Protégé Program Final Summer Report

Long-Term Dose-Controllable Drug Delivery

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Approved By: 

Chronic illnesses are long-term illnesses that require constant and ongoing medical attention for management [1]. This often requires repetitive injections and frequent hospital visits. Due to this, patients need repetitive injections to manage their illness. This project, "Long-Term Dose-Controllable Drug Delivery," was focused on improving drug delivery to help patients better manage their chronic illness with less injections. Previous work for long-term drug delivery has used liposomes, a phospholipid bilayer that has an aqueous core, in combination with light-activated materials such as gold nanoparticles and fluorescent dyes. Attaching gold nanoparticles into the liposomes allows for the lipid bilayer to temporarily melt from the localized heat created through laser irradiation which allows drug to be released. The dosage of the drug released can be controlled by altering the laser irradiation time and power to meet the necessary treatment requirements. However, the gold nanoparticles tend to overheat which can cause the liposomes to burst as well as localized too much heat can cause cell death and permanent deformation of the nanoparticles which makes the repeatability of drug delivery very difficult [2]. In addition to gold nanoparticles, indocyanine green, a fluorescent dye, has been encapsulated within the liposome's core. When the liposomes were activated by laser, the fluorescent dye would cause an increase in temperature, this would then temporarily melt the bilayer, releasing the encapsulated drug. The downside to using indocyanine green dye is that the half-life is only about 72 hours which is not ideal for the long-term aspect of this research project [3, 4, 5]. Croconium dye has a longer half-life of about 20 days which is 17 days longer than indocyanine green and has better photothermal efficiency which is important when irradiating with a laser for drug release [3]. In this research project, I focused around incorporating croconium dye into the liposome structure for long-term dose controllable drug release.

The overall goal of this research project was to improve light-activated drug delivery with croconium dye-conjugated reverse phase evaporation (RPE) liposomes. The main goal was broken into the four smaller goals of (1) conjugating croconium dye to stearylamine, (2) synthesizing drug-loaded RPE liposomes, (3) incorporating the conjugated stearylamine-croconium dye to the RPE drug loaded liposomes, and (4) releasing the encapsulated drug from the RPE liposomes by irradiating them using an 808 nm laser.

The first goal of conjugating croconium dye to stearylamine was necessary to avoid competition between the dye and the drug which would result in less drug being encapsulated. It was also necessary to conjugate to avoid accidental croconium dye leakage when the liposomes are irradiated. Conjugating croconium dye to stearylamine was accomplished by taking presynthesized croconium dye and dissolving it in dimethyl sulfoxide (DMSO). Once dissolved, EDC was added to the same vial. Once the EDC was added, the solution was stirred for three hours on a stir plate. After three hours, sulfo-NHS and stearylamine were dissolved in DCM and then added to the vial with the croconium dye, EDC, and DMSO. This solution was then stirred overnight. The DCM was then evaporated off and only conjugated stearylamine-croconium dye and DMSO remain. After all the DCM was evaporated off by placing in a fume hood, distilled water (DI) was added to the sample for further precipitation of the conjugated product and to help in washing the sample. The sample was then centrifuged at 10,000 g. The supernatant was then removed and discarded. Centrifugation was repeated by adding additional water until the supernatant was clear. This step was important because it washed out the excess DMSO and byproducts created from the EDC/NHS reaction. The remaining pellet was the conjugated stearylamine-croconium dye. The sample was then lyophilized overnight to remove all remaining water. The isolated stearylamine-croconium dye product was then collected. Fourier transform infrared (FTIR) spectroscopy was then performed to confirm that stearylamine-croconium dye

was created. A successfully conjugated product should have peaks at similar wavelengths for both stearylamine and croconium dye, as seen in Figure 1. The resulting FTIR spectroscopy reading (*Figure 1*) shows how the conjugated stearylamine-croconium dye had similar peaks in the FTIR scans. Circled in red are the similar peaks between croconium dye and the conjugated stearylamine-croconium dye product. Circled in yellow are the similar peaks that stearylamine and the conjugated stearylamine-croconium dye product share. This spectroscopy scan confirms that the conjugation method was successful and that the conjugated product can be used in the synthesis of RPE liposomes.

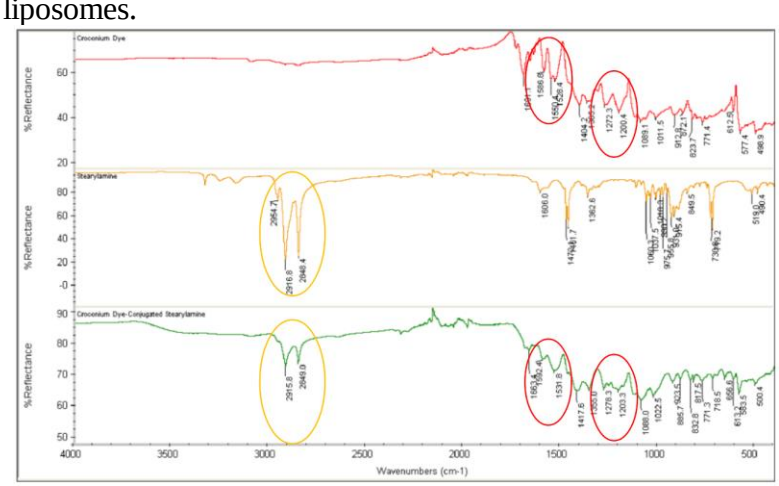

Figure 1: FTIR spectroscopy of croconium dye (red), stearylamine (yellow), and conjugated stearylamine-croconium dye (green).

The goals of synthesizing drug--loaded RPE liposomes and incorporating conjugated stearylamine-croconium dye were both accomplished using the following liposome synthesis method. The liposomes that were created in our research project have a composition of DSPC, DSPE-PEG-5000, cholesterol, and the conjugated stearylamine-croconium dye in a 50:5:35:10 ratio. The DSPC, DSPE-PEG-5000, and cholesterol stock solutions were made by dissolving the solid chemical and 1 mL of chloroform. Solutions were then combined and placed on a rotovap to remove chloroform and create a thin film of lipids. The stearylamine-croconium dye was dissolved in methanol in a centrifuge tube. After rotovapping, the thin film was rehydrated using a 1:2 ratio of the stearylamine-croconium dye dissolved in methanol solution and chloroform. The rehydrated lipids were then placed in an ice bath on and stirred while dexamethasone sodium phosphate (dex) dissolved in DI water was added drop wise. The solution was then bath sonicated to create a water-in-oil emulsion. After sonication, the liposomes were placed back on the rotovap in a 30° C bath to remove the remaining organic solutions. The resulting solution was the conjugated RPE liposomes. The sample was then collected and vortexed to help break up the liposomes that might be clumped together. A PD-10 column was performed on the liposomes to remove excess dex or any unincorporated stearylamine-croconium dye. Dynamic light scattering (DLS) spectroscopy was performed on the liposomes to check the size of the liposomes created. In addition, the ultraviolet-visible (UV-Vis) spectroscopy was performed from 200-300 nm and from 600-850 nm to ensure that dex and stearylamine-croconium dye was incorporated into the liposomes. The liposomes were then washed using dialysis tubing with DI water. After dialysis, the liposomes were centrifuged at 5000 g to concentrate the liposomes back to their original volume. The washed liposomes were then ready for irradiation for drug release.

The synthesis method for creating RPE stearylamine-croconium dye liposomes was deemed successful due to tests run on the UV-Vis spectrometer. Dex will be seen at a

wavelength of 240 nm and the conjugated stearylamine-croconium dye will be seen at 779 nm [4]. Figure 2 shows that dex was incorporated successfully into the aqueous layer of the liposome and can be used for drug release trials. Figure 3 shows the incorporation and presence of the conjugated stearylamine-croconium dye in the synthesized RPE liposomes shown by the peak at 780 nm. This supports the fact that the conjugation of the conjugated stearylamine-croconium dye was successful.

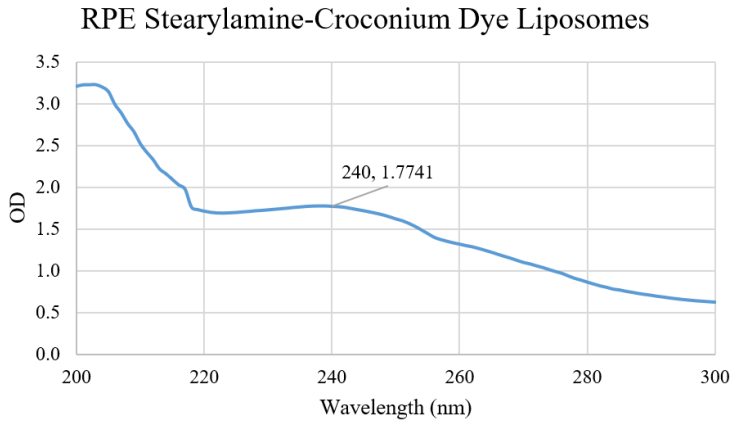

Figure 2: Ultraviolet-visible spectroscopy reading of the presence of dexamethasone sodium phosphate in conjugated RPE stearylamine-croconium dye liposomes.

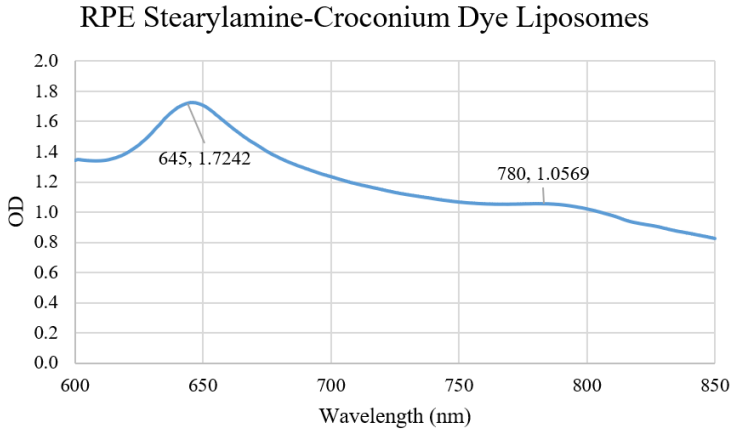

Figure 3: Ultraviolet-visible spectroscopy reading of the presence of conjugated stearylamine-croconium dye in conjugated RPE stearylamine-croconium dye liposomes.

The goal of releasing dex from the liposomes through laser irradiation was accomplished by separating the synthesized liposomes into 3 groups: non-irradiated, 1 minute irradiated, and 2 minutes irradiated. The non-irradiated group received no irradiation and was the control group. The 1-minute irradiated sample was placed under the 808 nm laser and irradiated for 1 minute. The 2-minute irradiated sample was done the same way as the 1-minute irradiated sample, but it had a 1-minute cool off period between the first and second irradiation periods. The laser irradiating the liposomes should activate the conjugated stearylamine-croconium dye and cause a localized phase change on the liposome bilayer that allows for the dex to be released. The samples were then left overnight for Dex to further release and for the liposomes to return to a closed system. The next day the liposomes were centrifuged and the filtrate was collected. To check if dex was released, UV-Vis was taken from 200-300 nm of the filtrate. If dex was

released, then there would be a peak at 240 nm on the UV-Vis [6]. The peak should be higher for more irradiation time because in theory, the longer the liposome was irradiated, the longer the liposome can release drug, resulting in more drug being released.

Drug Release was seen in a batch of RPE stearylamine-croconium dye conjugated liposomes with methanol. Upon taking the UV-Vis of the liposomes, the trend of the longer the liposomes are irradiated, the more dex that should be released held true. For the non-irradiated liposomes, a total of 43.7 µg/mL of dex was released. The 1-minute irradiation liposomes had a total of 53.1 µg/mL of dex released. And the 2-minute irradiated liposomes had a total of 58.7 µg/mL of dex released. These results indicate a successful drug release to the trend of the longer the liposomes are exposed to an 808 nm laser, the more dexamethasone sodium phosphate will be released from the liposomes.

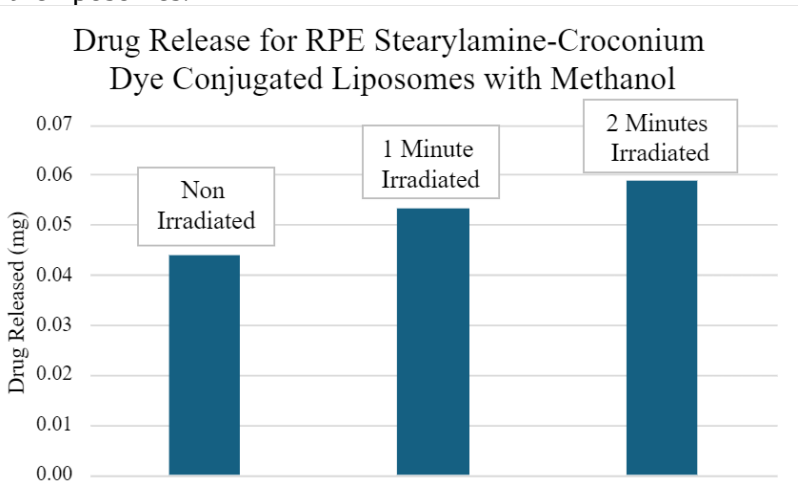

Figure 4: Drug Release from RPE Stearylamine-Croconium Dye Dex Loaded Liposomes using the methanol synthesis for liposome synthesis.

While most of the goals mentioned previously have been accomplished, there can always be improvements. Even though there have been preliminary results showing successful drug release, there has only been one successful trial. This indicates that some changes need to be made to the drug release protocol or the liposome conjugation protocol. To improve efficiency, one recommendation for future work would be to use a 650 nm laser for irradiating the liposomes because the conjugated stearylamine-croconium dye liposomes also show a peak around this wavelength. At certain pH's, the peak has a higher optic density which means that the 650 nm laser could heat up the liposomes more than the 808 nm laser which, in turn, could result in more dex releasing. Another suggestion would be to incorporate croconium dye into the liposomes by attaching croconium dye to alternative lipids [7].

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