Cell-free protein expression in a microchannel array with passive pumping

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We report in vitro (cell-free) protein expression in a microfluidic device using passive pumping. The polystyrene device contains 192 microchannels, each of which is connected to two wells positioned in a 384-well microplate format. A larger droplet of an expression solution was placed at one well of each channel while a smaller droplet of a nutrient solution was at the other well. Protein expression took place in the larger droplet and we found the expression yield in the expression solution is enhanced due to the replenishment of the nutrient solution supplied by passive pumping via the channel. The pumping pressure was generated from the difference in the surface tension between two different sized droplets at the two wells. We demonstrated expression of luciferase in the device and the expression yield was measured using luminescence assay. Different experimental conditions were investigated to achieve maximum protein yield with the least amount of reagents. Protein expression yields were found to be dependent on the amount of the nutrient solution pumped, independent of the amount of the expression solution within the experimental conditions studied. A higher feeding frequency or delivery rate of the nutrient solution resulted in higher protein expression yield. The work demonstrated the feasibility of using the microchannel array for protein expression with the following advantages: (1) simultaneous production of the same protein with different conditions to optimize the expression process; (2) simultaneous production of different proteins for high-throughput protein expression with high yield; (3) low reagent cost due to the fact that it consumes 125–800 times less than the amount used in a protein expression instrument commercially available.

Introduction

Proteins are important to life, since they regulate most molecular and cellular functions. As a result, it is often necessary to produce proteins for studying their molecular structures and functions. A variety of protein expression (biological synthesis) systems have been developed to synthesize proteins, including E. coli, yeast, insect, and cell-free protein expression systems.1

The cell-free protein expression method is appealing for high throughput protein production, because it has an ability to generate milligram quantities of proteins in hours and produce proteins that cannot be expressed in vivo due to their toxic effect on the physiology of the cell.2 Proteins expressed in vitro have been shown to have the same structures and characteristics as those produced in vivo, making cell-free protein expression a viable technique.1 In addition, cell-free protein expression simplifies protein purification and allows expression of multiple functional proteins in parallel.

Different configurations have been investigated to maximize the amount of proteins produced in cell-free protein expression systems.2,3 One is the batch process, in which the protein synthesis machinery and other reagents are mixed together in one container. The second one is the continuous flow configuration, in which nutrients (e.g., amino acids and adenosine triphosphate (ATP)) are continuously pumped to the expression container while byproducts (e.g., hydrolysis products of triphosphates) are removed with the pumped fluids.3 This approach leads to higher protein expression yield than the batch process because protein synthesis will not terminate earlier due to fast depletion of energy sources (ATP) and the removal of the byproducts eliminates possible inhibition of the biochemical reactions. Continuous supply of the nutrients for high-yield protein expression is similar to supplying nutrients to biological cells for their long-term viability. An alternative to the continuous flow configuration is continuous-exchange cell-free (CECF) protein expression, in which the addition of nutrients and the removal of byproducts are achieved by exchanging the solutions in two chambers separated by a dialysis membrane.5

Protein expression using the CECF configuration has also been implemented in microfluidic devices and miniaturized chambers.1,2 A dialysis membrane was used to separate reaction and nutrient chambers fabricated in poly(methyl methacrylate) or polydimethylsiloxane.3 A bilayer to separate transcription and translation reagents has also been studied in a microplate.4 Microreactors have also been fabricated in a silicon wafer to carry out cell-free protein expression.15,16 Water-in-oil emulsion is another approach to achieve compartmentalization of two solutions for in vitro protein synthesis.17,18

In this work, we implemented cell-free protein expression in a microchannel array that has characteristics of continuous flow and CECF configurations. The ports of the device are in a format of a 384-well microplate with 192 microfluidic channels.
connecting pairs of ports. When a larger droplet of the protein expression solution is placed at the outlet of a channel and a smaller droplet of the nutrient solution at the inlet, passive pumping occurs driving the fluid from the inlet through the channel to the outlet because of the difference in the size of these two droplets and their surface tensions.\(^{19-21}\) This pumping mechanism allows for continuous supply of nutrients from the inlet to the outlet where protein expression takes place.\(^{22}\) The byproducts are also diluted, reducing possible inhibitory effects. The array format allows simultaneous production of the same protein with different conditions, facilitating screening for the optimum expression conditions. It also enables simultaneous production of different proteins for high-throughput protein production, matching high-throughput gene discovery. We utilized the device for the expression of luciferase and studied the effects of different experimental conditions on the protein expression yield. The work demonstrated the feasibility of using the microchannel array for high-throughput protein expression with high expression yield. More than 2 orders of magnitude of savings in the reagent cost can be achieved since the microchannel array consumes 125–800 times less than the amount used in a protein expression instrument commercially available.

### Experimental

#### Reagents and materials

RTS 100 wheat germ kits were purchased from Roche (Manhein, Germany). Each kit consists of wheat germ lysis buffer, reaction mix, feeding mix, amino acids, and methionine. T7 luciferase DNA vector, luciferase assay reagent, and nuclease free water were obtained from Promega Corporation (Madison, WI, USA). Purified water for rinsing devices was obtained from a Barnstead Nanopure water system (Dubuque, IA, USA).

#### Device fabrication

The microchannel arrays were fabricated by Edge Embossing, LLC (Medford, MA, USA) using the following process flow. The desired microstructures were created in an acrylic master using CNC milling. Next, a soft elastomer tool was cast from the acrylic master and resin pellets of polystyrene (PS 168 N, Frantschach Rothrist AG) were placed in the soft tool cavity. A combination of temperature and pressure were applied to ther-moform the resin. The embossed polystyrene part was then exposed to a 20% solution of dichlormethane for 1 min, rinsed in ethanol, and then placed in contact with a Nunc OmniTray. A second temperature and pressure cycle was then performed on the assembly to induce solvent-assisted thermal bonding.

#### Protein expression

Luciferase was produced using a RTS 100 kit. A protein expression solution and a feeding (nutrient) solution were prepared by following the protocols recommended by the manufacturer. The protein expression solution consists of 15 \(\mu\)L wheat germ lysis buffer, 15 \(\mu\)L reaction mix, 4 \(\mu\)L amino acids, 1 \(\mu\)L methionine, 2 \(\mu\)L T7 luciferase DNA vector, and 13 \(\mu\)L nuclease free water. The nutrient solution contains 900 \(\mu\)L feeding mix, 80 \(\mu\)L amino acids, and 20 \(\mu\)L methionine. The production of luciferase using this kit has been implemented in conventional containers or devices as described previously.\(^{11,12}\)

To implement protein expression in the passive pumping device (Fig. 1), a channel was first rinsed once with purified water. Protein expression solution (4–8 \(\mu\)L) was then pipetted into the outlet of the channel; the solution flowed through the channel towards the inlet due to pipetting and the capillary force. The inlet was then filled with an appropriate amount of the nutrient solution using a pipette. Note that the amount of the nutrient solution at the inlet was always less than the amount of the expression solution at the outlet so that passive pumping took place.

In addition to using a pipette, we also employed a syringe pump for experiments that required a continuous supply of the nutrient solution. A syringe pump (UltraMicropump II) from World Precision Instruments Inc. (Sarasota, Fl) was connected to the solution droplet at the inlet via a syringe needle. Note that the syringe pump is for continuous delivery of the nutrients, not for pumping it through the channel. The delivery speed (33–1000) nL/s was set to maintain the droplet size at the inlet smaller than that at the outlet.

At the end of every experiment, the droplet of the expression solution at the outlet (as well as most solution in the channel) was collected using a pipette. There was no droplet of the nutrient solution at the inlet at the end of every experiment since the inlet droplet disappeared within a couple of seconds (due to passive pumping to the outlet). The amount collected depended on the amount of the initial expression solution, the amount of nutrient solution pumped, and the duration of the experiment. A fixed amount (5 \(\mu\)L) of the collected sample was used to assay the concentration of luciferase expressed in each experiment.

The assay was performed using a Mithras microplate reader (Berthold Technologies, Germany). Each of the collected samples (5 \(\mu\)L) was added to a well in a standard 384-well microplate. The plate was then placed in the reader, which was programmed to inject 35 \(\mu\)L of luciferase assay reagent, followed by a 2 s shake. The luminescence signal of the following 10 s was measured and reported by the reader, and it is correlated to the amount of luciferase.
Results and discussion

Device design and protein expression

A picture of the device is shown in Fig. 1a, in which there are 192 channels and 384 wells. Each pair of two adjacent wells is connected by one channel. The distance between the well centers is 4.5 mm, following the microplate standards defined by the Society for Biomolecular Screening (SBS) and accepted by the American National Standards Institute.

As indicated in Fig. 1b, one well of each channel is designated as an inlet while the other well is an outlet. A larger droplet is first placed in the outlet, followed by a smaller droplet in the inlet. As explained previously, the difference in the size of the two droplets at two wells generated a pumping pressure because of the surface tension in their curved surfaces. As a result, a flow was produced in the channel from the inlet to the outlet, as illustrated in Fig. 1b. Note that the flow was continuous; it stopped when the inlet droplet shrank to a slightly curved shape that was at equilibrium with the outlet droplet.

When the protein expression solution was placed in the outlet, protein synthesis took place as shown in Fig. 1c. Protein expression consisted of two steps: a DNA template consisting of a coding sequence is transcribed into a messenger RNA; and the RNA is then translated into the corresponding protein. The transcription and translation steps were coupled together and occurred in the same reaction mixture. When the nutrient solution was added into the inlet either intermittently or continuously, nutrients were supplied through the channel to the outlet. Continuous supply of the nutrients enabled the protein expression to continue for a long period of time, in an analogy to supplying nutrients to biological cells for their long-term viability.

Fig. 2 shows the luciferase expression yield in the passive pumping device. The yield is indicated by the luminescence signal in relative light units (RLU) per second from the microplate reader when 5 μL of the collected droplet at the outlet was assayed using luciferase assay reagent as described in the Experimental section. The error bars were obtained from four repeat experiments in different channels in the same device, indicating the channel-to-channel variation as well as the inherent protein expression variation. In these experiments, 6 μL of the protein expression solution was initially pipetted into the outlet, followed by filling 4 μL of the nutrient solution. After passive pumping of the nutrient solution from the inlet to the outlet and 10 min of reactions, another 4 μL of the nutrient solution was added into the inlet using a pipette; this step was repeated every 10 min. A total of 12 μL of the nutrient solution was added into the inlet while the whole experiment lasted for 30 min. Note that the droplet size of the nutrient solution at the inlet was always smaller than the droplet size of the expression solution at the outlet so that the passive pumping took place. Both droplets were exposed to air, so that evaporation occurred at the same time. However, evaporation was contained to a large degree since the device was covered with a lid that came with the device.

For comparison, we did the same experiments in a microcentrifuge tube. The same amount (6 μL) of the protein expression solution was used; and the reaction was allowed to last for the same period of time (30 min). And 5 μL of the expression products was assayed in the same way. As shown in Fig. 2, the expression yield in the microcentrifuge tube was 4.9 times lower than in the device. The results suggest that the additional nutrients supplied by passive pumping in the device prolonged the biochemical reactions and resulted in higher protein expression yield. Note that simply mixing 6 μL of the expression solution with 12 μL of the nutrient solution in a tube led to a 4.1 times increase in expression yield (compared to the same amount of the expression solution in a tube without the nutrient solution). Incorporation of a dialysis membrane in a well-in-well device described previously could result in a 121 time increase in expression yield, though a much larger amount of nutrient solution (200 μL) and a larger amount of expression mix (10 μL) were used in that case.

Nutrient solution amount

We then quantitatively studied the effects of the amount of nutrient solution on the protein expression yield. The protein expression solution initially pipetted into the outlet remained the same at 6 μL. However, the amount of nutrient solution initially added into the inlet and added consecutively afterwards was 1, 2, 3, or 4 μL at a frequency of once every 10 min (i.e., a total of 24 μL was added in one hour for the 4 μL treatment). They were added into the inlet using a pipette. Each experiment lasted for one hour; the same reaction time was used for all experiments. Fig. 3 shows the protein expression yield as a function of the amount of the nutrient solution. The result indicates that the protein expression yield exponentially increased with the amount of the nutrient solution in the range of the experimental conditions studied. The larger the amount of the nutrient solution, the greater the quantity of the nutrients for protein expression, and the higher the protein expression yield.

It is worth noting that the amount of the nutrient solution could not be significantly larger than 4 μL without causing the droplet at the outlet to deform, disrupting the passive pumping mechanism. In addition, too large amount of the nutrient solution could lead to a larger droplet at the inlet than at the outlet, causing the fluids to move in the opposite direction, i.e. from the outlet to the inlet through the same passive pumping mechanism.

To address the concern of adding too much solution at once and to maximize the effect of the nutrient solution amount, we
This journal is either the passive pumping devices (i.e., the number of adding the nutrient solution per unit time) on the protein expression yield. In all experiments, the total amount of nutrient solution is the same over the same period of time. However, the amount added each time and the time interval between feedings were different. Fig. 5a shows the protein expression yield as a function of the feeding frequency. The studied feeding frequencies are 0.0067 Hz (once every 2.5 min), 0.0033 Hz (once every 5 min), and 0.0017 Hz (once every 10 min). The nutrient amounts are 1 μL, 2 μL, and 4 μL, respectively. As a result, the total amount of the nutrient solution added was 24 μL in one hour for all three cases. The results in Fig. 5a suggest that protein expression yield is higher at a higher feeding frequency while there is no significant difference between low frequency feedings.

The increase in the expression yield at a higher feeding frequency is likely a result of increased mixing when the nutrient solution was used is a characteristic of the reactions, rather than the device.

To investigate if the trend observed is a result of the device, we studied the same experiments in microcentrifuge tubes. Different amounts of the nutrient solution were added to the same amount (6 μL) of the expression solution. The mixture was also allowed to react for 30 min and the luminescence was then measured. Fig. 4b shows a similar trend as in Fig. 4a, suggesting that the decrease in the protein expression yield when too much nutrient solution was used is a characteristic of the reactions, rather than the device.

The phenomenon can be explained by the increased dilution of the protein synthesis machinery when too much nutrient solution was added. Keeping the reactions for a long period of time while allowing the additional nutrient solution to diffuse slowly into the expression mixture will not significantly dilute the expression synthesis machinery at any moment, resulting in higher protein expression yield. However, too much nutrient solutions at once will dilute the expression synthesis machinery, likely slowing the reaction kinetics. This explains the difference between Fig. 3 (the nutrient solution supplied over time) and Fig. 4 (when the syringe pump was supplying the nutrient solution too fast).

Another plausible explanation is related to the different roles played by catalysts, for example, magnesium ions (Mg²⁺). It has been reported that the amount of Mg²⁺ required for transcription (DNA to mRNA) is higher than for translation (mRNA to proteins) in the wheat germ expression system. As a result, the Mg²⁺ concentration in the expression solution is higher than in the nutrient solution in the kits, reflecting the fact that transcription takes place prior to translation. Adding too much nutrient solution to the expression solution reduces the concentration of Mg²⁺, resulting in the reduction in the transcription yield and accordingly the overall expression yield. It is possible to solve the issue by adding an extra amount of Mg²⁺ to the nutrient, but it will likely have adverse effects on translation. These discussions signify the advantage of using the device to carry out protein expression because it allows the additional reactants to pump slowly into the reaction mixture, keeps the reactions for a longer period of time, and leads to higher protein expression yield.

Feeding frequency and delivery rate

Since the amount of the nutrient solution has an effect on the protein expression yield, we studied the feeding frequency (i.e., the number of adding the nutrient solution per unit time) on the protein expression yield. In all experiments, the total amount of nutrient solution is the same over the same period of time. However, the amount added each time and the time interval between feedings were different. Fig. 5a shows the protein expression yield as a function of the feeding frequency. The studied feeding frequencies are 0.0067 Hz (once every 2.5 min), 0.0033 Hz (once every 5 min), and 0.0017 Hz (once every 10 min). The nutrient amounts are 1 μL, 2 μL, and 4 μL, respectively. As a result, the total amount of the nutrient solution added was 24 μL in one hour for all three cases. The results in Fig. 5a suggest that protein expression yield is higher at a higher feeding frequency while there is no significant difference between low frequency feedings.

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solution is frequently supplied. At a higher feeding frequency, a smaller amount of nutrient solution was supplied in a shorter time interval, resulting in a smaller droplet at the inlet that caused an increase in the pumping pressure and the flow velocity according to Berthier and Beebe. A higher flow velocity enhanced the degree of mixing. More mixing is known to improve the reaction kinetics and increase cell-free protein expression yield.

To confirm the effect of the feeding frequency, we also investigated using the syringe pump as the feeding approach. The delivery rates of the nutrient solution ranged from 33 nL/s to 1000 nL/s, and the delivery time varied to maintain that the total nutrient solution pumped was 12 μL. Protein expression reactions were allowed for 30 min after the initiation of delivery. Fig. 5b shows the protein expression yield as a function of the delivery rate. The result indicates that protein production yield increased initially with the delivery rate of the nutrient solution. Similarly, the observation can be explained by the increase in mixing at a higher delivery rate.

**Expression solution amount**

Since one of the goals of this research is to achieve the maximum protein production with the least amount of reagents and cost, we studied the effects of the amount of the expression solution on protein production. Different amounts of the expression solution were placed in the outlets while the same amount of the nutrient solution was used in the inlets. The nutrient solution (12 μL) was added using a syringe pump with a delivery rate of 1000 nL/s. Fig. 6 shows the protein expression yield as a function of the amount of the expression solution. The results indicate there is no significant difference among experiments when different amounts of expression solution were used, at least within the range of the experimental conditions we used. Statistical analysis using ANOVA (analysis of variance) indicates that the luminescence signals in Fig. 6 are the same at the 95% confidence level for different amounts of the expression solution. The results are significant since we can use a smaller amount of the expression reagents for protein production as long as enough amount of the nutrient solution is used.

It should be useful to compare the amount of the reagents used in this device with those required in a commercial CECF machine called Rapid Translation Systems (RTS). In this work, we used 5–8 μL of the expression solution; this volume is 125–200 times less than 1 mL of an expression solution in RTS 500. Similarly, the volume of the nutrient solution is 12–24 μL, which is ~400–800 times less than 10 mL of a feeding solution in RTS 500. The decrease in the volume of both expression solution and nutrient solution will significantly reduce reagent consumption when using cell-free protein expression for high-throughput assays. As a result, the device will be extremely useful in situations where a large number of proteins need to be screened and the amount of proteins produced in the device is sufficient for assays.

Comparison of the results in Fig. 6 with that in Fig. 3 indicates that the amount of nutrient solution has a greater effect on the protein production than the amount of expression solution. This finding is in agreement with incorporation of microfluidic channels for supplying nutrients, as well as in agreement with the reports using continuous flow or CECF configurations.

**Conclusion**

Production of luciferase using a cell-free expression system was demonstrated in a microchannel array device. Cell-free protein expression systems address the challenges encountered in cell-based methods, including reagent and facility cost, time and labor consumption, and difficulty in separating proteins of interest from other cellular proteins. This protein production method will be useful in proteomics studies, drug discovery, and fundamental scientific and biomedical research.

Passive pumping in a microchannel is a method meeting the requirements of cell-free protein expression. The nutrient
solution in a smaller droplet is passively pumped through a channel to the protein expression solution in a larger droplet, achieving continuous replenishment of nutrients. As a result, protein expression yield is enhanced by ~5 times. Using luciferase expression as an example, we studied the effects of different experimental conditions on the protein expression yield. We found that the expression yields are dependent on the amount of nutrient solution supplied, and independent of the amount of expression solution within the experimental conditions studied. A higher feeding frequency or a higher delivery rate of the nutrient solution resulted in higher protein expression yield. These results will help achieve maximum protein yield. In addition, we showed more than 2 orders of magnitude of savings in the reagent consumption than a protein expression instrument commercially available.

The protein expression device is in a 384-well microplate format, which is compatible with commercially available reagent dispensers and microplate readers. As a result, the method and device are adaptable to high-throughput protein production. In addition, we demonstrated an acceptable repeatability among the experiments in different channels. Therefore, the device may provide a way to achieve protein expression in parallel, with high yield and low cost.

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References