A SELF-DISPENSING MICROFLUIDIC CHIP WITH THE OSMOTIC DEWATERING METHOD FOR NANOVOLUME CHEMISTRY AND PROTEIN CRYSTALLIZATION

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ABSTRACT

We present a self-powered and osmosis-based microfluidic crystallizer (SOMC) for multiplexed nanoliter-scale crystallization experiments by combining a sample with multiple different dewatering rates. Powered by pre-evacuation of its PDMS substrate, SOMC does not require any external pumps, connections, or tubing to transport fluid. In addition, by integrating a row of dialysis channels which are filled with different concentration of salt solution, SOMC can simultaneously screen a wide range of supersaturation for rapid optimization of protein crystallization. The feasibility of this crystallization platform is demonstrated using the model proteins of lysozyme.

KEYWORDS: Crystallization, Nanovolume, Osmotic Dewatering, Self-Dispensing

INTRODUCTION

In recent years, a variety of microfluidic devices have been developed and applied to nano-volume crystallization screening [1]. However, most of the devices suffer from a certain complexity of the pumping and valving of fluids with multi-layer channel networks or sophisticated liquid handling instruments [2, 3]. Moreover, the screening strategies in these devices mainly depend on varying the ratio of protein to precipitant, which offer limited capabilities for controlling supersaturation levels, and thus yield low crystallization hit rates.

In this paper, we report a simple microfluidic device which incorporates the attributes of automation, high throughput, osmotic pressure gradient and kinetic control for rapid screening and optimization of protein crystallization conditions. This device consisting of PDMS and glass uses degassed PDMS as an internal pumping source to dispense nanoliter liquid into arrays of microwells. This passive pumping method allows simple and reliable fluid handling without requiring external power and tubing. Meanwhile, an array of osmotic microchannels filled with different concentration salt solutions is integrated under the addressable crystallization microwells. Each well is in contact with an osmotic microchannel via a semipermeable PDMS membrane through which only water and other low-molecular-weight organic solvents can pass, but not salt, polymer, or protein. This enables the rate of supersaturation in a solution to be easily, rapidly, and precisely varied.

Figure 1. Schematic of the microdevice. (a) Exploded 3D-view; (b) Top view.

EXPERIMENTAL

Microdevice. A schematic diagram of the SOMC is shown in Figure 1. The device, made of PDMS, consists of a flow channel layer, a thin membrane layer, and a dewatering control layer. The flow channel layer contains a set of eight parallel
microfluidic channels. Each channel is connected with a row of storage wells which are located to the side of the microchannel and a pump chamber which located to the end of the microchannel. The dewatering control layer has twelve independent, straight microchannels. These microchannels are filled with different concentration salt solution. The thin membrane layer serves as a semi-permeable barrier to separate the stored protein drops from the lower osmotic pressure solution and control loss of water from a crystallization drop for increasing supersaturation.

The device was fabricated through the well-established multi-layer soft lithography process. Firstly, two master molds were fabricated by standard photolithography techniques on 3” silicon wafers. The flow channel layer was prepared from a multi-layer SU-8 process consisting of two-level features: 15µm high features for the flow channel and 100µm high features for the storage wells and the pump chambers. The dewatering control master is a single-layer mold consisting of 200 µm high features. After the completion of the master molds, patterned PDMS layers were fabricated using the procedure developed previously [4]. Briefly, prepolymer of PDMS was cast onto the master with a frame for holding the prepolymer, and then a PET film was place on the top of the frame with PDMS prepolymer for fabricating double-side flat PDMS pad. The cured PDMS slabs were peeled off from the masters, and punched for inlet/outlet ports. Thin membrane layer was spun on silicon wafers and cured. After that, the flow channel PDMS layer and the dewatering control PDMS layer were aligned face to face with sandwiched PDMS membrane layer. Finally, the three layers were assembled by plasma bonding and placed on a glass wafer.

**Operation.** All outlets of the microdevice were sealed with adhesive tape. Then the device was placed in a vacuum desiccator, and degassed at 10 kPa for 1h. After the microdevice was brought back to atmosphere, a two-step dispensing process was performed. Firstly, twelve droplets of salt solution with different concentration were added separately by micropipette to the inlets of the dialysis channels of the SOMC. After all dialysis channels had been filled with salt solution, aliquots of eight different protein samples were then dispensed into the protein inlets and also aspirated into the microchannels and microwells by the degassed PDMS. Once all microwells had been filled with protein solution, a few drops of paraffin oil were added into the protein inlets. The oil follows the aqueous plug, removes the aqueous phase in the main channel and thereby isolates the aqueous phase in the wells. After the completion of dispensing process, each reservoir was sealed with a drop of paraffin oil for preventing evaporation.

**Crystallization experiments.** For the experiments described here, lysozyme (40 mg/mL in 0.02 M sodium acetate buffer, pH 4.6) was used as model protein to investigate the application of the microfluidic device in protein crystallization. After introducing and dispensing the mixture of the protein and the precipitants, the device was preserved in a refrigerator at 4 °C. The microwells were examined by an inverted microscope (IX-51, Olympus, Japan) at regular intervals of one hour. Optical micrographs were obtained using a CCD camera (DP70, Olympus, Japan).

**RESULTS AND DISCUSSION**

**Nanoliter Liquid Dispensing.** First, we tested the self-dispensing ability of the degassed SOMC with a food dye. When aliquots of dye solution were added in all the inlets of the SOMC, the air flow to the PDMS microchannel was blocked, and thus a negative pressure was generated inside microchannels due to the air dissolution into pre-evacuated PDMS substrate. As a result, the dye solution was automatically sucked into the microchannels and gradually filled up the whole vacancy of the closed system. Figure 2a clearly shows that the entire crystallization well was filled with the solution and no trace of air bubbles was observed after completion of the loading process. Subsequently, oil was introduced into the main channel to remove the excess liquid, and droplets of precise volumes were left in the crystallization microwells (Figure 2b).

**Permeation of Water.** To simultaneously screen a wide range of supersaturation for rapid optimization of protein crystallization, an array of addressable storage microwells incorporated with a row of dialysis channels which are filled with different concentration of salt solution (i.e. different osmotic pressure solution) are employed. A dewatering experiment was performed to ascertain that the SOMC had the controllability over the water contents of the drop. As shown in figure 3,
aqueous droplets gradually shrank due to water diffusion through PDMS membrane from droplets of low concentration to reservoir containing high concentration salt. Furthermore, it also indicates that the dewatering rate of drop stored in a crystallization well increases as the concentration of the salt solution filled in the dialysis channel increases.

**Protein crystallization.** To illustrate the power and practicability of this device, we applied it to high-throughput screening of protein crystallization conditions. Micrographs of protein crystals formed in the microdevices are shown in Figure 4. Obviously, crystal has tendency to form twins and irregular shape at the high dewatering rate. It is supposed that the low dewatering rate favors the growth of good single crystals.

![Figure 3. Water loss sequence under different osmotic dewatering rates.](image)

![Figure 4. Lysozyme crystal growing sequence under different osmotic dewatering rates.](image)

**CONCLUSION**

In summary, we have developed a simple microfluidic device integrated with self-dispensing function and gradient control of the solute concentrations inside arrays of droplets, and illustrated the feasibility of the device by applying it to protein crystallization. The device has a simple micropipette-accessible interface and requires no external power and connections for dispensing samples and controlling evaporation. Thus this power-free monolithic device is well-suited for rapid, flexible and economic screening of protein crystallization conditions.

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