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Faculty Mentor: Prof. Basar Bilgicer

Project Title: Engineering Multifunctional Nanoparticles for Targeted Drug Delivery in Cancer

Skills Acquired: Liposomal Nanoparticle Formation, Liposomal Extruder Purification (LEP), High Performance Liquid Chromatography (HPLC), Spectrophotometer Analysis, Thermocycler Use, Cytotoxicity Assays, Cell Biology Techniques

Project Summary:

The proteasome inhibitor carfilzomib is commonly used in the treatment of cancers like multiple myeloma (MM), mainly because its pro-apoptotic effects are stronger in malignant cells than in healthy cells. However, dose-limiting toxicity reduces the full potential of this drug. To overcome this limitation, we employ the use of targeted, drug-loaded liposomal nanoparticles which are intended to increase the efficacy of drug delivery to malignant cells and reduce system toxicity. Nevertheless, the drug loading for these nanoparticles remains low, leaving room for significant improvement. Increasing the drug loading reduces the amount of particles that need to be administered to the patient. In this project, the overall goal was to find the liposome formulation which yielded the highest amount of Carfilzomib encapsulation. To achieve this, the formulation of the liposomes was varied using different lipid molecules at different ratios as well as the size of the particles to maximize the amount of carfilzomib loading. All particles were formulated at 5 mol % mPEG-2000, which helps to aid the "stealth" effects of the particles *in vivo*. These particles were prepared by hydrating a lipid film, and were sized using standard extrusion techniques. The drug loaded particles were purified using the liposome extrusion purification (LEP) method developed in our lab, and the amount of drug encapsulated was measured using high performance liquid chromatography (HPLC). The formulations that yielded the highest drug content were evaluated in terms of their efficacy via *in vitro* MM cell viability studies. Thus, we identified the most effective formulation for encapsulating carfilzomib payload, which in turn will be used to engineer the next generation of targeted nanoparticle drug delivery systems.

From the studies performed this summer, two dual-lipid liposome formulations in particular have been found to increase drug loading significantly as compared to previously used, single-lipid formulations. We examined formulations which varied the carbon tail length of the bulk lipids at 75:25 and 50:50 ratios, as well as varying the particle size between 50 nm and 100 nm diameters. As can be seen in Figure 1 below, most of the lipid combinations yielded a steady downward trend as the molar percentage of drug formulated was increased. The only exceptions to this trend were the DSPC:DMPC and DSPC:DPPC combinations formulated at a 50:50 ratio; these combinations seemed to show a plateau effect as the molar formulation percentage was increased. This is promising because the combinations of phospholipids used in these nanoparticles have lead to statistically increased drug retention as compared to previous single-lipid formulations. This trend was only observed only in the 100 nm particles; the 50 nm particles did not display the same trend, which may be a result of the biophysical properties induced by increasing the curvature of a two-lipid liposome system that has the potential to form lipid rafts.

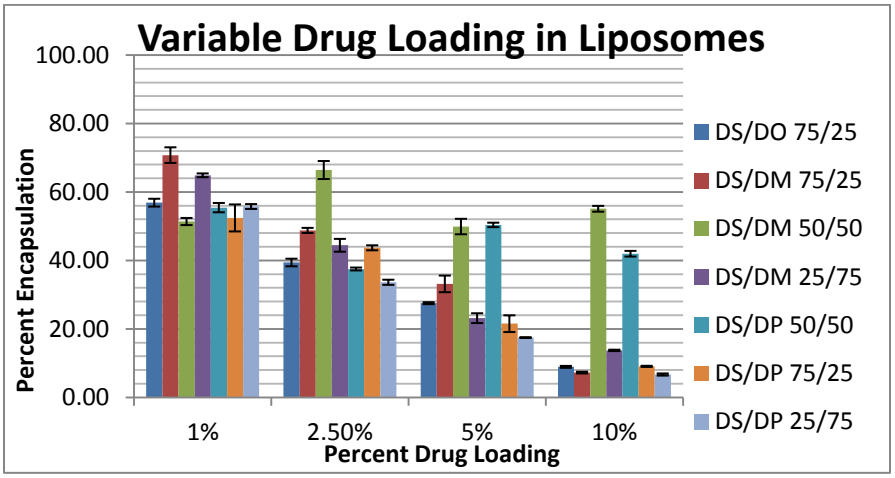

Figure 1. This graph displays the drug retention values found for the 100 nm particle formulations.

After the success of the DSPC:DMPC combination was demonstrated, the formulation was then subjected to further testing in a non-targeted liposomal drug cytotoxicity assay. Two cell lines were tested: MM.1S and H929. The liposomes were compared to a free carfilzomib drug control, which yielded the results in Figure 2. From this data, we have determined that the IC_{50} for these non-targeted particles is on the order of ~40 nM, varying some between the two cell lines tested. This is a higher IC_{50} than the free drug alone (~25 nM, depending upon cell line), which is to be expected.

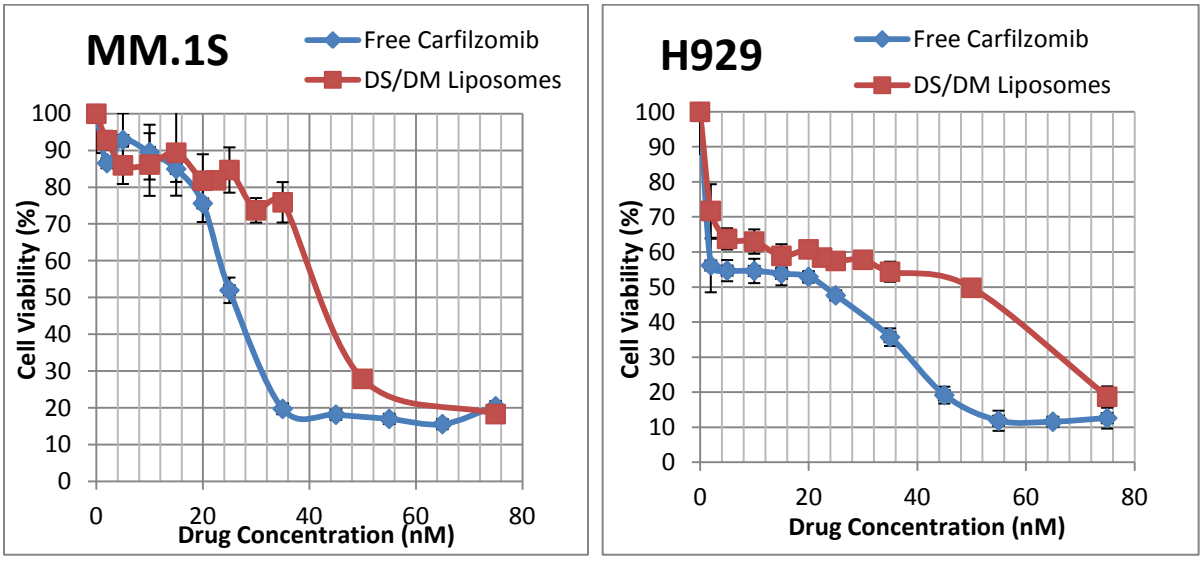

Figure 2. The results of the cytotoxicity assay are displayed for both cell lines.

In conclusion, more detailed study of this combination, including the incorporation of targeting ligands, is likely to be completed *in vitro* to further discover the therapeutic benefits of this promising liposomal formulation.