained by the Mie theory, varying ϵ_{eff} in this shell-like structure produces optical resonances at different wavelengths, where the condition of resonance can be obtained from the approximation reported by Kerker^[46] as follows in Equation (2):

$$q^3 = \frac{2(\epsilon_m + \epsilon_{eff})^2 + \epsilon_m \epsilon_{eff}}{2(\epsilon_m - \epsilon_{eff})^2} \quad (2)$$

where $q = R/(R+d)$ is the ratio of core diameter to that of the imaginary shell (Figure 5a).

This distinctive type of tunability is demonstrated in Figure 5b. The increasing amount of tetrachloroaurate ions reduced in the presence of these 100 nm diameter liposomes shifts the plasmon resonance position to the longer wavelengths. In Figure 5b, these experimentally obtained spectra are superimposed with spectra obtained from the Mie theory, using fill factors selected to match the maxima of the experimental curves (see Experimental Methods).

2.4. Spectrally Controlled Release

Pulsed illumination at the wavelength matching the resonance of gold-coated liposomes leads to release of encapsulated fluorescein. Figure 6 illustrates the on-resonant illumination time required to obtain 80% content release from liposomes resonant at 760 nm for the six laser pulse widths tested: 0.2, 0.5, 1, 5, 20, and 200 μ s. The 0.5 and 1 μ s pulse widths required the least illumination time to achieve 80% release; the 200 μ s pulse required approximately 50% more time than these pulses to reach 80% release. As the laser diode was pulsed using a constant current source at a 10% duty cycle, the illumination time is directly proportional to the amount of energy delivered; therefore, the 200 μ s pulse width required about 1.5 times as much energy as the 0.5 and 1 μ s pulse widths to achieve 80% of total content release.

A train of 0.5 μ s pulses was subsequently used to study the kinetics of light-induced content release from the plasmon resonant gold-coated liposomes and demonstrate the spectral selectivity of this process. As shown in Figure 7, full release of encapsulated fluorescein was achieved for an illumination time of 3 minutes when gold-coated liposomes with a plasmon resonance peak at 760 nm were illuminated with coincident 760 nm laser light. In contrast, 3 minutes of 760 nm illumination induced less than 13% release from liposomes with a plasmon resonance peak at 1210 nm and no release from uncoated liposomes. Figure 8 demonstrates a corresponding trend when the liposome preparations were illuminated with 1210 nm laser light. Illumination of liposomes having a coincident resonance peak at 1210 nm resulted in full release at 4 minutes; only 15.5% release was induced from liposomes resonant at 760 nm at 4 minutes. In contrast to 760 nm illumination, some release, about 5%, was observed from uncoated liposomes after 4 minutes of 1210 nm illumination, most likely

due to the absorption of water at this wavelength. Together, these experiments illustrate the ability to release content in a temporally and spectrally controlled manner by matching the wavelength of illuminating light to the varied optical resonances of 100 nm diameter liposomes. The negligible release from uncoated liposomes suggests that liposomal encapsulation of fluorescein is stable over the duration of the experiment and that nonspecific release from gold-coated liposomes not resonant with the light source is caused by partial overlap of the extinction spectra at the 760 and 1210 nm wavelengths (Figure 5b).

The pulsed illumination method is a crucial consideration for maintaining the spectral selectivity of release. As shown in Figure 9, spectral selectivity is dependent on laser pulse width. When the two liposome preparations with plasmon resonance maxima at either 760 or 1210 nm are combined and illuminated with 760 nm laser light, the release from off-resonant liposomes (1210 nm) can be suppressed by the use of 0.5 μ s pulses, whereas 200 μ s pulses produce significantly greater off-resonant release. Consequently, it appears that shorter illuminating pulse widths improve the spectral selectivity of release.

2.5. Heat Equation Modeling

While complete release may require a long sequence of more than 10^6 laser pulses, computational analysis of the propagation of thermal changes that follow just a few pulses reveals important aspects of the photothermal release process in gold-coated liposomes. To evaluate the propagation of heat away from a single gold-coated liposome, we used numerical methods to evaluate the heat equation in spherical coordinates:

$$\frac{\partial T}{\partial t} = \alpha \frac{1}{r^2} \frac{\partial}{\partial r} \left(r^2 \frac{\partial T}{\partial r} \right) + \frac{q}{m_{Au} c_{p,Au}} \quad (3)$$

where T is the temperature, r is the radial distance from the gold-coated liposome, α is the thermal diffusivity of water, q is the calculated laser power experienced by each liposome, m is the calculated mass of gold on one liposome, and c_p is the specific heat of gold. In this model, the liposome was treated as a point source and it was assumed that the gold on the surface of the liposome absorbed 100% of the laser radiation. The liposome itself was assumed to not have absorbed any of the laser radiation. The thermal diffusivity used for water was $1.3 \times 10^{-3} \text{ cm}^2 \text{ s}^{-1}$ and the specific heat used for gold was $129 \text{ J kg}^{-1} \text{ K}^{-1}$. To mimic our experimental conditions, heat distribution was modeled for illumination with a source operating at a 10% duty cycle and the initial temperature, T_0 , was set at 4 °C. The location of the liposome, and subsequently the source of heat from laser irradiation, was set at $r = 0$. The boundary conditions were set to account for heat transfer due to both convection and radiation at the system boundary, $r = 10 \mu\text{m}$,

$$\left. \frac{\partial T}{\partial r} \right|_{r=10\mu\text{m}} = -\frac{h}{k}(T - T_0) - \frac{\sigma}{k}(T^4 - T_0^4) \quad (4)$$

where h is the convective heat transfer coefficient of water, σ is the Stefan-Boltzmann constant, k is the thermal conductivity of water, T is the temperature at the boundary, and T_0 is the ambient temperature, which, in this case, is the initial temperature of the sample.

Heat equation modeling indicates that release is achieved through the cumulative response of gold-coated liposomes to multiple laser pulses. As shown in Figure 10, a single pulse of any duration and energy used in this experiment is incapable of bringing the liposome to a temperature where leakage will occur. Rather, heat must accumulate locally, or at the

liposome, as in the case with shorter pulses (0.2 – 1 μs), or globally, through the heating of the entire sample volume, as seen with longer pulses (5 – 200 μs). This trend is evidenced by the experimental results in Figure 9. Longer pulses result in more heat extending to nearby liposomes, resulting in unspecific content release. Therefore, longer pulse or continuous illumination should be avoided to achieve spectral selectivity. Shorter illuminating pulses will confine the greatest temperature increases to the proximity of the liposome and will avoid increasing the temperature of adjacent, off-resonant liposomes.

Figure 10 also demonstrates that shorter pulses lead to faster heat accumulation at the gold-coated liposome; as shown over the selected time durations, it requires 40% less time, and subsequently 40% less energy, for the 1 μs pulses to surpass the cumulative temperature reached by the 20 μs pulses at the liposome. Consequently, it appears that the general trend leading to more efficient release is the use of a train of short pulses, which will cause both less heating of surrounding media and more rapid heat accumulation at the liposome.

Release with the 0.2 μs pulse width did not follow the general trend of shorter pulse widths inducing more efficient release. We examined the characteristic time required for the development of a quasi-stationary temperature distribution within a zone of radius r , the thermal confinement zone, which is often estimated from the formula

$$t_p = \frac{r^2}{4\kappa} \quad (5)$$

In this model, t_p is the pulse width of the illumination source and κ is the thermal diffusion coefficient within the thermal confinement zone.^[47] Using the thermal diffusivity of water, $1.3 \times 10^{-3} \text{cm}^2 \text{s}^{-1}$, the calculated thermal confinement zone for a 0.2 μs pulse is close to the size of the gold-coated liposomes. It appears the confinement of thermal changes at this short pulse width prevents the entire liposome from reaching thermal equilibrium, which may affect thermal release from liposomes. Therefore, this model's treatment of the gold-coated liposome as a point source does not provide a satisfactory explanation of content release initiated by short pulses having a 0.2 μs or shorter width.

3. Conclusions

The gold-coated liposomes discussed here present a unique combination of degradability^[9] and tunable optical properties,^[10] enabling their potential use in delivery of diagnostic and therapeutic agents, in microfluidics and micron scale chemical reactions, in various sensing techniques and in cellular interfacing. In this work, we demonstrated spectrally selective release from gold-coated liposomes by means of pulsed illumination using wavelengths of light matching their plasmon resonance band. This spectral multiplexing of release was achieved with liposomes having the same colloidal properties: diameter, surface coating, and zeta potential. Experimental evidence and analysis of heat distribution shows that this ability to selectively release content can be optimized through consideration of the pulse width used for illumination. Analysis of the heat equation also revealed that release occurs through the accumulation of heat from a series of laser pulses. The use of multiple, low intensity laser pulses allows for the spatial control of release, while avoiding unnecessary heating of the surrounding medium. In the context of biological and medical applications, gold-coated liposomes will enable spatially and temporally controlled release of multiple agents from liposomal carriers using a physiologically safe light-delivery system.

4. Experimental

Liposome Preparation

Liposomes were prepared from synthetic lipids using a lipid composition similar to one previously demonstrated to exhibit temperature-sensitive controlled release;^[43] the logic supporting this composition is that the instability that occurs during the gel to liquid-crystalline phase transition of lipids sufficiently perturbs the liposome membrane to induce the leakage of contents. The membrane was composed of dipalmitoylphosphatidylcholine (DPPC), monopalmitoylphosphatidylcholine (MPPC), and dipalmitoylphosphatidylethanolamine-[N-methoxy(polyethylene glycol)-2000] (DPPE-PEG2000, all lipids from Avanti Polar Lipids; Alabaster, AL) in a 90:10:4 molar ratio. MPPC was included to facilitate membrane leakage at the phase transition temperature and DPPE-PEG2000 was included to improve colloidal stability.

The proper proportions of dry lipids were dispersed in chloroform and dried by convection with N₂; this process was followed by overnight evaporation under vacuum. Dry lipids (60 mM lipid concentration) were then dispersed in phosphate buffered saline (PBS) containing fluorescein (5 mM). Liposomes were prepared by the standard freeze-thaw cycle method and subsequent extrusion through 100 nm polycarbonate membranes, as detailed in previous publications.^[9,10] Following extrusion, of the liposome preparation (10 mL) was subjected to at least two stages of dialysis against PBS (2 L) at 4 °C using cellulose membranes with a 100,000 molecular weight cut-off (Spectrum Laboratories; Rancho Dominguez, CA) to remove excess fluorescein. All liposome preparations were stored at 4 °C to minimize content leakage.

Reduction of Gold

The process for the reduction of gold onto the surface of liposomes was similar to the technique previously reported.^[9,10] To summarize, aqueous solutions of gold chloride (100 mM) and of ascorbic acid (500 mM) were prepared. These solutions were added to the previously prepared liposome sample diluted with PBS (1 mL, 20 mM). For resonance wavelengths matched to the 1210 nm laser diode, the gold chloride solution (38 µL) was added and gently swirled until uniformly distributed; this was followed by the addition of the ascorbic acid solution (57 µL) and gentle swirling until color, a feature characteristic of the presence of plasmon resonance, developed. This preparation demonstrated a broad extinction band centered at 1210 nm. Samples with a resonance wavelength peak corresponding to the 760 nm laser diode source were prepared in much the same manner, with lower amounts of gold chloride solution (24 µL) and ascorbic acid solution (36 µL) used to generate a preparation with a resonance peak centered at 760 nm. Following reduction, each sample was dialyzed against PBS at 4 °C.

Extinction Spectra

Extinction spectra of gold-coated liposomes were taken with a Cary 5 spectrophotometer in double beam mode. Samples of uncoated liposomes, prepared at the same time and in the same manner as those used for coating with gold, were used in the reference beam. Samples were diluted (5 mM lipids) to match spectrophotometric range of the instrument.

Spectral Modeling

The dimensionless extinction efficiency can be calculated as

$$Q_{ext} = - (2/\alpha^2) \sum_{n=1}^{\infty} (2n+1) \text{Re}(a_n + b_n)$$

where $\alpha = 2\pi b/\lambda$, with b being the radius of the shell (Figure 5a illustrates that $b=R+d$), λ the wavelength, and a_n and b_n the scattering coefficients.^[48] For spectral modelling, Q_{ext} was approximated by retaining only the leading term of a power series representing a_1 (an approximation that is valid for $\alpha < 0.3$):

$$a_1 = \frac{2}{3} i \alpha^3 \frac{(\epsilon_s - \epsilon_m)(\epsilon_c + 2\epsilon_s) + q^3(2\epsilon_s + \epsilon_m)(\epsilon_c - \epsilon_s)}{(\epsilon_s + 2\epsilon_m)(\epsilon_c + 2\epsilon_s) + q^3(2\epsilon_s - 2\epsilon_m)(\epsilon_c - \epsilon_s)}$$

where ϵ_c , ϵ_{Au} and ϵ_m are dielectric constants (or, generally, functions) of the core, the shell, and the surrounding medium, and $q = R/(R+d)$ with R being the radius of the core.^[45] The shell was treated as a monolayer of metallic particles isotropically dispersed within a dielectric matrix and its dielectric function was estimated using the Maxwell Garnet theory. To obtain simulated spectra with maxima at 760 and 1210 nm, the fill factor was 0.85 and 1, respectively. To account for the reduced mean free electron path in the metallic particles, the size-dependent dielectric function of gold was obtained in concurrence with the Drude theory.^[49]

$$\epsilon_{Au}(d) = \epsilon_{Au,bulk} + \frac{\omega_p^2}{\omega^2 + i\omega\gamma_{bulk}} - \frac{\omega_p^2}{\omega^2 + i\omega\gamma(d)}$$

with the size-dependent damping constant estimated as follows:

$$\gamma(d) = \gamma_{bulk} + \frac{2v_F}{d}$$

where γ_{bulk} is the bulk metal damping constant, v_F is the Fermi velocity, and d is the diameter of the individual metallic particles forming the composite shell. Finally, ω_p is the plasmon frequency and $\epsilon_{Au,bulk}$ is the dielectric (complex) function of bulk gold.^[50] Gold particles were treated as suspended in water and the dielectric properties of the supporting lipid bilayer were ignored; therefore $\epsilon_c = \epsilon_m = 1.78$ and represents the dielectric constant of water at optical frequencies. The diameter of the core (liposomal template) was assumed to be 100 nm and the thickness of the shell was estimated at 2 nm.

Particle Sizing and Zeta Potential

A Zeta Sizer Nano-ZS particle sizer from Malvern Instruments was used to measure the size and zeta potential of intact (as prepared) uncoated and gold-coated liposomes. Liposomes degraded by the addition of 10% Triton X-100 in a 1:9 ratio of Triton X-100 to the suspension media were also sized.

Sample Imaging

Uncoated and gold-coated liposomes were photographed 1, 3, 7, and 14 days after fabrication at a 5 mM lipid concentration.

Light-Induced Content Release

Light-induced content release from uncoated and plasmon resonant (gold-coated) liposomes were tested by shining a light beam generated by a laser diode (RPMC Lasers; O'Fallon, MO) onto a liposome sample. A droplet of a liposome suspension (30 μL , 10 mM) was retained in a semi-micro cuvette (1.5 mL volume; BrandTech; Essex, CT). Light from a laser diode was focused onto the volume of the liposome droplet using a 4.51 mm focal length aspheric lens (Thorlabs; Newton, NJ) to achieve a power density of approximately 0.127 W mm^{-1} . Laser diodes were driven by a constant current, square-pulse source (ILX Lightwave; Bozeman, MT) operating at 10% duty cycle.

To evaluate changes in light-induced content release due to varying pulse width, the liposomes exhibiting a plasmon resonance peak at 760 nm were exposed to a 760 nm laser diode (on-resonant illumination). The laser diode was driven at 1 W power (at maximum) and at frequencies of 0.5, 5, 20, 100, 200, and 500 kHz; therefore, the tested pulse widths were 200, 20, 5, 1, 0.5, and 0.2 μs . Samples were exposed to pulses of laser light for varying durations of time in increments of 1 min, for up to 8 minutes.

For specific release, the 760 nm and the 1210 nm liposome samples were each individually exposed to pulsed light from the 760 nm and 1210 nm laser diodes. Laser diodes were driven using approximately 0.8 W of power and at a frequency of 200 kHz to obtain a 0.5 μs laser pulse width. Samples were exposed to laser light for varying durations of time in 1 minute increments.

As confirmation of our ability to avoid global heating and subsequently induce the individual release from liposomes having various plasmon resonances, we illuminated a liposome sample containing both liposomes resonant at 760 nm and liposomes resonant at 1210 nm in a 1:1 ratio with a 760 nm laser diode. The off-resonant, 1210 nm liposomes encapsulated self-quenched fluorescein while the on-resonant, 760 nm liposomes contained no dye. The liposomes were exposed to pulsed laser light according to the same scheme used for specific release.

Fluorescence spectra were recorded immediately after illumination to monitor the release of self-quenched fluorescein from liposomes; an increase in fluorescence intensity at the emission peak of fluorescein served as evidence of content release. The droplet of the irradiated solution (30 μL , 10 mM) was diluted with PBS (1970 μL) for measurement. Fluorescence emission spectra were collected over the range of 200 to 800 nm using a diode array spectrometer (Ocean Optics; Dunedin, FL). The excitation source was a 470 nm LED and emission spectra were taken with SpectraSuite Software. The maximum value of the emission spectrum for each illumination event was recorded and the percent release was determined using the following equation:

$$\%release = \frac{I - I_0}{I_T - I_0}$$

where I is the maximum intensity of fluorescence emission (generally near 515 nm) for an individually measured sample, I_0 is the maximum fluorescence for an untreated sample, and I_T is the fluorescence maximum in the event of complete release, which was determined by replacing 10% of the PBS diluting solution with an aqueous 10% Triton X-100 solution.

TEM Imaging

A Phillips CM-12 transmission electron microscope (TEM) operating at an accelerating voltage of 100 keV was used to observe the morphology of the liposomes. The three

samples imaged were a control with no gold coating, and two gold-coated samples with a plasmon resonance peak at 760 nm, one before irradiation and one after irradiation with the 760 nm laser light source. Sample preparation followed the Liposome Preparation and Reduction of Gold procedures described earlier. The 760 nm irradiation procedure followed that described for specific light-induced release; a droplet of gold-coated liposomes (30 μ L, 10 mM lipids) was illuminated for 3 minutes using a 760 nm laser diode driven at 0.8 W of power and at a frequency of 200 kHz to obtain a 0.5 μ s laser pulse width.

Liposome samples were diluted (100 μ M lipid concentration). Each sample was prepared for TEM by placing a droplet of the liposome solution (5–6 μ L) on a mica-carbon support film; then, the film was floated onto a solution of water and 8% ammonium molybdate, a stain introduced to visualize the surface of lipid bilayers. A nickel grid was subsequently used as a deposition surface and the excess solution was wicked away using filter paper.

Heat Equation Modeling

MATLAB was used to numerically solve the heat equation in spherical coordinates. The liposome was treated as a point source and convection and radiation conditions were applied to the boundary.

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Figure 1.

Sizing data for the intact and degraded product of gold-coated liposomes resonant at 760 nm (a and c, blue) and at 1210 nm (b and d, green). The average size for intact gold-coated liposomes was around 98 nm for both measured resonances. The degradation products of gold-coated liposomes resonant at 760 nm have an average size of about 3 nm, while the degraded products of gold-coated liposomes resonant at 1210 nm have an average size around 6.2 nm.

Figure 2.

a) Images of uncoated liposomes (left), gold-coated liposomes resonant at 760 nm (middle) and gold-coated liposomes resonant at 1210 nm (right). Uncoated and 760 nm liposomes demonstrate consistent and uniform color, while 1210 nm liposomes show inhomogeneous and lighter color at 7 and 14 days. b) Sizing of uncoated liposomes (grey), gold-coated liposomes resonant at 760 nm (blue) and gold-coated liposomes resonant at 1210 nm (green). Numbers were collected using a Malvern Zeta Sizer and represent Z-average diameters. Error bars represent the full width at half maximum of sizing data peaks. Uncoated and 760 nm liposomes maintain their sizes over the observation period, whereas 1210 nm liposomes appear to increase their size after 3 days, likely due to aggregation. c) Zeta potentials of uncoated liposomes (grey), gold-coated liposomes resonant at 760 nm (blue) and gold-coated liposomes resonant at 1210 nm (green).

Figure 3.

Fluorescence measurements for 100% release from uncoated (grey, left) and gold-coated liposomes resonant at 760 nm (blue, middle) and 1210 nm (green, right). The respective fluorescence intensities of untreated samples were subtracted from the 100% release intensities to obtain a measure of dye encapsulation. Values are the average and error bars are the standard deviations of three measurements. Minimal content loss was observed after the gold coating process.

Figure 4. Negative stain transmission electron micrographs of (a) liposomes before deposition of gold (b) gold-coated liposomes before laser irradiation and (c) gold-coated liposomes after irradiation with 760 nm laser light. Radiation conditions mimicked those of specific light-induced release; gold-coated liposomes were irradiated for 3 minutes using a 760 nm laser diode operating at 0.8 W of power and delivering 0.5 μ s laser pulse widths. Scaling is the same for all images and the scale bar represents 1 μ m.

Figure 5.

(a) The schematic model of gold-coated liposomes used in the Mie calculations. In this model, gold reduced in the presence of liposomes forms discrete spherical gold clusters. Here, R is the liposome, or core, radius, d is the diameter of individual gold clusters, equal to the thickness of the imaginary shell partially filled with gold clusters, ϵ_{Au} is the dielectric constant of gold clusters, and ϵ_m is the dielectric constant of the surrounding medium.

(b) Extinction spectra of liposome preparations: uncoated liposomes (grey), gold-coated liposomes with a plasmon resonance peak at 760 nm (blue), and gold-coated liposomes with a resonance peak at 1210 nm (green). Experimentally obtained spectra (solid lines) are overlaid with simulated spectra obtained from the Mie theory (dotted lines). All experimental samples were prepared and measured with equal quantities of lipids in solution and, therefore, presumably an equal number of liposomes per unit volume. Liposomes were loaded with fluorescein; the presence of fluorescein in the liposomes is evident by the peak at 485 nm. The relative broadening of the experimental resonances in comparison to simulated resonances can be attributed to the size distribution of liposomes produced by the extrusion method.

Figure 6.

Light-mediated release from gold-coated liposomes resonant at 760 nm using a 760 nm laser diode operating at pulse widths of 0.2, 0.5, 1, 5, 20, and 200 μs . Points are the average and error bars are the standard deviations of three trials. Shorter pulse widths generally elicited more rapid content release from gold-coated liposomes.

Figure 7.

Light-mediated release from uncoated liposomes (grey) and plasmon resonant liposomes resonant at 760 nm (blue) and at 1210 nm (green) using a 760 nm laser providing 0.5 μ s pulses. (a) In the schematic of the anticipated outcome, 760 nm illumination elicits content release from gold-coated liposomes resonant at 760 nm while uncoated and gold-coated liposomes off-resonant with the light source retain their content. (b) In the experimentally obtained data, points are the average and error bars are the standard deviations of three trials. Full content release was observed in on-resonant, gold-coated liposomes after a 3 minute or longer exposure to the laser light. At 3 minutes, approximately 13% release was observed in off-resonant gold-coated liposomes and no release was observed in uncoated liposomes.

Figure 8.

Light-mediated release from uncoated liposomes (grey) and plasmon resonant liposomes resonant at 760 nm (blue) and at 1210 nm (green) using a 1210 nm laser providing $0.5 \mu\text{s}$ pulses. (a) The schematic of the anticipated outcome shows that 1210 nm illumination causes content release from gold-coated liposomes with a resonance peak at 1210 nm while uncoated and gold-coated liposomes off-resonant with the light source retain their content. (b) In the experimental data, points are the average and error bars are the standard deviations of three trials. On-resonant gold-coated liposomes with a resonance peak centered at 1210 nm exhibited full release at exposure times of 4 minutes. At this same time, off-resonant gold-coated liposomes with a resonance peak centered at 760 nm exhibited an average of 15.5% release and uncoated liposomes exhibited an average of less than 5% release.

Figure 9.

Light-induced release from liposomes resonant at 1210 nm induced by a 760 nm laser diode (off-resonant conditions) operating at one of two different pulse widths: 0.5 μ s (dotted) and 200 μ s (dashed). Points are the average and error bars are the standard deviations of three trials. The illuminated sample consisted of 50% liposomes with 5 mM encapsulated fluorescein and a resonance peak at 1210 nm, and 50% liposomes without fluorescein and a resonance peak at 760 nm; the total lipid concentration of the sample was 10 mM. Light-induced release using a 0.5 μ s pulse width resulted in a release curve similar to the off-resonant release from gold-coated liposomes using the 760 nm laser diode (Figure 7).

Figure 10.

Simulated changes in heat distribution due to varying pulse width. The heat equation was modeled in spherical coordinates for a single gold-coated liposome experiencing illumination with two different pulse widths: (a) 1 μs and (b) 20 μs . The gold coated liposome is placed at distance = 0. The graphs represent evolution of temperature over time, in the radial distance up to 10 μm from the liposome. The time scales in both simulations were selected so that the final temperatures at distance = 0 are about equal. A 10% duty cycle was applied for both 1 and 20 μs pulses, and subsequently pulses appear at frequencies of 100 kHz and 5 kHz, respectively. Note that it requires 240 μs for the 1 μs pulse train (equivalent to 24 pulses) and 400 μs for the 20 μs pulse train (2 pulses) to reach equal temperatures at the position of the liposome. Also note that as pulse width increases, thermal changes spread over greater radial distances and heat accumulation at the particle becomes less efficient.