CENTRIFUGAL MULTIPLEX FIXED-VOLUME DISPENSER (C-MUFID) ON A DISPOSALBE PLASTIC LAB-ON-A-DISK FOR BIOCHEMICAL ASSAYS

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ABSTRACT

In the present study, a centrifugal multiplex fixed-volume dispenser (C-MUFID) was developed on a disposable plastic lab-on-a-disk for biochemical assays. Multiplex metering and dispensing of sample with fixed-volume were achieved throughout the C-MUFID based on microfluidic theoretical verification. Biochemical assays were also carried out with help of dispensing chamber, serpentine micromixer, and reaction chamber.

KEYWORDS: Centrifugal force, Lab-on-a-disk, Dispenser, Biochemical assay

INTRODUCTION

Lab-on-a-disk (LOD) has been attracting attention for clinical diagnostics because of its remarkable advantages. The LOD does not require pneumatic pumps, but only a single rotational motor for its operation. It is possible to control serially integrated elements and realize multiplex analysis of radial arrays [1, 2]. To achieve biochemical assays in terms of development as a miniaturized system, multiplex metering and dispensing of sample with fixed-volume should be performed as well as effective micromixing [3-4]. Mark et al. obtained good multiplex dispensing on the LOD by aliquoting structure and centrifuge-pneumatic valve. However, further operations, such as mixing, separating, or extracting, could not be applied to that system due to the closed (unvented) chamber at the end of the metering channel [4].

DESIGN AND THEORY

Figure 1 shows a schematic design of the C-MUFID with geometric parameters. The C-MUFID comprises an inlet chamber, a circumferential feed channel, connecting metering channels with passive microvalves, vented dispensing chambers, and waste chamber. Initially loaded sample in inlet chamber fills into feed channel and metering channel based on pressure derived on the bottom of inlet chamber under centrifugation at an angular velocity of ω_1 (Fig. 2(a)-(b)). At a higher angular velocity, ω_2, the dosed sample is discharged to waste chamber except the samples inside the metering chambers due to pinning mechanism of passive microvalve (Fig. 2(c)). Finally, the remained samples with fixed-volume (1 μl) by adjusting dimensions of the chamber is delivered to the dispensing chamber at an angular velocity of ω_3 higher than ω_2 (Fig. 2(d)).

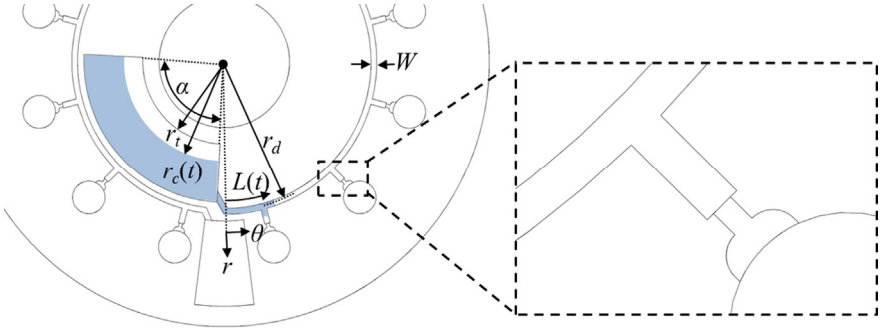

Figure 1. Schematic of the C-MUFID with geometric parameters. C-MUFID comprises an macro inlet chamber, a circumferential feed channel, branching metering channels, passive microvalves connected with vented dispensing chambers, and waste chamber. Height of all the structure is 1 mm except the passive microvalve (H_{valve} = 300 μm).

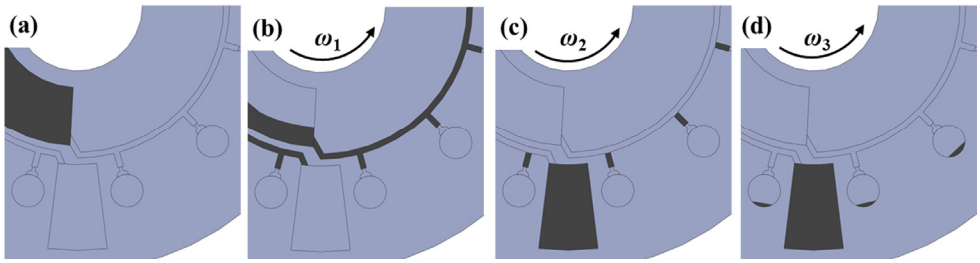

Figure 2. Schematic procedure of sample metering and dispensing by the C-MUFID: (a) sample loading (by pipetting at stationary state), (b) sample dosing to feed channel and metering channel, (c) sample metering, and (d) sample dispensing ($\omega_1 < \omega_2 < \omega_3$).

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In order to confirm that the loaded sample was able to fill totally into the feed channel and metering channel within appropriate processing time (~ 1 min) under given geometry and angular velocity, an approximate equation of the filling flow front advancement, $L(t)$, into the rectangular feed channel was derived based on Navier-Stokes equation with proper boundary conditions as below:

$$L(t) \sim \frac{\alpha}{2W} \frac{A(t) + \left(r_d^{\,2} - r_t^{\,2}\right) B(t)}{B(t) + C(t)} \tag{1}$$

$$A(t) = t\sigma \cos\theta_c \left(\frac{1}{W} + \frac{1}{H}\right), \quad B(t) = \frac{t}{2}\rho\omega, \quad C(t) = \frac{\mu U \alpha}{2W}\left[\frac{\rho}{\mu} - t\left(\frac{1}{W^2} + \frac{1}{H^2}\right)\right] \tag{2-1, 2-2, 2-3}$$

where, σ, θ_c, ρ, and μ are surface tension, contact angle, density, and dynamic viscosity of sample liquid, respectively. U represents approximate mean downchannel velocity of fluid flow. Based on the given value of parameters, the filling flow front advances up to the end of feed channel within 1 min.

FABRICATION AND EXPERIMENTAL

The plastic LOD containing the C-MUFID was fabricated by CNC-micromilling on a polycarbonate (PC) substrate, followed by thermal bonding with a cover plastic substrate. Oxygen plasma treatment was then carried out on the surface of the feed channel and metering channel for enhancing filling velocity of sample flow. To confirm the metering performance of the C-MUFID, volume of metered sample was measured by image processing method. Captured image right after the metering step was compensated to recognize the area occupied by metered sample. Each volume could then be calculated by multiplying the area by the channel height.

Figure 3 shows schematics of a LOD containing the C-MUFID connecting with half-opened dispensing chamber, serpentine micromixer, and reaction chamber. Throughout the centrifugal force based serpentine micromixer (CSM), effective mixing could be achieved because of transvers secondary flows and three-dimensional stirring effect based on the centrifugal force and induced Coriolis force [5]. Structured substrate was manufactured by injection molding using PC, then sealed with a flat PC substrate by high-pressure thermal bonding. Multiplex biochemical assays were carried out using standard serum with albumin and glucose reagents. After the dispensing of metered reagent, each reagent was dosed into the half-opened dispensing chamber at stationary state of the LOD. By rotating the LOD, serum and reagent was mixed together throughout the serpentine micromixer.

RESULT AND DISCUSSION

Figure 4(a) shows measurement result of each volume of metered sample for 5 different chamber. Average volume (1.01 μl) was similar with target fixed-volume with small standard deviation (0.046).

As shown in Figure 4(b), multiplex biochemical assays were performed with meaningful colorimetric results. Color of each reagent significantly changed after mixing and reacting with the serum. It might be mention that quantitative analysis for these color change of the reagent could be achieved by means of colorimetric analysis or spectrophotometry.

CONCLUSION

In this study, multiplex fixed-volume metering and dispensing were successfully achieved based on the developed C-MUFID. Geometric design and metering performance of the C-MUFID were confirmed by microfluidic theoretical verification and experimental image analysis, respectively. Multiplex biochemical assays were also achieved on the LOD containing C-MUFID with appropriate micromixer and chambers.

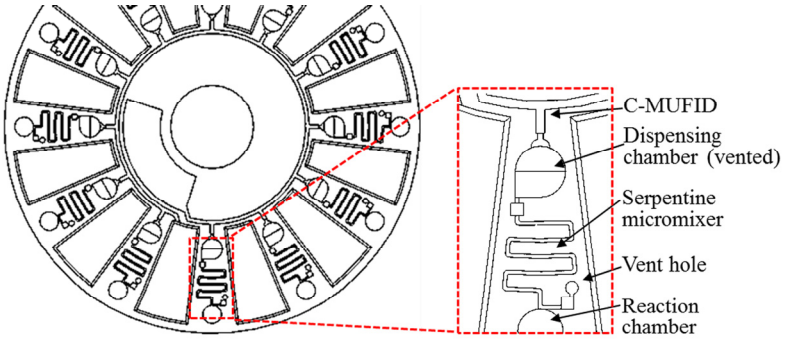

Figure 3. Schematics of the LOD containing the C-MUFID, half-opened chamber, serpentine micromixer, and reaction chamber.

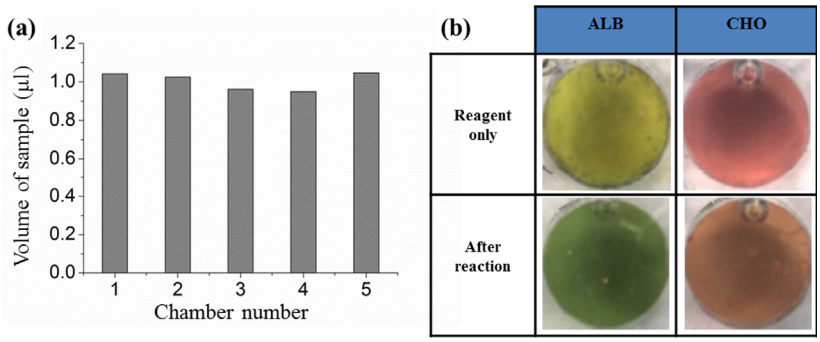

Figure 4. (a) Measuring result of each volume of metered sample for 5 different chamber (Average volume = 1.01, Standard deviation = 0.046). (b) Color change of each reagent after mixing and reacting with the metered serum.

ACKNOWLEDGEMENTS

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