MICROFLUIDIC DEVICE FOR COMBINATORIAL PROTEIN REFOLDING
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ABSTRACT
We have developed a microfluidic device that has the unique capability of automating the combinatorial process of finding solution conditions for protein refolding. The microfluidic approach offers potential advantages of automation and cost-effectiveness as opposed to conventional techniques of hand-pipetting or using robotic systems. The device is a multi-layered Poly(dimethylsiloxane) (PDMS)-glass device with automated controls for reagent aliquoting and mixing. Refolding experiments have been performed on the protein β-galactosidase and refolding yield has been quantified on-chip using fluorescein di-β-D-galactopyranoside (FDG), a caged-fluorescent molecule. The main impact of this work is on greatly reducing the cost of realizing protein pharmaceuticals.

KEYWORDS: Protein Refolding, β-Galactosidase, Combinatorial, Microfluidic

INTRODUCTION
This work presents a microfluidic device that has the unique capability of automating combinatorial processes like protein refolding and bioconjugate synthesis. Conventionally, these processes are done by hand-pipetting or using robotic systems. The microfluidic approach offers the advantages of automation, cost-effectiveness, compatibility with optical detection, and a million-fold reduction in sample volumes.

In this work, we develop a device designed specifically for protein refolding applications. Protein refolding has been a bottleneck in the production of biopharmaceuticals on a large scale. In order to maximize the production of proteins, bacterial hosts are induced with promoters, resulting in the production of aggregated proteins. The process of refolding, which involves the conversion of these inactive aggregated proteins into their functional native state, is a highly empirical process. Determination of solution conditions to properly refold a protein is a combinatorial process which can be automated using our microfluidic device.

β-GALACTOSIDASE REFOLDING
Refolding experiments are performed on the protein β-galactosidase, which is an essential enzyme in the human body that catalyzes the hydrolysis of β-galactosides like lactose into monosaccharides. Denaturing studies on the protein have shown that the protein can be completely denatured by solubilizing in 8M urea [1]. The solubilized protein along with the denaturants is then diluted in a renaturing medium containing 1.4M urea and Mg$^{2+}$ ions that aid refolding [1]. The refolding yield is quantified on-chip using the nonfluorescent substrate fluorescein di-β-D-galactopyranoside (FDG) which is hydrolyzed to fluorescein by the protein. A
combination of artificial chaperones can be combinatorially explored to improve the refolding yield in subsequent trials.

Reagent aliquoting and mixing is achieved using microfluidic control [2]. The device consists of two layers of PDMS on a glass slide. The schematic of the channel layout is shown in Figure 1. The layout consists of input channels for protein and reagent solutions, an annular mixer and an output channel for the refolded protein. High pressure air is passed through control channels to control fluid flow in flow channels. The device is integrated with a control unit which enables automation of the processes (Figure 2).

Figure 1(a): Schematic of the channel layout showing flow and control channels (b): Peristaltic pump action in the device. (c): Valve action in the device. (1 – 5 mm thick flow layer made of PDMS; 2 - 30 μm thin control layer made of PDMS; 3 – Glass).

RESULTS AND DISCUSSION

Peristaltic pumps present on each flow channel allow precise control of the amount of fluid through the channel. The mixing time of reagents in the annular mixer is calculated from dye-water mixing experiments as 45s (figure 3). The volume injection rate determined by monitoring the interface between water and dye in a flow channel is calibrated against the frequency of actuation of the control channels as shown in figure 4.

The refolding protocol and the fluorometric assay for β-galactosidase are optimized for best on-chip signal intensity. The fluorescence intensity is then calibrated as a function of β-galactosidase concentration in a PDMS microchannel (figure 5). Finally, the active protein content in denatured and refolded protein samples is quantified on-chip using the obtained calibration curve (figure 6).

Figure 2: Microfluidic device integrated to an eight-valve manifold through tygon tubing. The size of the glass slide to which the PDMS layers are bonded is 2"x3". This manifold is controlled through a Fluidigm circuit which is operated using a LabView interface.

Figure 3: Snapshot of the device during mixing of water and dye.
Figure 4: Volume injection rate in a flow channel versus frequency of actuation of the control channels.

Figure 5: Calibration curve of β-galactosidase concentration versus fluorescence intensity in a PDMS microchannel.

Figure 6: Micrograph of device filled with denatured and refolded β-galactosidase samples. Activity is quantified using a fluorescence-based assay. The bar graph shows the fluorescence intensities of the denatured and refolded samples (n=3).

CONCLUSIONS

In conclusion, by integrating the control capabilities with the PDMS device, we can facilitate automation of combinatorial protein refolding protocols. Successful devices will greatly reduce the cost of producing protein pharmaceuticals.

REFERENCES
