Optical Trapping of Gold-Coated Liposomes

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ABSTRACT

To address unmet needs in the area of controlled delivery, we introduced a nanocomposite material, plasmon resonant gold-coated liposomes. We previously showed that gold-coated liposomes display plasmon resonance that is tunable in the near-infrared range and are capable of light-induced content release. The plasmon resonant structure facilitates rapid content release when illuminated with laser light at wavelengths that correspond to the spectral position of the resonance band. Here, we demonstrate optical trapping of gold-coated liposomes that allows for controlled movement of these liposomes within a biological sample. Furthermore, we computationally refined this experimental system with the potential for delivery and localized release of an encapsulated agent with high spatial and temporal resolution. Full development of this technology will enable accurate perturbation of cellular functions in response to released compounds, with applications in signaling pathway research and drug discovery.

Keywords: controlled release, plasmon resonance, liposome, nanoparticle, optical trapping

1 INTRODUCTION

Optical trapping is a powerful scientific technique that is gaining popularity in a variety of biological applications. While it was previously believed that metallic particles could not be trapped due to relatively large scattering and absorption forces [1], gold nanoparticles have recently been shown to be capable of trapping, due to the high polarizability of gold and concomitant gradient force. Indeed, gold nanoparticles have demonstrated more stable optical trapping in comparison to latex [1] and polystyrene [2] beads. Here we demonstrate successful optical trapping of a composite structure developed in our lab, plasmon resonant gold-coated liposomes.

We recently introduced a nanocomposite material, plasmon resonant gold-coated liposomes [3]. Plasmon resonant gold-coated liposomes are created via the deposition of gold onto the surface of 100 nm diameter thermosensitive liposomes and provide optical plasmon resonant properties similar to that of solid gold shells. Gold-coated liposomes have a plasmon resonance peak that is tunable in the near-infrared range and that these structures are consequently capable of light-induced release [4]. The thermosensitive liposome allows for the encapsulation of substances, including diagnostic and therapeutic agents. The plasmon resonant structure facilitates the rapid release of encapsulated contents via photothermal conversion when illuminated with laser light at a wavelength corresponding to the resonance band. In previous work we have shown that one can use the tunability of the plasmon resonance band to achieve spectrally selective content release using 760 and 1210 nm laser diode wavelengths [5].

Optical trapping may be used to spatially manipulate the location of gold-coated liposomes. When spatial manipulation is used in conjuction with the ability of gold-coated liposomes to encapsulate and release substances on demand, this setup may allow for the extracellular release of biologically active compounds at a subcellular resolution. Here we demonstrate successful optical trapping and controlled movement of gold-coated liposomes within a biological sample. We also use computational modeling to demonstrate that gold-coated liposomes can be used with a modified optical trap to independently control the trapping of and content release from gold-coated liposomes. Full development of this technology may enable accurate perturbation of cellular functions in response to released compounds, with applications in signaling pathways and drug discovery.

2 MATERIALS AND METHODS

2.1 Liposome Preparation

Liposomes were prepared from synthetic lipids using a lipid composition previously demonstrated to exhibit temperature-sensitive controlled release [6]. 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. The proper proportions of dry lipids were dispersed in chloroform and dried by convection with N_2; this process was followed by overnight evaporation under vacuum. Dry lipids were then dispersed in phosphate buffered saline (PBS). Liposomes were prepared by the standard freeze/thaw cycle method and subsequent extrusion through 100 nm polycarbonate membranes. Following extrusion, the liposome preparation (2 mL) was subjected to one stage of dialysis against PBS (2 L) at 4°C using cellulose membrane with a 100,000 molecular weight cut-off (Spectrum Laboratories; Rancho Dominguez, CA). Liposome preparations were stored at 4°C.

2.2 Gold Reduction

The process for the reduction of gold onto the surface of liposomes was similar to the technique previously reported [3]. 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). The gold chloride solution (20 µL) was added and gently swirled until uniformly distributed; this was followed by the addition of the ascorbic acid solution (30 µL) and gentle swirling until color, a feature characteristic of the presence of plasmon resonance, developed. Following reduction, the gold-coated liposomes (1 mL) were dialyzed twice against PBS (2 L) at 4 °C. Extinction spectra of gold-coated liposomes were taken with a Cary 5 spectrophotometer in double beam mode. Samples were diluted in PBS to yield 1 mM lipids for measurement.

2.3 Optical Trapping

For the trapping beam, we utilize a continuous wave 1064 nm TEM_{00} trapping beam (Ventus IR, Laser Quantum, United Kingdom) operating at 90 mW power. The 1064 nm beam allows for relatively safe use of the optical trap with biological samples. The trapping beam is coupled into an inverted microscope before the objective by a VIS/IR dichroic mirror. The trapping beam is focused through a 60x 1.42 NA PlanApo N oil immersion objective (Olympus, Center Valley, PA). The position of the optical trap is controlled by two beam steering lenses. Differential interference contrast (DIC) images of gold-coated liposomes within the optical trap were acquired through the same objective lens and collected continuously at 200 ms exposure times. All images were acquired using an air-cooled 512x512 pixel back-thinned EM-CCD digital camera (Hamamatsu, Bridgewater, NJ).

2.4 Computational Modeling

To model the temperature distribution resulting from the optical trapping of a liposome-supported gold shell, the heat equation was solved numerically in one dimensional spherical coordinates using FlexPDE (PDE Solutions, Spokane Valley, WA):

$$\mathrm{div}(K\times\mathrm{grad}(T)) + Q = c_p\times dT/dt \quad (1)$$

where T is the temperature change in K, K is the material thermal conductivity in W/nm×K, Q is the calculated laser power in W/nm^3, and c_p is the material heat capacity in J/K×nm^3. Liposome-supported gold shells were modeled as an aqueous phosphate buffered saline (PBS) core 46 nm in radius, surrounded by a concentric lipid bilayer shell 4 nm in thickness and a gold shell 3 nm in thickness. The gold was the only material absorbing light at an assumed 100% efficiency. The liposome supported gold shell was surrounded by PBS. Boundary conditions were set at a radius of 10 µm from the center of the particle, and defined as grad(T)=0. The thermal conductivity and heat capacity values used are provided in Table 1.

Laser power values were estimated assuming a 0.8 µm diameter focused spot size for a 1064 nm TEM_{00} laser operating at 100 mW. Laser pulses were modeled using square waves.

Material	ThermalConductivity W/nm×K	Heat Capacity J/K×nm^3
PBS	6.0×10^{-10}	4.180×10^{-21}
Lipid bilayer	1.4×10^{-10}	3.916×10^{-21}
Gold	3.2×10^{-7}	2.492×10^{-21}

Table 1: Thermal conductivity and heat capacity values used for materials in heat equation modeling. Values for PBS were taken from those of water and values for the lipid bilayer were taken from those of hexadecane [7].

3 RESULTS AND DISCUSSION

We are interested in using this technology to selectively perturb cellular processes in a spatially controlled manner. As shown in Figure 1, this system can be used to manipulate gold-coated liposomes in the presence of cells. For this manipulation, the cells were located between the objective and the trapped particle, as depicted in Figure 1a. The relative transparency of biological samples to 1064 nm light appears to preserve the characteristics (spatial light gradient) of the trapping beam and allows for optical manipulation above cells. The stable movement of a gold-coated liposome over a cell, as shown in Figure 1b, supports that this setup is a viable method for the manipulation of gold-coated liposomes in cell cultures. It appears we may move gold-coated liposomes to specific locations in proximity to cells, which is necessary for achieving high spatial control of cellular perturbations.

While a continuous wave laser, as typically used in optical tweezer setups, allows for stable trapping, metallic gold rapidly heats under continuous illumination [2]. As gold-coated liposomes are thermally sensitive, this may cause the release of contents encapsulated within the liposome in an uncontrolled manner. Figure 2 uses computational modeling to demonstrate how laser pulsing can be used in the optical trap configuration to control the release of contents from gold-coated thermosensitive liposomes. A train of 100 ns pulses at a frequency of 1 MHz does not cause an increase in the baseline temperature of the particle (Figure 2a). As water-soluble agents require microseconds to milliseconds to transverse a thermosensitive liposomal membrane, a sustained increase in the baseline temperature of the gold-coated liposome is necessary for release to occur. More rapid pulsing of 100 ns pulses at 5 MHz, leads to a significant and sustained particle baseline temperature increase of about 5.3 °C over a period of 10 µs (Figure 2b). As a result, the 1 MHz frequency pulse train enables stable trapping of gold

liposomes without inducing release, while the 5 MHz pulse train may be used to release encapsulated contents on demand.

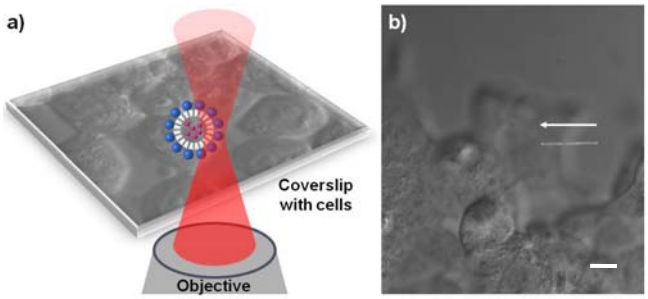

Figure 1: Movement of gold-coated liposomes over cells. (a) Cell monolayers are axially located between the focusing objective of the optical tweezer and the trapped particle. (b) Gold-coated liposomes can be trapped and moved in an axial plane above the extracellular membrane of cells. Scale bar represents 10 μm.

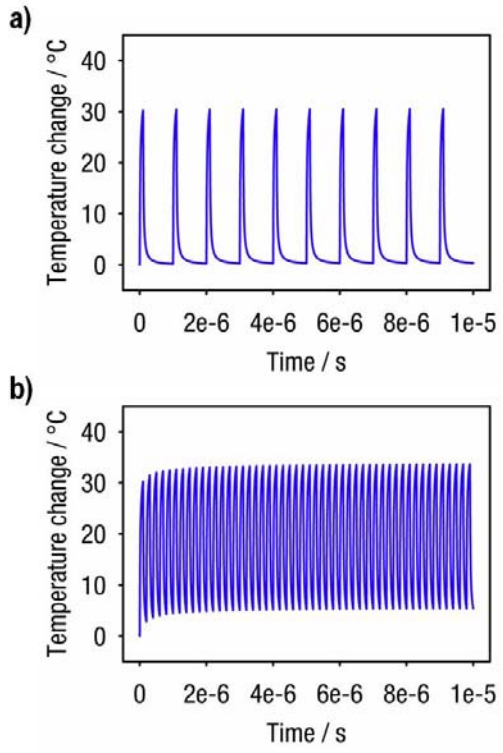

Figure 2: Computational analysis of the heating effects of optical trapping on a liposome-supported gold shell using a laser providing 100 ns pulses at a frequency of 1 MHz (a) or 5 MHz (b). The temperature profile at the center of each particle type as a function of time is plotted. Both pulsing schemes result in transient increases in particle temperature lasting about 100 ns. However, the 5 MHz frequency results in a sustained baseline temperature increase, which may be necessary to elicit liposomal content release.

As slower pulsing of the trapping laser may result in unstable trapping and particle escape, we experimentally examined trapping of gold-coated liposomes under the proposed pulsing regimen. We demonstrated that gold-coated liposomes were stably trapped and manipulated using a train of 100 ns laser pulses at a 1 MHz frequency, as can be seen in Figure 3. Therefore, trapping schemes that are computationally shown to allow for selective trapping and content release from gold-coated liposomes can be experimentally realized.

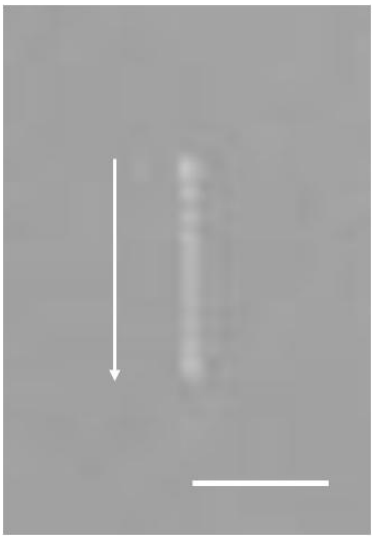

Figure 3: User defined movement of a gold-coated liposome using a pulsed trapping laser. White arrow shows the direction of particle movement. The gold-coated liposome is visualized using DIC; images represent the sum of images taken sequentially during optical manipulation every 0.2 s. The composite image represents a total time of 5 s. Laser pulsing consisted of 100 ns pulses at a 1 MHz frequency. Scale bar represents 5 μm.

4 CONCLUSION

In conclusion, we have shown optical trapping of gold-coated liposomes within a biological sample. We also used computational modeling to demonstrate how pulsing of the trapping laser can be used to independently control the trapping of gold-coated liposomes and the release of their content. The necessary pulsing scheme for this functionality is also experimentally feasible. This type of functionality can be used in many cellular studies, including examination of signal propagation in cellular networks and for the development of cancer therapies.

5 ACKNOWLEDGEMENT

The authors acknowledge financial support for this research by NIH (CA 120350 and HL007955) and NSF (CBET 0853921).

REFERENCES

[1] K. Svoboda and S. Block, Opt. Lett. 19, 930, 1994.

[2] Y. Seol, A. Carpenter and T. Perkins, Opt. Lett., 31, 2429, 2006.

[3] T. Troutman, J. Barton and M. Romanowski, Adv. Mater., 20, 2604, 2008.

[4] T. Troutman, S. Leung and M. Romanowski, Adv. Mater., 21, 2334, 2009.

[5] S. Leung, X. Kachur, M. Bobnick and M. Romanowski, Adv. Funct. Mater., 21, 1113, 2011.

[6] D. Needham, G. Anyarambhatla, G. Kong and M. Dewhirst, Cancer Res., 60, 1197, 2000.

[7] G. Bogatov, Y. Rastorguev and B. Grigor'ev, Chem. Technol. Fuels Oils, 5, 651, 1969.