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Cell Free Translation in Engineered Picoliter Volume Containers

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Abstract

Engineers seek to use biological design principles to manipulate information and import new functionality to synthetic devices. Such devices inspired by natural systems could, in turn, play a crucial role in allowing biologists to explore the effects of physical transport and extreme conditions of temperature and pH on reaction systems. For example, engineered reaction containers can be physically and chemically defined to control the flux of molecules of different sizes and charge. The design and testing of such a container is described here. It has a volume of 19pL with defined slits of 200nm. The device successfully contained DNA and protein molecules and is evaluated for carrying out cell-free protein synthesis. The effect of DNA concentration and slit size on protein yield is discussed.

I. Introduction

Synthetic biology is a relatively new discipline that can help to elucidate many of the mysteries of cell biology and enable us to develop new applications related to the fabrication of nanostructures and biomaterials, medicine, biosensing and energy production [1]–[3]. By combining genes, synthetic biologists are successfully inserting the equivalent of chemical factories into microorganisms that create new forms of cellular control and enhance our understanding of basic cellular operations.

Advancements in nanotechnology have created opportunities for synthetic biologists to match the scale of biological system components and create biologically inspired devices [1].

These small volume devices can establish favorable conditions for protein production and function. They can also be used to understand biological systems, study single molecule reactions and examine self organization at the cellular scale [1], [4]–[7].

Commonly, liposome vesicles are used as model systems to mimic the function of the cellular membrane. Since these vesicles are made of natural lipids, they are compatible with membrane proteins which can be used to facilitate material exchange [1], [3], [8], [9]. Water in oil emulsions have also been used to create small reaction volumes [2], [10]. However, the long-term stability of liposome vesicles and water in oil emulsion structures can be problematic [1]. Additionally, such systems are difficult to integrate with synthetic control mechanisms and devices, which leads to the need for more robust and reliable vesicles.

Microfabrication and nanotechnology based techniques can be used to create such robust reaction containers from inorganic materials. The small volume devices can be used for a variety of applications including: high throughput screening [11], enzyme kinetics [12], analysis of single cells [13] and cell-free protein synthesis [1], [11], [14], [15]. They can be patterned in different materials, shapes and pore sizes and can be physically or chemically modified to control the flux of molecules of different sizes and charges so that they mimic some of the characteristics of a natural cell. Such a silicon based device is described here. It can be used for studying biochemical reactions and the effects of scale and compartmentalization on reaction efficiency. The cylindrical container is 2 μm thick with 8 sets of 7 slits spaced around the perimeter. Slits are etched in the same processing step as the device and can range in size from 30–200 nm wide and 10 μm deep (Fig. 1 and 2). Devices can be created without slits as well. These small volume containers can be used to characterize reaction systems and material organization in a fluid environment. They can also be used to “self assemble” reaction systems. Further, the device allows for controlled transport between the local environment and the contained volume through the synthetic membrane. Cell-free transcription and translation reactions conducted in this container would be useful for producing functional proteins as needed for biomedical applications.

Reported here is the use of picoliter volume containers to conduct cell-free protein synthesis. Further, the optimal DNA concentration and the time required for maximal protein yield in the device is determined.

II. Materials and Methods

A. Device Fabrication

Reaction containers were fabricated using semiconductor processing techniques to define membrane characteristics. Feature fabrication was achieved using electron beam and optical lithography, followed by cryogenic etching and plasma enhanced chemical vapor deposition (PECVD) of silicon dioxide. Each fabrication run, results in a silicon wafer with 8 completed chips. Each chip consists of 2 channels with 15 containers per channel (Fig. 1A, B). For the experiments described here, the resulting containers have a volume of ~ 19 pL and are made with either no slits or 200nm slit sizes.

The Sylgard 184 silicone elastomer kit was used to prepare Polydimethylsiloxane (PDMS) which is used to seal the top of the devices. Sylgard 184 silicon base and the curing agent were mixed together in a Petri dish at a 10 to 1 ratio, respectively. The homogeneous mixture was degassed in a desiccator for ~ 30 min until the air bubbles were removed and was later placed in the oven at 70°C for 60–90 minutes. After baking, PDMS was cut into pieces in the shape of the chip and two holes were created on each end of the channel to allow for the input and output of the reaction mixture (Figure 1C).

B. Cell Free Translation

Escherichia coli (*E. Coli*) T7S30 Extract System for Circular DNA reaction kit (Promega TB219) was used for transcription/translation experiments according to the manufacturer's directions. For the template, enhanced green fluorescent protein (eGFP) gene was cloned into pDEST17 (Invitrogen) which allows expression of eGFP from a T7 promoter. The solution containing the DNA template and the cell free transcription/translation mix was mixed in a 1:1 ratio with 8 % glycerol and placed in the cell mimic using a glass tip with 2 μm orifice (World Precision Instruments, TIP2TW1). After the structures were loaded, the chip was covered with a 5 mm thick layer of PDMS. The channel was then filled with *E. coli* S30 cell free extract and small metabolites. The experiment was incubated at 37°C. EGFP fluorescence was visualized using a Zeiss Axioskop 2 FS Plus epifluorescent microscope.

A similar procedure was also followed for cell-free transcription/translation in containers containing no slits. In this case, the DNA template was mixed with *E. coli* T7S30 cell free extract and small metabolites in a 1:4 ratio, and a final 4 % glycerol concentration. The entire reaction mixture was then loaded into the device using a 2 μL glass microtip (World Precision Instruments, TIP2TW1). Several mimics were filled with *E. coli* T7S30 cell free extract and were used as negative controls. The chip was then covered with PDMS and fluorescence was measured. The experiment was incubated at 37°C.

In order to compare cell-free translation in the device with conventional scale reactions, cell-free translation was also conducted in a Costar 96 flat bottom well plate. The concentrations and temperature were kept the same as in the device reactions. The final reaction volume in the plate well was 50 μL and fluorescence was measured every 10 minutes using a Perkin Elmer HTS 7000 Plus BioAssay Reader.

C. Characterization

A GFP standard was prepared by diluting purified GFP protein to concentrations of 26.2, 13.1, 6.55, 3.275, 1.64, 0.82, 0.41, 0.205 and 0.103 μM GFP. Each of the GFP solutions contained 4 % glycerol concentration. These solutions were loaded in no pore devices and fluorescence was measured using identical settings for devices with no pores or with 200nm pores. GFP concentration produced in the cell-free transcription/translation reactions in the no slit and 200 nm slit size mimics was measured against this GFP standard.

For all the experiments, images were taken every 10 minutes using a dry 40 $\times$ objective at an exposure time of 500 milliseconds. A 3CC syringe with a 19 gauge needle was used to flow the reaction mix into the channel.

III. Results and Discussion

To test the ability of the microfabricated picoliter volume containers to carry out biochemical reaction systems, cell-free protein synthesis experiments using no slits and 200 nm slit size devices (Fig. 3) were carried out. Due to the low viscosity of the reaction mix and the small volume of the container (19 pL), we encountered several difficulties when loading the device. These difficulties consisted of overflowing the device at the time of loading, air bubble formation inside the device and quick drying of the sample immediately after loading. In order to overcome these difficulties, the reaction was mixed with different solutions that altered viscosity and evaporation time. The solutions that were evaluated include: polyethylene glycol (PEG), 1% alginate, glycerol, dimethyl sulfoxide (DMSO), agar, dimethylformamide (DMF) and several sucrose concentrations. All these solutions increased viscosity of the reaction mix making it easier to load the devices. Evaporation of the reaction mix after loading the device was also overcome. Protein production was also

evaluated and it was significantly decreased. In many cases it was completely inhibited. Alginate was the only solution of those tested that increased protein yield, however it was difficult to handle when loading the device as cross-linking of the mixture would often occur. The most useful solution which did not significantly decrease cell free transcription/translation was glycerol. There was a small decrease in protein yield when using glycerol, but the increased viscosity made it easier to load the device and air bubble formation was avoided. Therefore, several glycerol concentrations were tested and the lowest one which still allowed effective loading of the devices and avoided evaporation was determined to be 4% glycerol concentration.

Cell-free transcription/translation experiments were successful for small volume containers that contained no slits and 200 nm slits (Fig. 3). The reaction yield was consistent in the no slit devices and the optimal DNA concentration was determined. It was found that 90 ng/ μ L DNA concentration had the highest GFP yield (Figure 4) followed by 75 ng/ μ L. The other concentrations tested had a similar GFP yield of $\sim 0.1 \mu$ M. When compared to experiments carried out at the conventional scale (50 μ L), a plateau was reached at template concentrations greater than 60 ng/ μ L (Fig. 4).

Cell-free transcription/translation in 200nm slit size cell mimic devices was more difficult to achieve and typically GFP was produced throughout the entire microfluidic channel. This could be caused by (a) GFP leaking out of the device through the slits after it was produced or (b) the mRNA leaking out and translation occurring in the channel instead of only in the device. GFP observation in the channel was eliminated when the buffer was flowed through the channel using gravity. Under these conditions, GFP was detected only in the reaction chamber (Fig. 3a). A better understanding of the containment properties of the chamber is needed. Additional experiments with fluorescent beads or fluorescently tagged proteins of different sizes will need to be conducted in order to understand the containment properties of the device. An evaluation of containers with smaller slit sizes is also needed. Properties that limit the escape of mRNA from the device, contain GFP and allow transport of small molecules (i.e. amino acids and ATP) would be ideal.

IV. Conclusion

Nanotechnology based devices are finding inspiration from biological systems. Shown here are cell mimic devices that can be used to carry out basic biochemical reactions that are essential for cell survival. In the future this device can be used to probe and manipulate biological systems and to perform new functions. It can also be used to carry out enzymatic reactions in order to better understand molecular systems and self organization at the micro scale.

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Fig. 1.
Wafer with several chips and sealed device.

Fig. 2. Scanning Electron Microscope (SEM) images of the device (A) Container with no slits (B) Container with 200 nm slit size (C) 200 nm slits

Fig. 3.
Cell-free translation in cell mimic devices.
(A) Container with 200nm slit size (B) No slit container.

Fig. 4. Cell-free protein expression in (A) plate reader and (B) no slit device