Design and fabrication of automated sedimentation-based separation and siphon-based extraction for detection of allergic reaction on a centrifugal microfluidic disc

(Invited Paper)

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We report the design and fabrication of a novel and fully integrated polymer-based centrifugal microfluidic disc for rapid automatic allergens detection. All essential steps for a single test including flow valving, sequencing, mixing, separation, extraction, and sedimentation for final detection are automatically conducted within 30 min on a centrifugal microfluidic disc. Our design features a siphon-based valving and analyte extraction structure where the released analyte is separated and subsequently extracted by a siphon valve into the detection chamber. Incorporating the siphon valve and a series of capillary valves, we realize automated detection of allergic reaction on a centrifugal microfluidic disc.

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Immunoassay is an established technique for a wide range of biomedical applications. Traditional immunoassays, such as the enzyme-linked immunosorbent assay (ELISA), are conducted in laboratory[1]. They require tedious fluid handling procedures and expensive facilities, and also involve a lot of manual input. It often takes many hours to perform one assay. There is certainly a need to improve the efficiency. With the capability to perform simultaneous and identical functions in parallel layouts, immunoassay in a rotating disc is a promising way to realize many point-of-care applications by embedding many analysis functions which may be performed in laboratory. The transfer of immunoassay, even clinical diagnosis, to a compact portable "rapid-sample-to-answer" device is subjected to both academic and commercial efforts in recent years[2−4]. The conventional immunoassay to study allergic reactions is ELISA on histamine. Lai *et al.* reported a centrifugal microfluidic disc for ELISA[5]. The ELISA disc relies on surface modification to capture the detection antibody, which may lead to failure in a long term. With simplicity in operation, universality for various occasions, low-cost, and long-term stability as the objectives, we design and fabricate a centrifugal microfluidic platform for automated detection of allergic reaction based on internal sedimentation-based analyte separation and siphon-based extraction. Degranulation in basophils or mast cells is an indicator to allergic reaction. In this connection, we demonstrate the detection of acridine orange (AO) which is released from granules accompanied with histamine during degranulation when KU812 basophiles are subjected to stimulation by ionomycin.

The disc with 4 identical units, 12 cm in diameter and 2 mm in thickness is comprised of a PDMS slab microstructured by PDMS molding using an SU-8 2075 mold through photolithography and sealed to a flat PMMA disc. In our disc platform, the separation of basophiles and the released AO after degranulation is precisely controlled through a rotation-speed-controlled separation and extraction structure (Fig. 1). The structure is a U-shape hydrophilic microchannel (siphon valve) connected with a separation chamber and an outlet detection chamber. The flow control of the structure is based on the balance of centrifugal force and capillary force. The suspended particles in centrifugal fields can be extensively accelerated since the centrifugal net force $F_f = (2\pi f)^2 r_p V_p (\rho_p - \rho_l)$ induced on a particle of volume V_p at radial position r_p is proportional to the square of the rotational frequency f (ρ_p and ρ_l are mass densities of particles and liquid, respectively), particles with larger volume or higher density are driven outwards whereas smaller/lower density particles settle inwards. The U-shape microchannel bends towards the rotation center so that the capillary force and centrifugal force are in opposite directions. Above a threshold frequency f_{th}, the centrifugal force F_ω overcomes the capillary force, the liquid from the inlet microchannel is driven to the bottom of the separation chamber, and the overflow flows to the waste chamber rather than to the capillary siphon valve. Once the rotation is ceased, the liquid with smaller particles located in radially inwards than the siphon inlet will be separated and extracted into the right chamber. The threshold frequency depends on the channel geometry and dimensions, the radial position, and the physical properties of fluid. In this work, the structure has been tested using colored pigment solutions (Fig. 2). It is
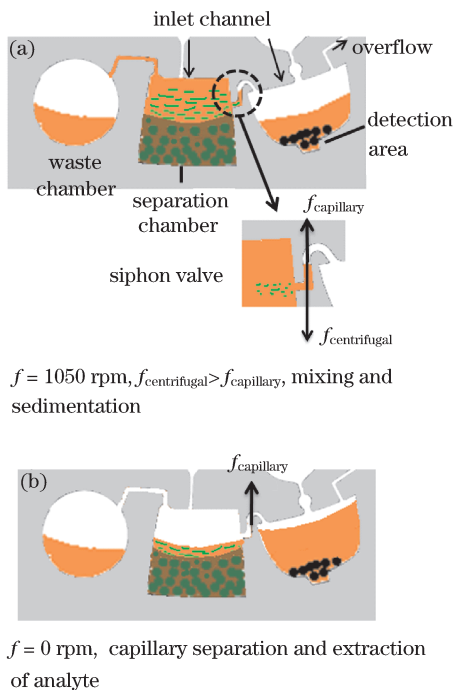
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$f = 1050$ rpm, $f_{\text{centrifugal}} > f_{\text{capillary}}$, mixing and sedimentation

$f = 0$ rpm, capillary separation and extraction of analyte

Fig. 1. Schematics of the separation and extraction strcture. (a) Particles with different sizes and densities (e.g., cells and small molecules) are separated with a shock interface at a pre-determined rotation speed; (b) liquid with small molecules is extracted to the detection chamber when the rotation is ceased.
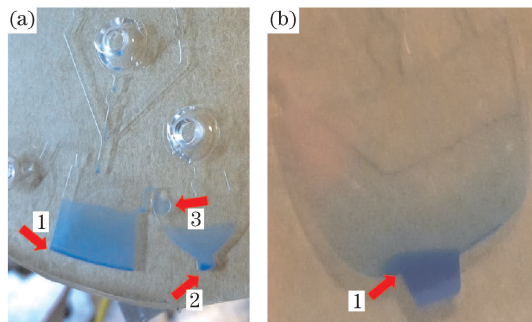

Fig. 2. Experimental sedimentation and siphon valving on the disc.

observed that the centrifugal force prevails the capillary force in the siphon valve at 1050 rpm. Arrows 1 and 2 in Fig. 2(a) show that large pigment particles deposit in the dead area of the left chamber, arrow 3 shows that the pigment solution is extracted to the right chamber after the rotation is ceased. Arrow 1 in Fig. 2(b) shows that large pigment particles deposit in the detection area, which is an attractive way to concentrate the analyte and strongly increase the detection sensitivity.

Incorporating a series of capillary valves, flow sequencing in an automated manner can be achieved. The increasing centrifugal force from the center toward the edge of the disc is used for flow sequencing which replaces the stepwise procedures. Capillary valve features a structure of hydrophilic microchannel expanding suddenly and is based on a balance between capillary pressure and centrifugation induced pressure. Briefly, liquid flowing through a hydrophilic microchannle can be trapped
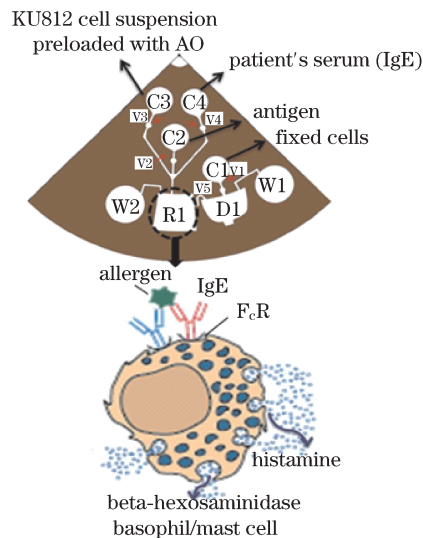

Fig. 3. Schematic of the structure for a single assay. C1, C2, C3, and C4: sample inlet chambers; R1: reaction and separation chamer; D1: detection chamber; W1, W2: waste chambers.
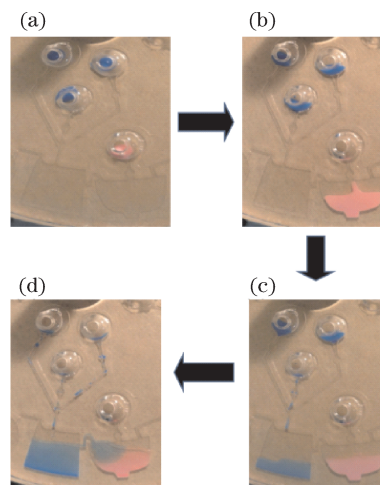

Fig. 4. Flow testing results using pigment solutions.

by the influence of surface tension and fluid/substrate interactions, the capillary valves are generated by introducing a sudden expansion in the channel diameter, such as the case in which a channel meets a reservoir. Sufficient fluidic pressure must be introduced by the centrifugal force to overcome the pressure of curved liquid surfaces and to wet the walls of the chamber. The theoretical burst frequency is the rotation speed when the centrifugal force overcomes the capillary force and bursts the fluid to the valve and rotation. The detailed calculation has been discussed by Chen et al.[6]. A small detection column is designed in the bottom of the detection chamber, so that the fixed cells are concentrated by the centrifugal force in the column based on sedimentation theory.

A single unit structure is depicted in Fig. 3. The flow sequence is designed in such a way that the preloaded reagents burst into the three capillary valves in the order of C1−C2−C3, C4 by increasing the rotation speed. Reliability of the device is of utmost importance. To evaluate
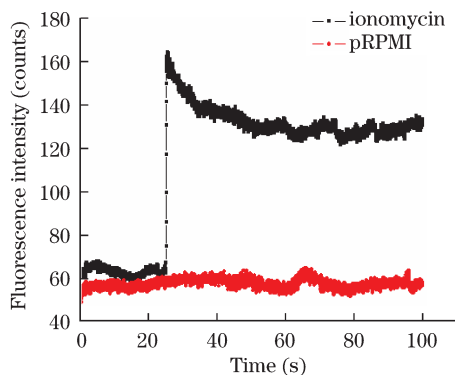
Fig. 5. Fluorescence signal increases significantly when the KU812 human basophil is stimulated by ionomycin (1 μmol/L).

the reliability of valves and repeated flow sequence, colored pigment solutions are used to test deviation of the burst frequencies used in the flow sequencing process. The average experimental burst frequencies from six samples of the three capillary valves (V1,V2,V3) are 531, 682, and 965 rpm, respectively (coefficient of variation CV<5%). The flow sequencing result is shown in Fig. 4 which agrees with the expected outcome. No large variation between the channel dimensions is noted. The threshold frequency of flow gating for the siphon valve is 1050 rpm. The analysis process is as follows. 1) Loading samples to the reservoirs. 2) Increasing the spinning speed rapidly to 1050 rpm with an acceleration speed of 10000 rmp/s. Cells and reagents are retained in chamber R1 for reaction. 3) Stopping the rotation after 20 min. The liquid with released AO and histamine accompanying degranulation located radially inwards than the siphon valve inlet starts to be extracted to the detection chamber D1, whereas the cells deposit in the dead end of the reaction chamber, thus separating the cells and the released AO and histamine. 4) The extracted AO are then stained to the fixed cells pre-released and preconcentrated to the detection area.

To demonstrate the feasibility of performing the monitoring of allergic response on the disc, we have hereby measured the fluorescent intensity in fixed cell in the detection chamber. The fluorescence signal is monitored with a photomultiplier tube (PMT) connecting with an oscilloscope (Agilent, USA) using a 473-nm diode-pumped solid-state (DPSS) blue laser (Lasever, USA) for excitation. As the AO stained living cells in the reaction chamber have fluorescence, we have to confirm that no living cells are pumped to the detection chamber. In the testing experiment, ionomycin, AO stained living cells,

and colorless pRPMI medium are loaded into chambers C2, C3, and C4, respectively while leaving C1 as blank. After a single run of the frequency protocol, the detection area is measured through PMT, and the results show that there is red fluorescence. While using pRPMI instead in chamber C2 to repeat the former test, there is no fluorescence in the detection area this time. It confirms that no living cells are pumped into the detection chamber and AO can be separated through the siphon structure. Microscopic observation also proves our results.

Figure 5 shows the fluorescence intensity changes before and after stimulation with ionomycin (1 μmol/L) of living KU812 cell suspensions on the disc. Colorless pRPMI medium is used as control solution for comparison. In the case of pRPMI, no fluorescence above the background is observed. When ionomycin (1 μmol/L) is introduced, we see that the fluorescence signal significantly increases after the stimulation. The staining of AO to fixed cell is within several seconds, the steep increase of the signal indicates that the staining may have already finished before the rotation is completely ceased. The fast decrease of the fluorescence intensity after reaching the peak may be due to photobleaching. The observation shows that the device is applicable for detection of ionomycin-mediated degranulation. Besides, we have also successfully performed the identical assays in the four units simultaneously in a single run, followed by serial reading out of the signals which shows the same results (data not shown).

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