Single-Step Manufacture of Affinity Nanodiscs for Drug Delivery

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INTRODUCTION

Nanoparticles (**NP**s) have been used as delivery carriers for pharmaceuticals to target tumors through the "enhanced permeability and retention" (**EPR**) effect. This method, when combined with the use of ligands (which actively target cancer cells) has shown enhanced efficacy. However, tedious sample preparation, low yields of reactions, and environmental unfriendliness inhibit a large-scale manufacturing of NPs. Self-assembly of NPs has also been observed and widely applied. The advantages of such spontaneous formation include robustness, well-defined products, and high throughput. However, knowledge of interplay among the molecular architecture, molecular interactions and kinetic pathways is required to control the final morphology of the NPs. In this proposal, we focus on developing a protocol to manufacture self-assembled lipid-based nanodiscs capable of entrapping hydrophobic pharmaceuticals and ligands to enhance their effectiveness of targeting cancer cells, based on the self-assembling principle. These nanodiscs are reasonably uniform with a diameter of (30 ± 5) nm and a lipid bilayered thickness of 5 nm. The knowledge obtained from this study enriches the current understanding of the aggregation behavior of amphiphiles and the protocol can be easily adapted to fit industrial scale production of NPs.

RESULTS

Nanodiscs have been successfully manufactured via self-assembling process in a mixture of long- (di-C_{14} phosphatidylcholine, **DMPC**) and short- chain (di-C_6 phosphatidylcholine, **DHPC**) lipids doped with small amount of di-C_{14} phosphatidylglycerol (**DMPG**) as shown in the transmission electron microscopic (**TEM**) image (the inset of **Fig 1**). The chemical compositions of planar and rim regions of the discs are presumably rich in long-chain and the short-chain lipids, respectively, because of their curvatures Small angle neutron scattering (**SANS**) data obtained from the mixture can be best fit using a monodisperse discoidal model, further indicating that the disc morphology is globally uniform in the aqueous solution (**Fig 1**).

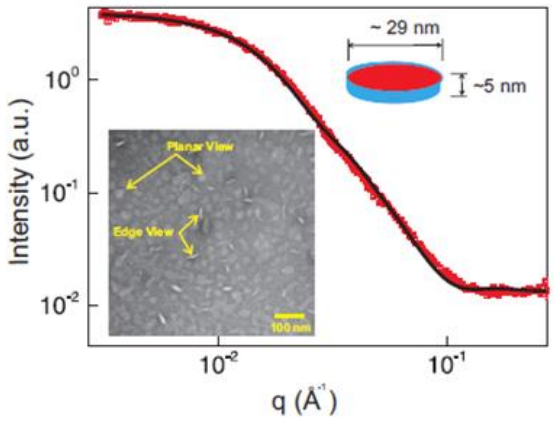

Figure 1 SANS data of DMPC/DHPC/DMPG mixtures (red symbols) and its best fit (solid curve) using monodisperse discoidal model. where q is the scattering vector defined as $4\pi\sin(\theta/2)/\lambda$, θ and λ being scattering angle and neutron wavelength, respectively. The inset is the negatively staining TEM micrograph.

The size of these nanodiscs increases upon dilution (as shown in **Fig 2a**), possibly due to the mismatch of solubilities of long- and short-chain lipids to water phase, elevating the line tension at the disc rim consequently promoting

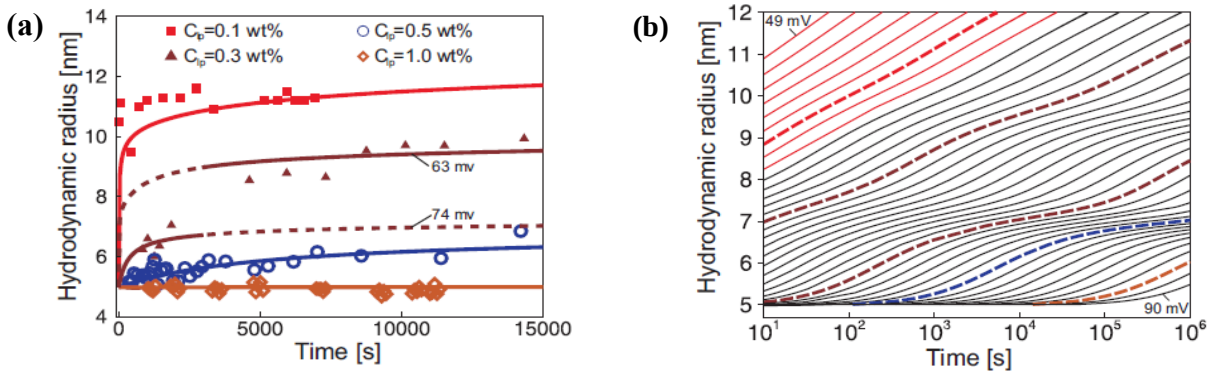

Figure 2 (a) The time-resolved growth of DMPC/DHPC/DMPG nanodiscs (monitored by dynamic light scattering) upon various dilutions and the solid curves represent the best fit using single parameter Smoluchowski discrete population model combined with DLVO. (b) The simulated growth mechanism of the nanodiscs with initial R_H = 5 nm based on the combined Smoluchowski and DLVO model at different surface potentials.

the disc coalescence. The growth mechanism has also been investigated theoretically and experimentally. Based on the Smoluchowski discrete population model and classic DLVO double layer theory, we are able to simulate the growth of nanodiscs with different surface potentials (**Fig 2b**). The fact that this method can describe the experimental data with a single parameter, i.e., surface potential (**Fig 2a**) confirms the disc coalescence mechanism is diffusion-limited caused by repulsive charge on the particles.

The stability of nanodiscs can be greatly enhanced as DMPC and DMPG are replaced by longer-chain lipids (i.e., di-C_{16} phosphatidylcholine, **DPPC** and di-C_{16} phosphatidylglycerol, **DPPG**), respectively and the discs' surface is modified by polyethylene glycol (**PEG**) using PEGylated-di-C_{18} phosphatidylethanolamine (**PEG-DSPE**). **Fig 3** illustrates the SANS data of the DPPC/DHPC/DPPG mixtures in presence and absence of PEG-DSPE, respectively. The effects of temperature (15 and 60 ^{o}C) and salinity (water and phosphate buffer solution, **PBS**) on the morphology were also investigated. The SANS result suggests that (1) PEG inhibits the structural transformation possibly due to the steric effect, (2) increased salinity results in elongated objects and (3) elevation of temperature leads to the closing-up of NPs into vesicular

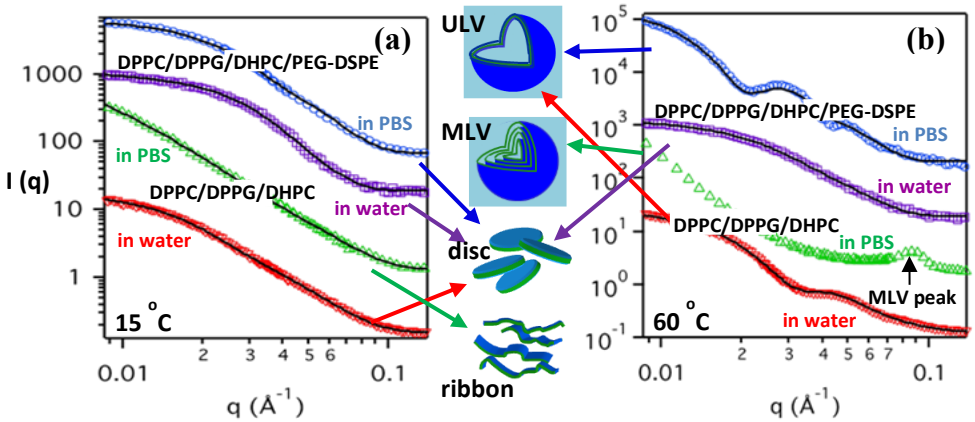

Figure 3 The SANS data (symbols) and best-fits (solid curves) of DPPC/DPPG/DHPC in water (red) and PBS (green) and DPPC/DPPG/DHPC/PEG-DSPE in water (purple) and PBS (blue) at (a) 15 ^{o}C and (b) 60 ^{o}C, The best fitting models are ULV, disc, and ribbons as pointed by arrows. The peak of multi-lamellar vesicle (MLV) is observed at 65 oC in the case of DPPC/DPPG/DHPC in PBS.

structures. The systematic study of the system provides insight to the interplay among the key parameters, allowing us to better control the morphology of the system.

The cell uptake of the nanodiscs has also been investigated in comparison with that of 100-nm extruded liposomes. **Fig 4a** shows the fluorescent confocal microscopic images of CCRF-CEM (T-lymphoblast) cells after being incubated with Nile Red containing nanodiscs and liposomes at 37 ^{o}C for 2 hours. It should be noted that the settings of optical configuration for both cases are identical, indicating that the cell uptake of nanodiscs is higher than liposomes. This result is further confirmed by fluorescent flow cytometry (**Fig 4b**), where the fluorescence peak from the cells in the case of nanodiscs is 3 ~ 5 times of that of liposomes.

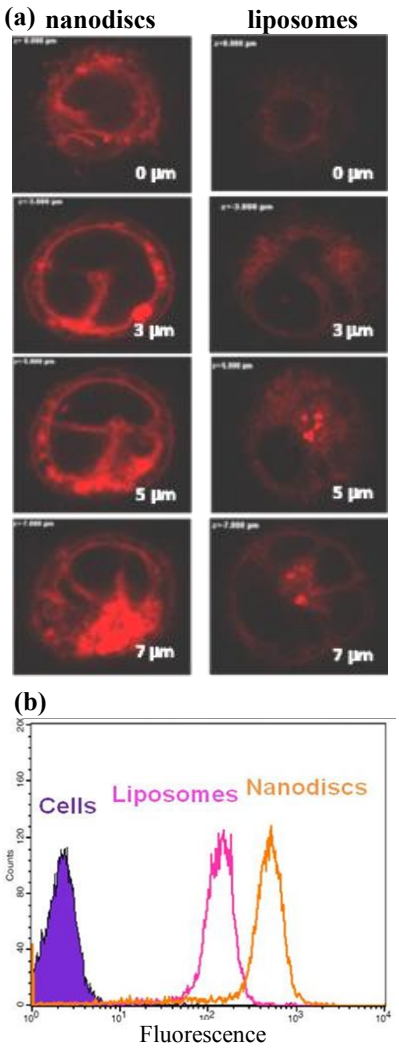

Figure 4 (a) The fluorescence confocal micrographs of the CCRF-CEM cancer cells after being incubated with Nile Red containing nanodiscs or liposomes at 37 ^{o}C for 2 hours. (b) the fluorescence flow cytometry results of the CCRF-CEM cells after being incubated with nanodiscs and liposomes, respectively.

SUMMARY

In this proposal, we have successfully developed the protocol for large-quantity manufacturing of uniform nanodiscs. These lipid-based nanodiscs are capable of entrapping hydrophobic molecules and their surface can be easily modified. Dilution may result in the coalescence of the nanodiscs forming larger-sized discs. The growth mechanism can be described by a diffusion-limited coalescence due to the Coulombic repulsion between the NPs. Incorporation of PEGylated lipid with the nanodiscs greatly enhances their stability, while elevation of temperature and increased salinity induce the instability of the nanodisc structure. The cell uptake study shows that the uptake of the nanodiscs by CCRF-CEM cancer cells is higher than that of the liposomes, indicating the uptake process is morphologically dependent. The future work will focus on further enhancing the cell uptake through incorporating targeting ligands with the nanodiscs based on self-assembling approach. Cytotoxicity of the nanodiscs to cells will also be investigated. The PI and co-PIs will also seek to assist the industry, who is interested in applying similar self-assembling principle to their manufacturing processes.

References

[1] For further information about this project link to http://www.ims.uconn.edu/~safn/SAFN_Research.html or email: mu-ping.nieh@ims.uconn.edu.