Light-Mediated, Multi-Step Release from Liposomes

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Statement of Purpose:

Temporally-controlled release of proteins from liposomes has great potential for applications in controlled cell differentiation and tissue engineering. A major hurdle in tissue engineering is accurately mimicking the temporal complexity of cytokine profiles during physiological processes such as angiogenesis. It has been shown that the sequential delivery of multiple growth factors such as basic fibroblast growth factor (bFGF) and platelet-derived growth factor (PDGF) promotes greater angiogenesis than when each growth factor is delivered individually or both are delivered simultaneously[1]. In order to create a more biomimetic environment, previous work in our lab has optimized the controlled, stepwise release of small molecules from liposomes loaded with gold nanorods. These liposomes undergo a phase transition at 41°C, releasing their contents through a more fluid-like membrane. Through the use of gold nanorods that absorb at separate, distinct wavelengths in the near-infrared region (**Figure 1A**), multi-step release can be achieved. We intend to adapt this liposome system to the release of macromolecules (proteins/growth factors) to promote vascularization after an injury such as myocardial infarction or ischemia. To induce a release mechanism suitable for macromolecules, we have implemented pulsed laser optics and 2-photon irradiation, as opposed to continuous-wave, and nanorods tethered to the surface of liposomes to induce liposome membrane disruption through microbubble formation and collapse. Here we demonstrate the feasibility of this release system.

Methods:

Liposome synthesis: Bovine serum albumin (BSA) and nanorods at varying concentrations were encapsulated inside DPPC liposomes using an interdigitation fusion method[2]. To tether nanorods to the liposome membrane, DSPE-PEG(2000)-Amine was incorporated into the DPPC lipid bilayer, and 2-iminothiolane-HCl was used to convert amine groups to thiols on liposome surfaces. Nanorod tethering was accomplished via the thiol linkages.

Loading and release: Quantification of BSA loading and release was completed using BCA (bicinchoninic acid) assays. Release was triggered using either a pulsed 748nm laser or 2-photon imaging at 840nm. Liposome membranes were stained with fluorescent CM-DiI.

Results:

The liposomes with encapsulated BSA and nanorods were able to load up to 118 μg/mL of BSA (**Figure 1B**). BSA was found to be stably loaded within the liposomes, as no release was detected after incubation at 37°C for up to 7 days. 2-photon irradiation and imaging of CM-DiI stained liposomes shows immediate disruption of the liposome membrane, mediated by the tethering of gold nanoparticles to the surface of liposomes (**Figure 2**).

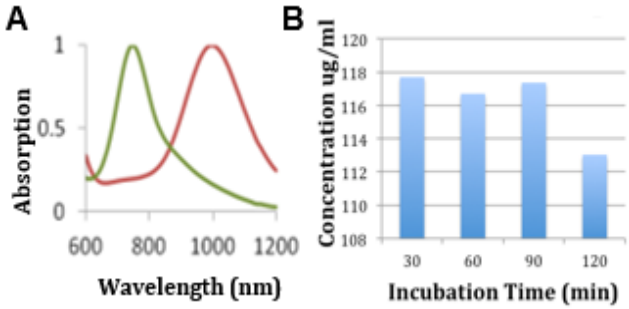

Figure 1. (A) Narrow absorption peaks of two different gold nanorods. (B) BSA loading concentration afte varying incubation times during synthesis; non-irradiated.

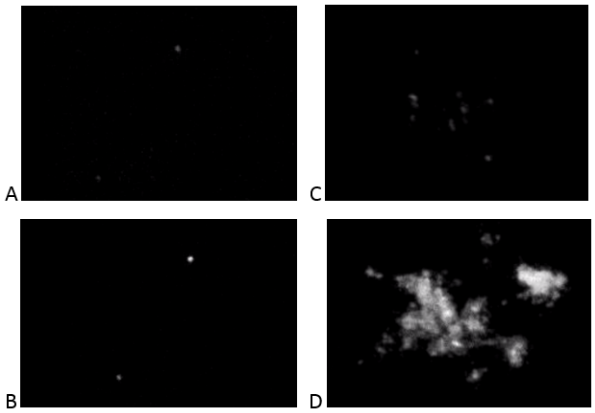

Figure 2. (A) DPPC liposomes without tethered nanoparticles before irradiation, (B) Fig. 2A after 2-photon irradiation, (C) DPPC-DSPE-PEG liposomes with tethered 760nm-responsive nanoparticles before irradiation, (D) Fig. 2C after 2-photon irradiation.

Conclusions: DPPC liposomes synthesized using interdigitation fusion allow for substantial protein loading compared to conventional loading techniques. We seek to utilize this high degree of loading for growth factor delivery to induce angiogenesis. Further, our multiple nanorod system will allow for sequential release of multiple growth factors based on the wavelength selected. Ultimately, our sequential release system will better mimic the temporal release profiles observed in biological systems. Future studies will be focused on: (1) optimizing laser optics, (2) increasing nanorod tethering efficiency, and (3) *in vitro* and *in vivo* studies of the effects of sequential release of growth factors from nanorod-containing liposomes.

References: [1] Tengood JE. Tiss Eng Pt A. 2011; 17:1181-1189. [2] Ahl PL. Bioch Et Bioph Acta-Biomem. 1994; 1195(2): 237-244.