Optimizing Liposomal Uptake and Content Release Using Glioblastoma Multiforme as a Model System

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Optimizing Liposomal Drug Delivery:

Liposomes are hollow vesicles of various sizes and morphologies composed of a phospholipid bilayer membrane. They can be used to encapsulate agents in targeted drug delivery systems where the drug only reaches the specific cellular pathway or type of cell it is intended to target. The liposome thus acts as a protective bubble, minimizing drug loss and reducing side effects, which can be especially valuable when using cytotoxic or immunosuppressive drugs.

The model system used to study optimization of targeted drug delivery is glioblastoma multiforme (GBM), a highly malignant cancer that metastasizes easily through soft brain tissue. A compound called imipramine blue (IB), which prevents actin polymerization, effectively localized tumor growth in GBM cells [1]. This greatly aids surgical removal and creates a more defined target for chemotherapy drugs. However, the half life of free IB in circulation is only 11 minutes, compared to 18 hours when packaged within liposomes. When treated with 160 nm liposomal IB followed by liposomal doxorubicin, a chemotherapy drug, rats with GBM had a survival rate of 100% and demonstrated no signs of regrowth [1]. The delivery of drugs to GBM cells is dependent on rapid vascularization around tumor growth which results in nonuniform vascular walls with pores measuring about 200 nm in radius. Since healthy vasculature only allows passive diffusion of particles about 10 nm in size, liposomes in circulation are only absorbed into tumorous regions.

Adjustments to the intrinsic properties of liposomes — size, phospholipids composing the membrane, the method of formation, and the thermodynamics of the structure — can significantly impact the overall drug delivery process. This study aims to find relationships between these variables and the rate of diffusion of liposomes, the rate at which they leak their contents, and their overall stability. This strategy preserves the low circulation time and diffusive potential characteristic of spherical liposomes without surface ornamentation, and doesn't require exogenous factors, like radiation, to induce drug release.

Methodology:

In past studies it was observed that the 160 nm liposomes were accumulating around the GBM vasculature [1]. Reducing the size should address this by allowing the drug to diffuse to the periphery of the tumor thereby increasing overall uptake. Liposomes less than 100 nm, or small unilamellar vesicles (SULVs), are generally unstable due to membranous stress from a high surface curvature [2]. To relieve this tension, a short chain phospholipid, dihexanoyl phosphatidylcholine (DHPC) was used to attenuate the longer chain phospholipids, dimyristoyl phosphatidylcholine (DMPC) and dimyristoyl phosphatidylglycerol (DMPG). Temperature and concentration of phospholipids primarily dictated the structure, size, and stability of the liposomes [2].

Figure 1 shows how SULV formation pathways rely on structural (disklike and ellipsoidal) precursors in solution, and the parametric ranges they require. The critical temperature is $T_c \approx 23°C$, the point at which the phospholipid chain melts. Lamellar sheets were formed at 45°C with 25% lipid wt., and diluted in one step to concentrations between 2.5% and 0.1% lipid wt. Initial dynamic light scattering (DLS) experiments indicated high polydispersity in size suggesting the formation of large liposomes with high thermodynamic stability. Extrusion through a 50 nm nuclepore membrane was predicted to significantly lower the yield, so this pathway was abandoned altogether.

The process was repeated at 4°C forming disklike bicelles at 1.5% to 0.75% lipid wt., and ellipsoids between 0.09% and 0.03% lipid wt. These solutions were heated to 45°C, extruded, and tested using DLS at 37.5°C. The intensity was measured at different angles, and cumulant analysis was used to determine the average decay rate, Γ, shown in Figure 2a, right, where q is the scattering vector and D is the diffusion coefficient. The Einstein-Stokes equation, Figure 2b is used to determine the average hydrodynamic radius, R.

(2a) $$\Gamma = -Dq^2$$

(2b) $$D = \frac{kT}{6\pi\eta R}$$

Figure 2

Radii of SULVs with Disklike and Ellipsoidal Precursors:

SULVs were successfully created ranging from 30-70 nm. Figure 3 shows that the average hydrodynamic radii of SULVs formed from ellipsoidal precursors range from 58.2 to 66.4 nm. Significant variation from the trendline indicates greater polydispersity, both in size and structure. The average radius was greater than 50 nm, even after extrusion, suggesting that these liposomes may have collapsed or aggregated. Of the disklike precursors, only the 0.75% lipid wt. solution yielded data as more concentrated solutions exhibited multiple scattering effects. This solution had an average hydrodynamic radius of 34.3 nm, as shown in Figure 4. Additional solutions of this precursor with varying concentrations will have to be tested to conclusively demonstrate that disklike bicelles consistently yield stable SULVs with radii less than 50 nm.

Future Work:

Future experiments will use rhodamine conjugated to liposome membranes to image their diffusion through cellular media. Liposomes packaged with rhodamine will also be used to test whether leakage rate is dependent upon vesicle size. Differential scanning calorimetry will show how phase-transition effects change with liposome size, phospholipids used, and on formation pathway. Cancer cells have greater cellular entropy due to a higher glycolitic rate. Lowering the liposomes' phase transition entropy by varying these intrinsic variables could cause significant pre-transition effects in liposome membranes and thus increase the rate of drug leakage within cancer cells.

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References:

[1] Munson, J. M., et al., Sci,Trans,Med,, 4(127), 127ra36-127ra36 (2012).

[2] Nieh, M.-P., et al. Langmuir, 21(15), 6656-61 (2005).

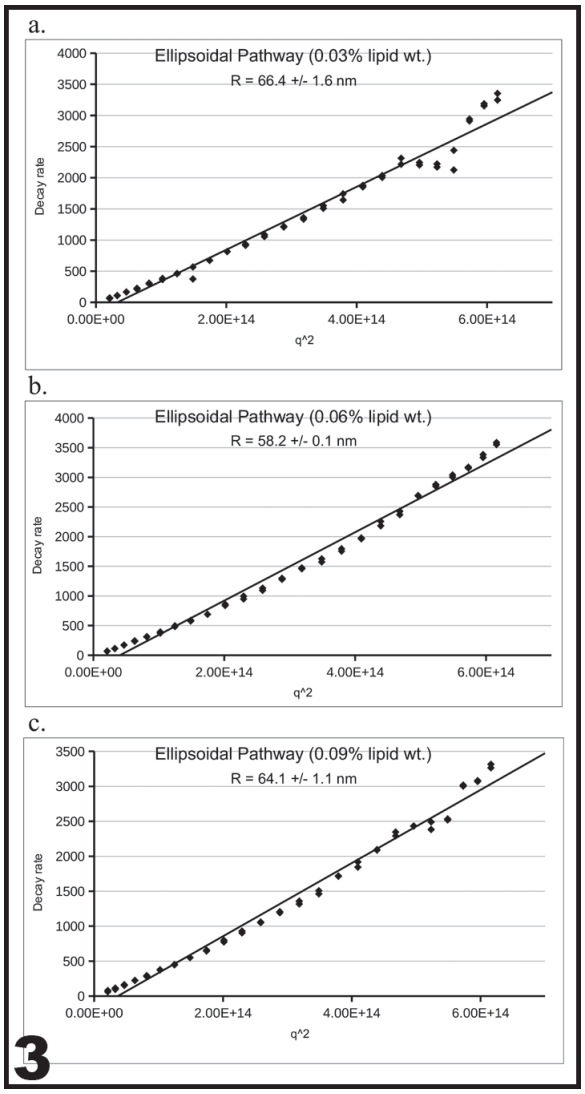

Figure 3: Average hydrodynamic radii of SULVs.

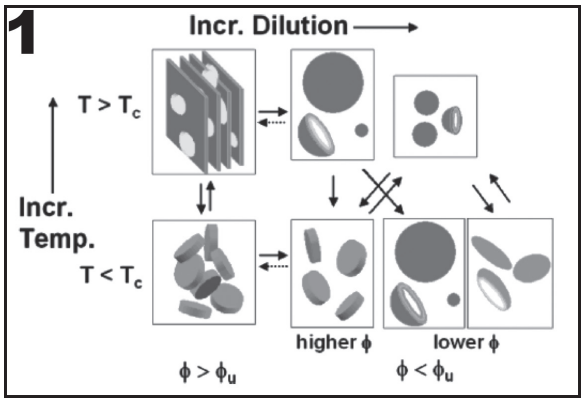

Figure 1: SULV formation pathways rely on structural (disklike and ellipsoidal) precursors in solution.

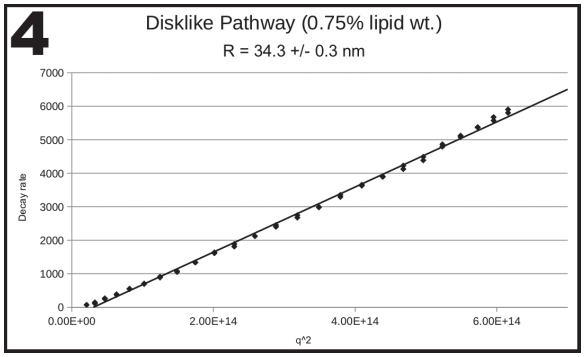

Figure 4: Solution with an average hydrodynamic radius of 34.3 nm.