Development of Microfluidic Device for Protein Synthesis and Modification

CNF Project Number: 2641-18

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Primary Source(s) of Research Funding: National Science Foundation

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Primary CNF Tools Used: Heidelberg DWL66fs mask writer, ABM contact aligner, Unaxis 770 deep Si etcher, Anatech resist strip, MVD 100, P10 profilometer, FilMetrics F50-EXR

Abstract:

Current biological production limits our ability to produce and study tailored biological therapeutics. Many important targets need post-translational modifications that are necessary for maintaining proper structure and function [1]. The cell naturally uses membrane-bound enzymes to do this in a regulated and compartmentalized way. We aim to create a microfluidic device that can recreate this cellular assembly line an in a synthetic system while still maintaining the natural biological environment. The first step in this process is the protein synthesis, which we have shown in this work. 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 local environment to efficiently produce a protein of interest. Future work will focus on the incorporation of the enzymes into a supported membrane in the device.

Summary of Research:

Previous work in cell-free protein synthesis has been done in static reaction conditions [2]. We aim to build on this body of work and build a platform with continuous flow protein synthesis in conjunction with the selective patterning of necessary enzymes to modify them in a precise way. To design the microfluidic device, first 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. 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. Photoresist on the channels 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.

Once the mold was fabricated, Sylgard 184 was poured over the mold and cured. This could then be removed and bonded to a glass coverslip by using oxygen plasma cleaning on both surfaces.

The first step in creating this device is the successful synthesis of a model protein. By collecting the cellular machinery, proteins can be produced in an *in vitro* environment [3]. We isolate a plasmid encoding the green fluorescent protein (GFP) and a cell lysate, and by mixing them together, we produce GFP.

As seen in Figure 1, we can inject each of them independently and through the mixing in the channels, GFP is produced as they proceed through the channels. The small volume of the microfluidic gives tighter temperature control by limiting heat transfer and enhances diffusion to give a higher rate of synthesis as compared to the test tube.

Future work will involve the synthesis of more relevant protein targets as well as the incorporation of enzymes into the supported membrane for modification of the proteins produced.

References:

[1] Defaus, S., Gupta, P., Andreu, D., and Gutiérrez-Gallego, R. Mammalian protein glycosylation - structure versus function. Analyst 139, 2944-2967 (2014).

[2] Swartz, J. R. Advances in Escherichia coli production of therapeutic proteins. Curr. Opin. Biotechnol. 12, 195-201 (2001).

[3] Mohr, B. P., Retterer, S. T., and Doktycz, M. J. While-you-wait proteins? Producing biomolecules at the point of need. Expert Rev. Proteomics 13, 707-709 (2016).

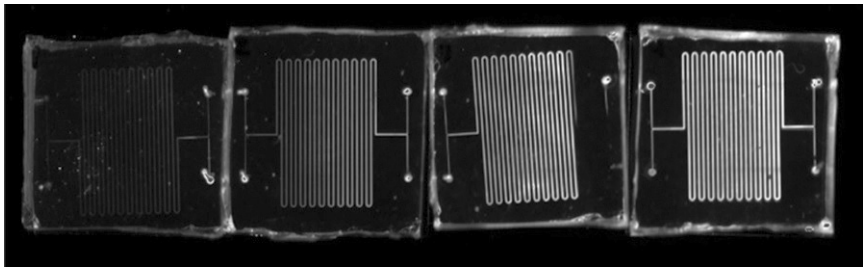

Figure 1: Series of microfluidic devices that were used to produce GFP in a cell-free continuous flow reaction. DNA and cell lysate are introduced independently on the left side and mix as they enter the first device. The increase in fluorescence across the image is seen as the protein increases in concentration through the length of the channels.