Optimization of Microfluidic Devices for Formation of Supported Lipid Bilayers

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Abstract:

Interactions between biological nanoparticles, like viruses, bacterial outer membrane vesicles, or oncogenic microvesicles, with cell membranes play important roles in the progression of disease. To get a better understanding of these nanoparticles, their interactions with supported lipid bilayers (SLBs) can be monitored using microscopy. SLBs act like cell membrane mimics and have the main features of cell membranes including proteins, lipids, and sugars. Forming SLBs within microfluidics enables control over the environment the nanoparticles are in, but bilayers formed this way tend to have plenty of defects. The goal of this study is to determine which microfluidic design results in bilayers with the fewest defects. Bilayers of 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) were formed in devices of width ranging from 50 to 2000 µm and height from 200 to 500 µm. The mobility of lipids in these bilayers was tested using fluorescence recovery after photobleaching (FRAP) characterization. Microfluidic devices containing bigger channels dimensions stimulated formation of POPC supported lipid bilayers with fewer defects and higher lipid mobility.

Summary of Research:

Researchers need tools to perform analytical assays on biological nanoparticles-cell membrane interactions in a quick and safe manner to monitor virus evolution for vaccine development, screen new antiviral drugs, and study fundamental membrane fusion processes [1]. Studying these nanoparticles in live systems can be difficult; however, studying with *in vitro* systems using supported lipid bilayers (SLBs) make it easier. SLBs act like membrane mimics. This biomimetic material preserves lipid mobility and the planar geometry removes many experimental complications imposed by live cells [1]. Using microfluidic devices for formation of SLBs enables control over the nanoparticles' environment, however the Daniel group's current microfluidic design results in bilayers with plenty of defects. To counter this problem, we created three new microfluidics designs (Figure 1).

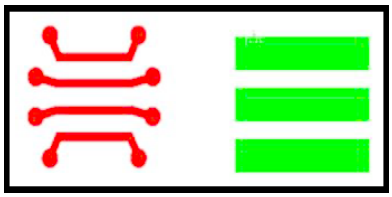

Figure 1: New designs for microfluidic devices (a) Design 1, Design 2; (b) Design 3.

For this study, Design 1 consisted of four channels of 5000 µm long, 500 µm wide, and 200 µm high. Design 2 had the same appearance and measurements as Design 1, except this device was 500 µm high. The last design, Design 3, consisted of three wider and longer channels, each one being 10,000 µm long and 2,000 µm wide by 200 µm. To test the quality of

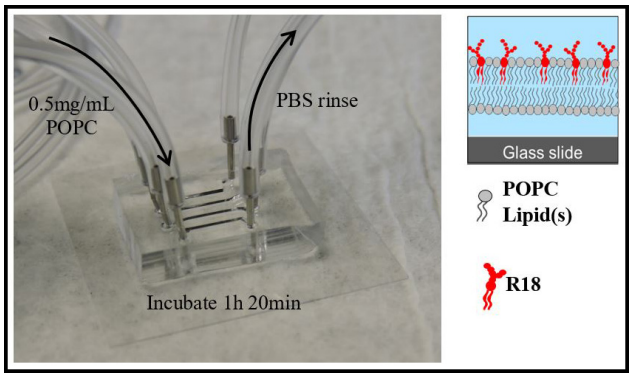

Figure 2: Formation of supported lipid bilayers (SLBs) with fluorescent label R18.

the bilayers formed in each microfluidic device, we examined the diffusion of the lipids within it, using fluorescence recovery after photobleaching (FRAP). In order to do this, 0.5mg/mL of POPC liposomes were added to either a polydimethylsiloxane (PDMS) well or injected into a microfluidic device and incubated for 1 h 20 min before rinsing it with phosphate-buffered saline (PBS) buffer (Figure 2).

Prior to the bilayer formation, the liposomes were fluorescently labeled with R18. This fluorescent label enabled the visualization of the lipid bilayers and was a probe for mobility measurements [1]. During the photobleaching experiment, the microscope shoots a laser light in one spot of the microfluidic channel, bleaching the R18 in it. Over time, the lipids will move around covering the bleached spot. The recovery data was fitted using a MATLAB code to calculate the diffusion coefficient using the two-dimensional solution of the Soumpasis equation:

$$D = \frac{w^2}{4t_{1/2}}$$

where w is the width of the bleached spot, and $t_{1/2}$ the time at which the bleached spot has recovered half of its original intensity. Also from this code we get a fractional recovery vs. time graph showing how the fluorescence intensity of the bleached spot changes over time, Fig. 3.

Results and Conclusions:

The images obtained from the FRAP analysis demonstrate that for bilayers formed in Design 1, the bleached spot did not recover. However, Design 3 demonstrated a fast fluorescent recovery. A comparison of the diffusion coefficient values (Figure 4) for all the microfluidic devices and PDMS well in a boxplot, showed that the closer values to 0.3 $\mu m^2/s$ corresponded to the PDMS well platform and microfluidics with Design 3. The average values were 0.302 and 0.298 $\mu m^2/s$, respectively. This confirms the information obtained from the microscopy imaging, wider channels dimensions stimulated the good formation of supported lipid bilayers.

Future Work:

Supported bilayers derived from cell membranes must be integrated to see if Design 3 still works for them as well.

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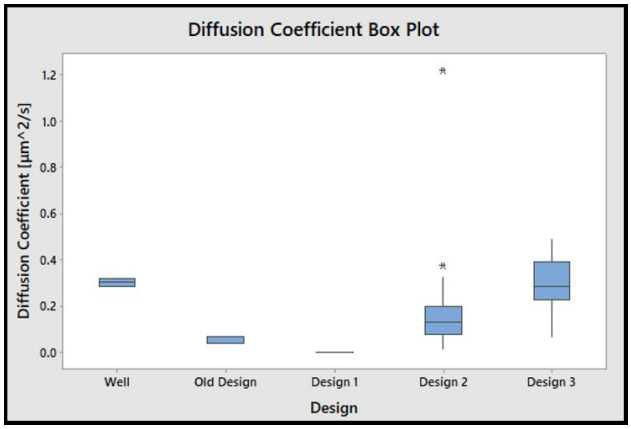

Figure 4: Comparison of diffusion coefficient of R18 in POPC supported lipid bilayers obtained from FRAP analysis under different experimental platforms.

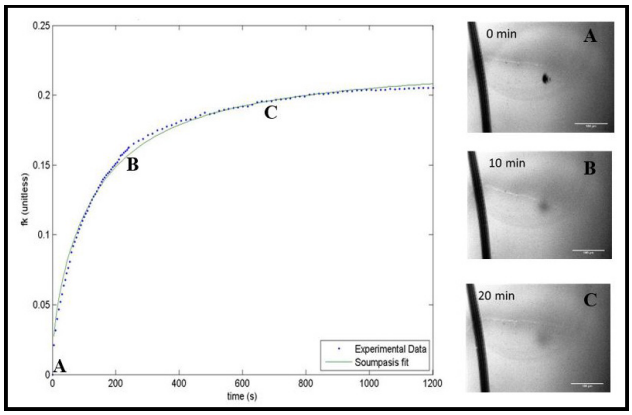

Figure 3: Diffusion of R18-labeled POPC supported lipid bilayer on a PDMS well with time after photobleaching.

References:

[1] Costello, D. A.; Hsia, C.; Millet, J. K.; Porri, T.; Daniel, S. Membrane Fusion-Competent Virus-Like Proteoliposomes and Proteinaceous Supported Bilayers Made Directly from Cell Plasma Membranes. Langmuir 2013, 29, 6409.6419.