Modification of Commercial Liposomal Drug Carrier by Plasmon Resonant Coating

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ABSTRACT

Multiple liposomal drugs have been successfully marketed as pharmaceuticals within the past two decades. Some of these clinically approved drugs, Doxil® in particular, have been shown to selectively localize within tumors due to the enhanced permeability and retention effect. However, a limitation exists with the inability to control content release once the drug gets to the desired location. Here we investigated the ability to apply a plasmon resonant coating to commercially available Doxil with the intention of spatially and temporally controlling doxorubicin release. While Doxil was successfully gold coated with a maximum plasmon resonant peak at 631 nm, the release at elevated temperature and on exposure to light was less than 10%. This result is similar to that observed for uncoated Doxil. Decreasing the pH had the same effect on both uncoated and plasmon resonant sample where a 10% increase in release was observed after samples were exposed to elevated temperature. Development of a highly efficient mechanism of light-activated drug release from Doxil will require a temperature-sensitive lipid composition.

Keywords: liposomes, doxorubicin, plasmon resonance, controlled release, Doxil

1 INTRODUCTION

Cancer continues to be one of the leading causes of death for individuals over the age of twenty five [1]. The knowledge that cancer has gone into remission is priceless and this is made possible through the use of multiple cancer medications including doxorubicin, 5-fluorouracil, and bevacizumab. However, the path to better health is compromised by the narrow therapeutic window of these drugs, where efficacious doses are accompanied by drug related, often life-threatening, side-effects. Hence, the demand for new methods of targeted delivery and controlled release of cancer therapeutics continues to be high. Doxil preferentially accumulates at the tumor site. This result is mainly due to the enhanced permeability and retention (EPR) effect as well as the inherent ability of these stealth liposomes to evade opsonization and remain in circulation for a long time (50 - 80 hours in humans depending on dose and tumor type) without significant leakage of the encapsulated drug [2]. The EPR effect is a consequence of the hypermeability of the tumor vasulature. It has been shown that delivery vehicles that are greater than 100 nm diameter and rely on the EPR effect are only able to get as far as the pervasculature space because this diameter prevents the passage of the vesicles through the extracellular matrix and deep into the tumor [3].

The accumulation of liposomes within or near the tumor site facilitates one of the conditions for improved efficacy whereby the drug can reach an efficacious dosage while avoiding normal tissues. However, increased drug concentration is ineffective if the drug bioavailability is low [4]. To release content from Doxil a collapse of the amonium sulfate gradient used for drug loading, as well as the loss of the lipid bilayer structural integrity, is needed [2]. Therefore, drug release is dependent on body enzymes and other biological processes and thus cannot be independently controlled. Our aim is to explore whether the application of a plasmon resonant coating could turn Doxil, and similar commercial drug formulations, into a light-activated drug delivery system. A successful realization of this goal would provide a method for doxorubicin release after accumulation in the tumor perivascular space, thereby increasing the drug bioavailability and allowing for drug diffusion deeper into the tumor with diminished side effects

The main focus of this study is to investigate the methods of formation of plasmon resonant coating on Doxil and its effect on the release of doxorubicin from Doxil. Within this report we show that this liposomal formulation is well suited for gold-coating and hence can be made plasmon resonant. Moreover, we demonstrate, the effect of pH-, temperature- and light-induced release of doxorubicin from Doxil.

1.1 Light-Induced Controlled Release

Previously, we have described a general process to coat liposomes with gold to form nanoshells [5]. These constructs possess a complex refractive index that allows plasmon resonance to occur in the visible and near infrared range. Moreover, the plasmon resonant coating facilitates the rapid release of liposome contents upon laser light illumination (Figure 1).

These gold-coated liposomes are capable of releasing their contents in a spectrally-controlled manner, whereby the content is released only upon illumination with a wavelength of light matching their plasmon resonance band (Figure 2) [6].

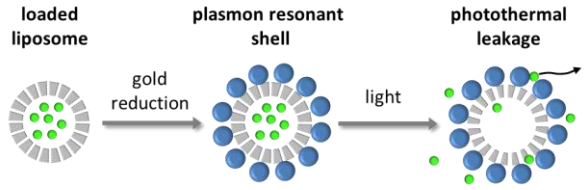

Figure 1: Schematic representation of uncoated liposomes, Au-coated optically resonant liposomes, and light-induced release

This provides the unique ability to control where and when liposomal contents are released. Therefore, the coupling of Doxil and plasmon resonance could potentially yield a product that could significantly reduce systemic toxicity by preferentially accumulating within tumors and releasing doxorubicin on demand by laser illumination.

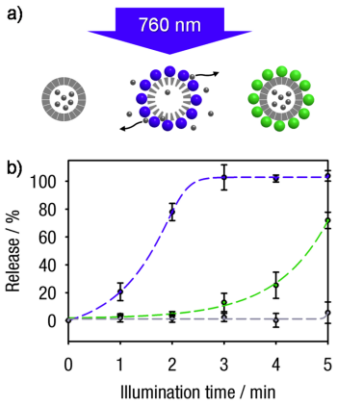

Figure 2: (a) Expected result of illumination with 760 nm diode laser. (b) Efficient release observed for liposomes resonant at the wavelength matching the laser light, 760 nm (blue), much slower for liposomes resonant at 1100 nm (green), none for uncoated and illuminated liposomes (grey) [6]

1.2 Doxil

Doxil, liposomal doxorubicin introduced in 1995, is the first liposome-based drug delivery vehicle approved by the FDA [7]. Currently this drug is being used in the treatment of recurrent ovarian cancer, multiple myeloma, and AIDS-related Kaposi sarcoma [8]. Doxorubicin (DXR) is an anthracycline antibiotic that promotes cell death by intercalating between DNA base pairs, inhibiting topoisomerase II, and creating reactive oxygen species. The latter of these three mechanisms is the main reason for the cardiotoxicity associated with DXR [9]. Encapsulating DXR in liposomes simultaneously reduced the cardiotoxicity and improved drug concentration at the tumor site [7,9].

2 MATERIALS AND METHODS

2.1 Reduction of Gold

Reduction of gold on to Doxil (Avanti Polar Lipids; Alabaster, AL) was carried out following a previously reported technique [10]. To summarize, aqueous solutions of gold (III) chloride (100 mM) and ascorbic acid (500 mM) were added to a 10 mM sample of Doxil to obtain a final concentration of 1.5 mM and 11.5 mM of gold (III) chloride and ascorbic acid, respectively. Each addition was followed by gentle swirling that was continued until a distinct color change was observed. Extinction spectra were collected using a dual beam Cary 5 spectrophotometer.

2.2 Doxorubicin Release from Liposomes

Doxorubicin release from Doxil® was monitored by fluorescent intensity at excitation and emmission wavelengths of 470 nm and 592 nm respectively. The release of 5 µl Doxil® in 1995 µl phosphate buffered saline (PBS) at 60 °C was observed over 60 mins in both uncoated and gold-coated samples. After the timed exposure to elevated temperature, the sample fluorescence intensity was measured using a diode array spectrometer (Ocean Optics; Dunedin FL). Subsequently, 5 µl of 10% *v/v* Triton X-100 was added, to simulate full release, and the intensity was collected again.

$$\%\ DXR\ release = \frac{I - I_0}{I_{max} - I_0} \quad (1)$$

The percent release of doxorubicin was calculated using equation (1) where I is the sample fluorescence intensity, I_{max} is the intensity after the addition of Triton X-100, and I_o is the intensity of the sample immediately after exposure to elevated temperature.

2.3 Light-Induced Release from Liposomes

Light-induced release of doxorubicin from uncoated and plasmon resonant Doxil was carried out using a previously described method [6]. Briefly, 10 µl of sample was added to a cuvette. The sample volume was then illuminated using a 760 nm laser diode coupled with a 4.51 mm focal length aspheric lens (Thorlabs; Newton, NJ). The laser diode was powered by an ILX Lightwave Pulse Current Source (Bozeman, MT) at a 10% duty cycle, 0.8 W power, and 200 kHz frequency.

Directly after illumination, samples were diluted with 1990 µl HEPES buffered saline (HBS) and fluorescence spectra were collected with a diode array spectrometer. Subsequently, 10 µl of 10% *v/v* Triton X-100 was added to the sample and flourescence intensity was measured again. The percent doxorubicin release was calculated using equation (1), as shown above.

3 RESULTS AND DISCUSSION

Our results demonstrate formation of plasmon resonant gold coating on Doxil. Figure 3 shows the absorbance spectrum obtained with gold (III) chloride and ascorbic acid concentrations of 1.5 and 11.5 mM, respectively. The maximum of the plasmon resonant peak is located at 631 nm, similar to those we observed earlier in liposomes of different compositions [10].

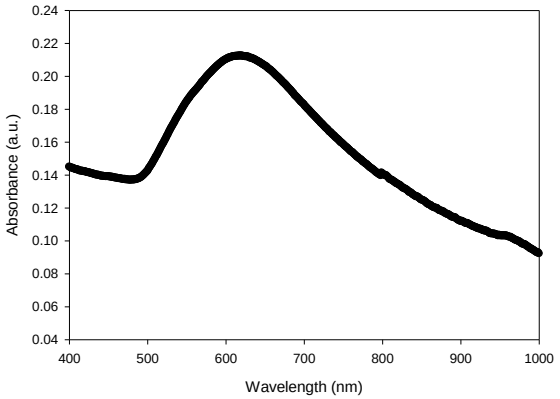

Figure 3: Plasmon-resonance spectrum of gold-coated Doxil®. The spectrum is obtained by subtraction of the spectrum of uncoated Doxil from that of gold coated Doxil.

We then compared the effects of laser illumination at 760 nm, elevated temperature (60 ^{o} C), and lower pH (4.7) on the release of doxorubicin from uncoated and plasmon resonant samples. Since the high concentrations of doxorubicin encapsulated in Doxil result in self-quenching of the characteristic fluorescence of the drug molecules, doxorubicin release from liposomes is conveniently monitored via the dequenching assay where the leakage is monitored by the increase in fluorescence intensity upon dilution in the extraliposomal space.

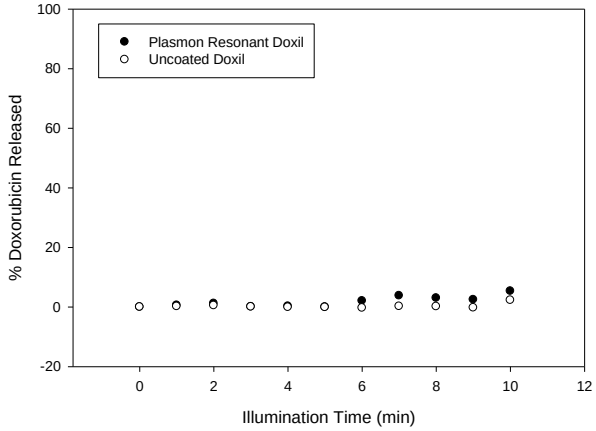

Figure 4: Light-induced release of doxorubicin from uncoated and plasmon resonant Doxil

The addition of a plasmon resonant coating to the surface of liposomes makes them potentially responsive to light. We hypothesize that, when illuminated at or near the maximum of plasmon resonance, Doxil may release its content through a phothermal conversion process whereby energy of light is converted into heat. When liposomes are heated to their phase transition temperature, leakage of contents can occur [10]. However, we observed only minimal release, up to 4%, upon laser light illuminations of gold-coated samples of Doxil (Figure 4).

We therefore investigated the response of Doxil to increasing temperature. Figure 5 shows the percent release of doxorubicin from both uncoated and plasmon resonant liposomes at 60 ^{o} C observed over the period of 1 hour. The results indicate that elevated temperature did not have an impact on release in either sample. This finding falls in line with earlier reports stating that the Doxil formulation is non-temperature sensitive [2,3,6].

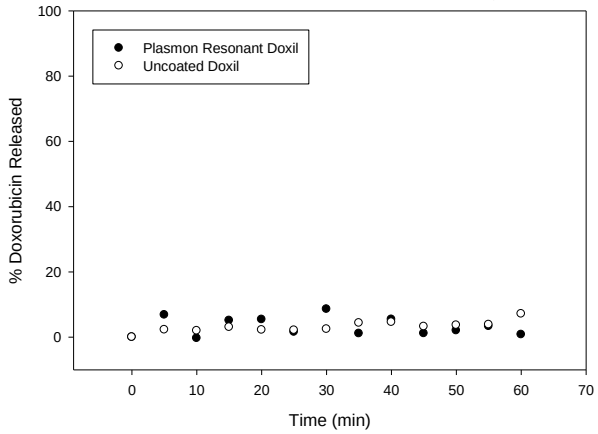

Figure 5: Release of doxorubicin from uncoated and plasmon resonant Doxil at 60 ^{o} C

We also explored the effect of pH on the release process. In Figure 6 we compare the effect of pH 4.7 and pH 7.4 on uncoated and plasmon resonant samples at 60 ^{o} C over 1 hour.

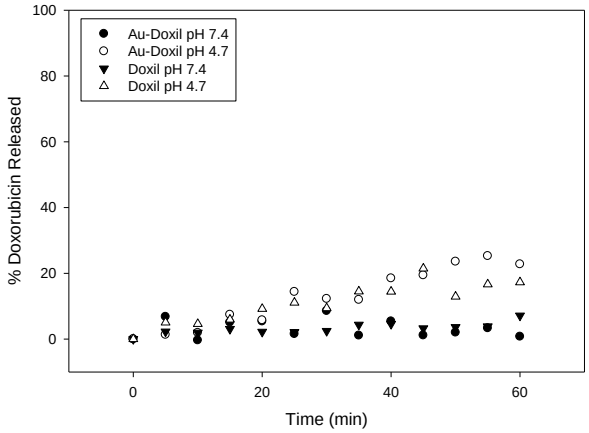

Figure 6: Effect of decreased pH and elevated temperature on doxorubicin release from both uncoated and plasmon resonant Doxil

The results indicate that pH plays a role in the release of doxorubicin. We see that at pH 7.4 both samples show no release, however, at pH 4.7, about 20% release is detected. The pH-sensitive release mechanism was evoked earlier to explain release of doxorubicin from Doxil within the lysosome [11].

4 CONCLUSION

The application of a plasmon resonant coating to the surface of liposomes imparts the ability to control the release of liposome contents. Functional plasmon resonant Doxil has the potential to reduce toxicity by enabling the spatial and temporal release of liposome contents. We were able to successfully reduce gold onto the surface of Doxil to give the drug a plasmon resonant characteristic. Testing both temperature and light-induced release assays on Doxil demonstrates only a minimal control of the release of doxorubicin from the liposomal core. On the other hand, a decrease in pH is shown to initiate doxorubicin release. These findings highlights the need for a liposomal formulation that can retain contents in physiological conditions but is also responsive to an external stimulus so that release can be initiated on demand. Future work will focus on encapsulating doxorubicin in a stable, temperature sensitive liposomal formulation and demonstrating controlled release in gold-coated, plasmon resonant samples.

5 ACKNOWLEDGEMENTS

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