Patterning of Native Proteins in Supported Lipid Bilayers

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Abstract:
Many in vitro experimental studies fail to study membrane proteins in their native cellular environment and instead use solubilized systems in which all cellular components are mixed together and removed from their lipid scaffold. To more directly study these proteins, while still decoupling them from other cellular processes, we aim to create a microfluidic system in which protein embedded cell blebs can be patterned and studied. A supported lipid membrane will keep key membrane bound proteins in their native environment, thus enabling us to study them as if they were still inside the cell. Since the flow characteristics, channel dimensions, and the local environment are readily controlled, this platform gives us a way to easily mimic and manipulate the native cellular environment.

Summary of Research:
The use of a supported lipid scaffold from cellular membrane lipids has been shown by our group and others to preserve protein function in their native environment [1]. Previous work has been done in wells and straight channel devices on the millimeter scale, but the extension of this platform to allow for selective patterning will enable us to more extensively study protein and lipid interactions. These are important in virus binding mechanisms, drug delivery applications, as well as in fundamental studies of membrane dynamics.

A negative mask for a prototype microfluidic design was created using the Heidelberg DWL66fs mask writer and used with the ABM contact aligner to pattern photoresist that was spun onto a silicon wafer. After development, the profile of the patterns was analyzed on the P10 profilometer, a snapshot of which can be seen in Figure 1. Optimization of the process was conducted to produce consistent and even films, as measured by the profilometer and the FilMetrics F50-EXR. Once this was done, the exposed silicon was etched using the Unaxis 770 deep Si etcher, with the final form shown in Figure 2. As can be seen, there is still photoresist on the channels, which was removed by oxygen plasma cleaning in the Anatech resist strip. A final hydrophobic coating (FOTS) was applied using molecular vapor deposition to allow for PDMS molds to be easily removed once cast. The final wafer can be seen in Figure 3.

Once the mold was fabricated, Sylgard 184 was poured over the mold and cured. This can then be removed and bonded to a glass coverslip by using oxygen plasma cleaning on both surfaces. Synthetic liposomes containing a fluorescent marker was passed into the channels and allowed to incubate, where they will adsorb to the exposed surfaces and form a bilayer structure. Fluidity is confirmed by using a laser to photobleach the dye and then measuring the diffusion rate as the spot recovers. This process can be seen in Figure 4. Future work will involve more complicated device designs as well as leveraging the microfluidic for biotechnology applications, as mentioned.

References:
2017-2018 Research Accomplishments

Biological Applications

2017-2018 Research Accomplishments

Figure 1: Image taken when measuring profile of exposed features. Depth of film was confirmed using FilMetrics F50-EXR.

Figure 2: Wafer after being removed from Unaxis 770 deep Si etcher.

Figure 3: Wafer after being stripped in the Anatech resist strip and coated with FOTS in the MVD 100.

Figure 4: Fluorescence recovery after photobleaching (FRAP) confirms that a supported lipid membrane was formed in the device.