Drug Delivery with Light-Activated Gold-Coated Liposomes

G.V. Orsinger*, S.J. Leung, S.S. Knights-Mitchell, J.D. Williams and M. Romanowski**

University of Arizona, Tucson, AZ, USA,
*orsinger@email.arizona.edu
** marekrom@email.arizona.edu

ABSTRACT

With the ultimate goal of delivering therapeutic compounds selectively to cancerous or diseased tissues and cells, we have presented liposome-based plasmon resonant nanocapsules. The liposome's lipid membrane allows for encapsulation of a small volume of soluble therapeutic or diagnostic compounds, while the surrounding plasmon resonant gold structure elicits rapid release of the liposomes' contents when illuminated by laser light at wavelengths corresponding to the spectral position of the plasmon resonance band. By depositing discrete gold nanoclusters onto the surface of 100 nm diameter thermosensitive liposomes, we harness the plasmon resonance of a gold pseudo-shell while maintaining degradability to a size compatible with renal clearance. In this work we describe optimization of the liposome design which combines stable encapsulation at physiological temperatures and gold coating responsive at near infrared wavelengths. The described biocompatible composite delivery vehicle enables encapsulation and selective release of therapeutic or contrast agents upon application of a light stimulus for applications in biomedical imaging, diagnostics, therapeutics and targeted drug delivery.

Keywords: drug delivery, liposomes, plasmon resonance, 5-FU

1 INTRODUCTION

The inherent low therapeutic index of cancer chemotherapeutics is a significant problem in effectively treating and managing cancer. Toxicities from potent chemotherapeutics lead to severe adverse side effects and early patient withdrawals from therapy, possibly before effective dose has been reached. To exhibit a high therapeutic index, systemically administered drugs must either have very little effect on non-target tissues or have a much greater affinity for the diseased or target tissues over healthy tissues. Nanoparticle-based drug delivery systems can be used to augment the pharmacokinetics and biodistribution of therapeutic agents to better treat disease [1]. Specifically, liposomes exhibit clinical utility in extending the half-life of cancer chemotherapeutics, as demonstrated in the FDA-approved liposome-encapsulated doxorubicin formulation, Doxil. We aim to advance the use of liposomes as drug delivery vehicles for on-demand release pharmaceutical agents at target tissues.

With the ultimate goal of delivering therapeutic compounds selectively to cancerous or diseased tissues and cells, we have presented liposome-based plasmon resonant nanocapsules [2,3]. More recently, we have demonstrated that the plasmon resonance of gold-coated liposomes is tunable in the near infrared range providing the ability to mediate light-induced release of model compounds, such as fluorescent dyes, encapsulated in gold-coated liposomes matched to different wavelengths as depicted in Figure 1a [4]. Compound release occurs when liposomes' thermosensitive lipid composition is heated above the lipid main phase transition temperature, via photothermal heating. However, our previous experience with thermosensitive liposomes prepared according to the Needham and Dewhirst recipe [5] and subsequently coated with gold indicated leakage occurring at sub-physiological temperatures [3]. Here, we demonstrate a gold-coated plasmon resonant liposome drug delivery system reengineered to work at and above physiological temperature, therefore enabling in vitro and in vivo drug delivery studies. We show that this system is compatible with encapsulation and release of anticancer drugs such as 5-fluorouracil (5-FU).

2 MATERIALS AND METHODS

2.1 Preparation of Gold-coated Liposomes

Thermosensitive liposomes were formulated from synthetic lipids using two different compositions representing low and high crystalline-to-liquid phase transition temperatures. The lower temperature composition, similar to one previously demonstrated for temperature-sensitive content release [5], was composed of dipalmitoylphosphatidylcholine (DPPC), monopalmitoyl-phosphatidylcholine (MPPC), and dipalmitoylphosphatidyl-ethanolamine-N-[methoxy(polyethylene glycol)-2000] (DPPE-PEG2000) in a 90:10:4 molar ratio. The higher temperature composition was composed of distearoyl-phosphatidylcholine (DSPC) and distearoylphosphatidyl-ethanolamine-N-[methoxy(polyethylene glycol)-2000] (DSPE-PEG2000) in a 95:5 molar ratio (all lipids from Avanti Polar Lipids; Alabaster, AL).

For each composition, lipids were dissolved in chloroform, convection dried with N_2 and remaining solvent was removed under vacuum. Dried lipids were introduced to either 3 mM carboxyfluorescein in phosphate buffered saline (PBS) or 50 mM 5-FU in PBS to reach a final lipid concentration of 20 mM. Liposomes were prepared by freeze-thaw cycling and then extrusion through 100 nm porous polycarbonate membranes. Free carboxyfluorescein or 5-FU was removed via dialysis using 100,000 MW cut-off cellulose membranes (Spectrum Laboratories; Rancho Dominguez, CA). Resulting liposome size distributions were measured with a Malvern Zetasizer Nano ZS.

To form the plasmon resonant gold pseudo-shell on the surface of the carboxyfluorescein- or 5-FU-loaded liposomes, 100 mM gold chloride followed by 500 mM ascorbic acid was added to the liposomal suspensions. This process is similar to the technique previously reported by Troutman et al. in 2008 [2]. The presence of the desired plasmon resonant structure was immediate visualized by a change in solution color. Peak resonance was confirmed via absorption spectra obtained on a Cary 5 spectrophotometer.

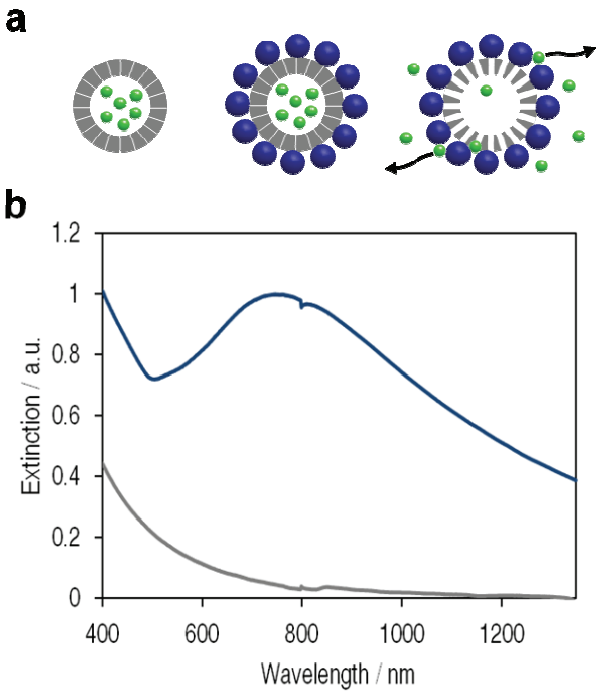

Figure 1: Light-mediated release from uncoated (grey) and plasmon resonant DSPC:DSPE-PEG2000 (95:5) liposomes resonant at 760 nm (blue). (a) Uncoated liposomes encapsulating a molecular agent (left), gold-coated plasmon resonant liposomes (middle), and content release from gold-coated liposomes resonant at 760 nm via illumination with 760 nm light (right). (b) Extinction spectra of uncoated liposomes (grey) and gold-coated liposomes tuned to peak resonances at 760 nm (blue).

To demonstrate stability at physiological temperature, samples were examined in a dialysis assay. Suspensions of gold-coated liposomes were loaded into 1 mL 100,000 MW cut-off dialysis cartridges and placed into a 5 mL reservoir filled with PBS and a small magnetic stir bar. The entire apparatus was placed on a stir plate in a 37 °C incubator. Dialyzate samples were acquired at various time points for up to 72 hours.

For carboxyfluorescein experiments, sample aliquots were excited with a 470 nm LED and the resulting fluorescence signal was collected using a back thinned TE-cooled CCD spectrometer. Measured fluorescence intensity at the 520 nm peak for each sample was converted to carboxyfluorescein concentration based on a standard curve. For 5-FU samples, absorption spectra from 200 to 350 nm for each sample aliquot were obtained on a Cary 5 spectrophotometer. The measured peak absorption value at 265 nm, characteristic of 5-FU presence, was converted to 5-FU concentration based on a standard curve. These assays allow for detection of carboxyfluorescein and 5-FU down to single nM and μM concentrations, respectively.

3 RESULTS AND DISCUSSION

Gold coated DSPC:DSPE-PEG2000 (95:5) liposomes produce a plasmon resonance band with a maximum located at 760 nm (Figure 1b). This plasmon resonance band and its tunability is similar to that obtained in gold-coated DPPC:MPPC:DPPE-PEG2000 (90:10:4) liposomes described earlier [2].

We examined liposomal encapsulation stability of carboxyfluorescein and 5-FU at 37 °C to mimic physiological conditions. Carboxyfluorescein, a standard fluorescent reporter of liposomal encapsulation, provides a sensitive marker of leaked content. 5-FU, a commonly used chemotherapy agent for colorectal, pancreatic, and skin cancers, provides a pharmacologically relevant marker of retained drug content. We encapsulated these two compounds in two different thermosensitive lipid compositions - a previously tested composition based on DPPC and a new composition based on DSPC. The rationale for using a DSPC-based lipid composition is that its higher transition temperature will result in more stable encapsulation under physiological conditions. Compositions also incorporated gold coating to facilitate photothermal conversion by laser illumination in future experiments. The following results demonstrate carboxyfluorescein and 5-FU exhibit quite different encapsulation stabilities under similar experimental conditions.

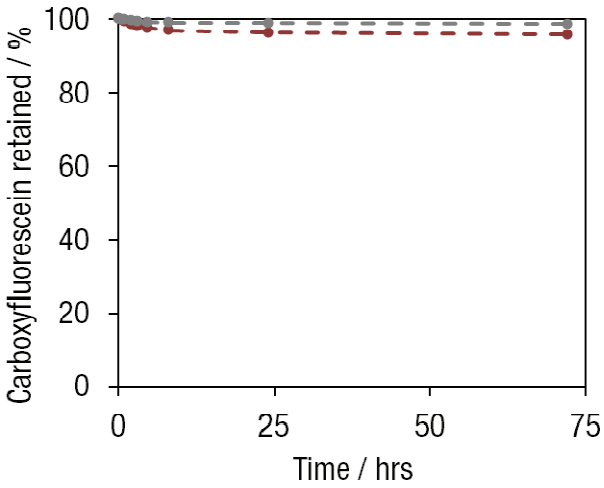

Figure 2: Stability of carboxyfluorescein encapsulation in DSPC:DSPE-PEG2000 (95:5) at physiological temperature (37°C) in uncoated liposomes (grey) and liposomes resonant at 760 nm (red). After 24 hours of exposure to 37°C, gold-coated liposomes retained 95% of the initially encapsulated carboxyfluorescein.

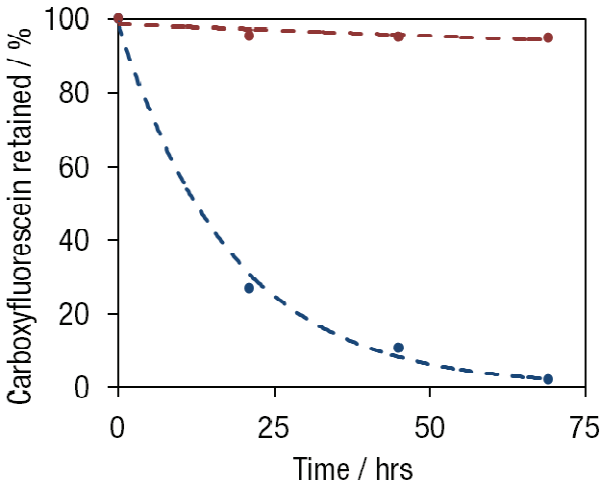

Figure 3: Stability of carboxyfluorescein encapsulation at physiological temperature (37°C) in gold-coated DPPC:MPPC:DPPE-PEG2000 (90:10:4) liposomes (blue) and gold-coated DSPC:DSPE-PEG2000 (95:5) liposomes (red). After 72 hours of exposure to 37°C, DSPC-based liposomes retained more than 95% of encapsulated carboxyfluorescein while DPPC-based liposomes did not.

When exposed to a temperature of 37 °C for 72 hours, uncoated and gold-coated DSPC-based liposomes encapsulating carboxyfluorescein retained 99% and 96% of their original content, respectively (Figure 2). Alternately, DPPC-based gold-coated liposomes retained only 2% of starting carboxyfluorescein content (Figure 3).

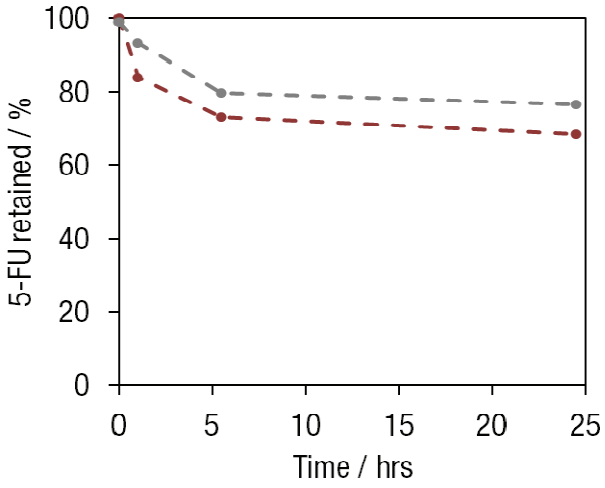

Figure 4: Stability of 5-FU encapsulation in DSPC:DSPE-PEG2000 (95:5) at physiological temperature (37°C) in uncoated liposomes (grey) and liposomes resonant at 760 nm (red). After 24 hours of exposure to 37°C, gold-coated liposomes retained 80% of the initially encapsulated 5-FU.

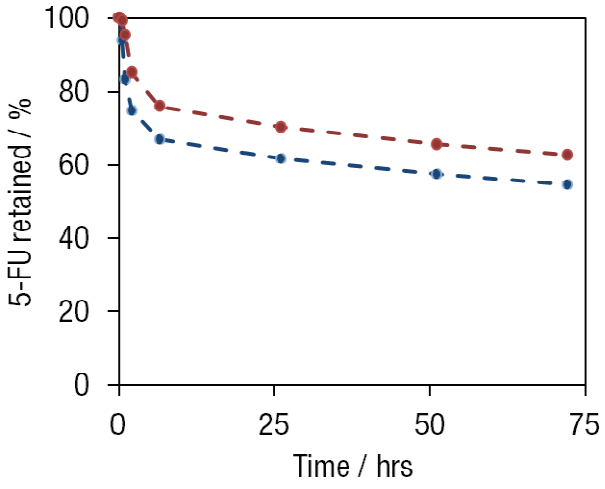

Figure 5: Stability of 5-FU encapsulation at physiological temperature (37 °C) in gold-coated DPPC:MPPC:DPPE-PEG2000 (90:10:4) liposomes (blue) and gold-coated DSPC:DSPE-PEG2000 (95:5) liposomes (red). After 72 hours of exposure to 37°C, DSPC-based liposomes retained more 5-FU than DPPC-based liposomes.

After 24 hours of exposure to physiological temperature, uncoated and gold-coated DSPC-based liposomes encapsulating 5-FU retained only 77% and 69% of their original 5-FU content, respectively (Figure 4). Over 72 hours, DSPC-based and DPPC-based gold-coated liposomes retained 65% and 55% of starting 5-FU content, respectively (Figure 5).

The results indicate that while the DSPC-based composition provides a generally more stable encapsulation than DPPC-based liposomes, carboxyfluorescein encapsulation is more sensitive to lipid composition than 5-FU. This suggests that the mechanism of permeability is different between lipid compositions and molecule-dependent. This could be attributed to differences in molecular properties of encapsulated drugs, such as their charge and the presence of ionizable groups, their size, solubility, or the partition coefficient. While the DSPC-based liposome composition provides more stable encapsulation at 37 °C for both carboxyfluorescein and 5-FU, a specific design of the delivery system should reflect properties of a particular drug to be encapsulated.

In summary, we demonstrated the feasibility of using an alternative gold-coated liposomal formulation, DSPC:DSPE-PEG2000 (95:5). By retaining encapsulated contents more effectively at physiological temperatures while maintaining plasmon resonant properties associated with gold coating, these liposomes are preferred for on-demand release of imaging, diagnostic, and therapeutic agents in tissues.

4 ACKNOWLEDGEMENTS

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