A SELF-LOADING, SELF-METERING AND SELF-MIXING MICROFLUIDIC REACTOR ARRAY FOR BIOCHEMICAL SCREENING

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ABSTRACT
A power-free, self-contained microfluidic reactor array is developed for high-throughput analyses. This device needs neither external power nor complex instruments for fluid handling. Thus, it offers an easy-to-use and inexpensive way to perform multiple nanoliter-volume distinct reactions in parallel format. We demonstrate the power of this device by testing it for rapid screening of protein crystallization conditions.

KEYWORDS: Power-free, Microfluidics, Reactor array, Screening

INTRODUCTION
Recently a variety of microfluidic reaction systems have been developed and applied to chemical engineering and biotechnology. However, most of the systems suffer from a certain complexity of the pumping and valving of fluids with multi-layer channel networks or sophisticated liquid handling instruments [1]. Here we present a self-contained and power-free microfluidic reactor array (MRA). This system, based on the combination of capillary effect and degassed PDMS pumping, allows automatically delivering multiple nanoliter sample and reactants simultaneously to the reaction chambers for biochemical screening.

EXPERIMENTAL
Design. A schematic diagram of the proposed MRA is illustrated in Figure 1. It consists of three major components: an array of glass capillary tubes, a microfluidic chip and a PnP PDMS pump. The capillary tube array is designed to automatically load multiple samples or reagents in parallel via capillary action. The PnP PDMS pump acts as a negative pressure source to drive fluid in the microfluidic chip [2]. The microfluidic chip, as shown in Figure 1b, contains 24 parallel microstructure units that implement 24 simultaneous metering and mixing reactions. Each unit contains a pair of metering structures designed to combine two fluidic samples with a ratio of 1:1. All units are connected with a common feeding channel via their respective centre metering channels. In addition, a shallow and narrow connection channel is present at the end of each metering channel. Besides connecting the metering channel to the mixing channel, the connection channel also acts as a passive stop valve for flow control.

Figure 1: Schematic diagram of the MRA. (a) Exploded 3D-view. (b) Top view.
Fabrication. Devices were fabricated using soft lithography. Briefly, SU-8 masters were fabricated by standard photolithography. Then Sylgard 184 was mixed in a 10:1 (w/w) ratio of resin to crosslinker and poured over the masters. After curing, the PDMS slabs were peeled off from the masters and assembled for building MRA. Typical structure depths used in the device are: feeding channel, metering channel, and reaction chamber 100 μm; connection channel 15 μm; venting channel 5 μm; pump chamber 100 μm.

Operation. A step-by-step procedure for operating the proposed MRA is shown in Figure 2. First, a PDMS chip mounted with a capillary tube array was turned upside down and all tips of the capillary tube array were aligned and immersed in an array of wells filled with reagents. Under the action of capillary force, all capillary tubes were spontaneously primed with corresponding reagents. After the loading of reagents, the chip was turned over and a pre-degassed modular PDMS pump was placed on its top. Then, a drop of liquid sample was pipetted into the centre inlet port of the microfluidic chip. Under the negative pressure created by the pre-degassed PDMS pump, a number of nanoliter-sized droplets can be accurately dispensed and mixed with the aid of specific channels. After the completion of metering and mixing operations, the PDMS pump was removed from the top of the microfluidic chip to allow easy observation.

![Figure 2](image)

**Figure 2**: Schematic illustration of the operation of the MRA: (a) parallel loading of different reagents into the microfluidic chip with the capillary tube array, (b) mounting of the PnP PDMS pump onto the microfluidic chip, (c) formation of a closed microfluidic system after a droplet of sample solution was loaded into the centre inlet port of the microfluidic chip and the aspiration of the liquid into the microchannel under the negative pressure created by the PnP PDMS pump, and (d) removal of the PnP PDMS pump after the completion of self-metering and -mixing.

RESULTS AND DISCUSSION

Liquid operation. To demonstrate the functionality of the MRA, we first tested its self-loading, self-metering and self-mixing ability with two dyes. Under the action of capillary force and negative pressure produced by the PnP PDMS pump, a series of liquid-handling operations: loading, metering, and mixing, was carried out in the microfluidic chip. Figure 3 shows snapshots of the metering and mixing of two dye solutions in the MRA. Liquids were automatically introduced into the feeding channels and metering channels under the driving force of the PnP PDMS pump. When the advancing liquid fronts reached the entrances of the connection channels, they stopped due to larger reverse capillary pressures existing at these narrow channels. Owing to the dynamic nature of the driving pressure generated by the PnP PDMS pump [2], there is a delay interval before the negative pressure created by the PnP PDMS pump exceeds the pressure barrier presented by the connection channel and pushes the liquid into the connection channel. During this interval, air was introduced to remove the excess liquid in the feeding channels, and droplets with precise volumes were left in the metering channels. With a further increase of the negative pressure in each reaction chamber, the two metered droplets overcame the capillary pressure barrier and entered the mixing channel. Next, the mixed droplet was pushed into the reaction chamber for subsequent detection and analysis. To keep the mixed droplet in the reaction chamber for addressable analysis, several venting side channels were included to allow release of the negative pressure after the completion of self-mixing. Finally,
the PnP PDMS pump was peeled off from the top of the microfluidic chip and a drop of paraffin oil was added into the centre inlet port to seal the mixed droplets and prevent evaporation.

**Protein crystallization.** To evaluate the feasibility of the MRA for biochemical screening, we further applied it to the screening of protein crystallization conditions. With the parallel, discrete nanoliter microfluidic system, the traditional microbatch protocol is simplified: multiple precipitants are loaded by the capillary force simultaneously and automatically, the protein sample is introduced by a single pipetting, and the protein sample and multiple precipitants are metered, dispensed, and mixed using a combination of capillary hydrophobic valving and degassed PDMS pumping, producing an array of droplets with each droplet representing an independent crystallization experiment. Lysozyme, a representative model protein, was chosen as a test sample. Crystal growth was observed in the MRA (Figure 4). The size of crystals is typically ~100 µm, which is large enough for X-ray diffraction studies.

**CONCLUSION**

In summary, taking advantage of capillary action as a parallel loading pressure source, a pre-degassed PDMS slab as a fluid-driven pressure source and microchannel contractions as passive valves, we have developed a self-contained and power-free microfluidic platform for biochemical screening. This platform can potentially be used for multiple (bio)chemical analyses and high throughput screening in individual laboratories or remote locations.

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**REFERENCES**


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