A Disk-Shaped Microfluidic Device for High Accuracy and Simple Operation of Enzyme Immunoassay

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A disk-shaped microfluidic device (lab-on-a-Disk) was developed to allow the high accuracy, reproducibility, and simple operation of heterogeneous enzyme immunoassay (EIA). The amount of reagents concerning with the assay is strictly required for the accuracy of the results. However, the significant miniaturization of sample and reagents volume forms the droplets of such solutions and results the increase of difficulty for the microfluidic manipulation: volume control, prevention of evaporation, and transport of droplets. In addition, the amount of immobilized immuno-reactant, such as standard antigen and antibody, critically influence the obtained results in a competitive EIA. Therefore, we have developed a feasible cross-linker and microdispenser using a centrifugal force. Because centrifugal force can act on all of the droplets on a disk, it is considered that a lab-on-a-Disk is suitable for parallel processing on a microfluidic device. In this study, the centrifugal and capillary forces were examined for microfluidic control in the multi-step process (e.g., antigen-antibody reaction, bound/free separation, detection) in EIA, in order to automatically carry out each step of EIA according to the rotational speed of the lab-on-a-Disk.

At first, we examined the relationship between the rotational speed, the channel profile, and the position of the microfluidic reservoirs from the center of rotation to manipulate sample solutions into each reaction reservoir through microchannels sequentially, i.e., retain in a reservoir or flow into a subsequent reservoir. A glass bead with immobilized antigen on its surface was injected into a reservoir for a competitive antigen-antibody reaction, and applied to a specific surface in a heterogeneous assay. The single glass bead was strictly sized in 1 mm in diameter and applied to each assay. To uniformly immobilize standard antigen upon the bead, we synthesized End-capped PolyCarboDiimide (E-PCD), which was polymer of highly reactive carbodiimide group.

E-PCD was successfully synthesized and it was feasible to be a mass synthesis and long preservation. To estimate the reproducibility of immobilization, the beads were immersed in the cross-linker solution and then dried for the E-PCD coating. The standard antigen was covalently cross-linked with the beads with high reproducibility. The RSD value was 3.5%, which was equal to the variation of the surface area on the beads.

In addition, we build up the sample dispensers on the lab-on-a-Disk in order to complete a high-reproducible assay. The lab-on-a-Disk of 4 inches in diameter was fabricated of inexpensive polydimethylsiloxane (PDMS) and polystyrene sheet (Fig.1). Using measuring microchannels, 200 nl of solutions could be dispensed exactly. In particular, the shapes of menisci in the measured droplets remarkably affected to the reproducibility in the dispensed volume. Therefore, we build on trapezoidal overhang into measuring channel to reduce the meniscus sizes. As the results, the solution was dispensed into each 200 µL with the variation of 5.5 % in RSD.

Finally, the new design of lab-on-a-Disk which consisted of sample dispenser following 7 flow channels was fabricated. As a standard sample, secretory immunoglobulin A (sIgA), which is a candidate marker of mental stress. The sample and HRP-labeled anti-sIgA antibody was mixed in the inlet on the lab-on-a-Disk. The solution was dispensed into the following reservoirs at 350 rpm. Then solution was reacted with standard sIgA on the surface of sIgA-immobilized bead. After the competitive antigen-antibody reaction on the bead, the floating immuno-complex in the solution was removed to waste reservoir. The entrapped HRP-labeled antibody on the sIgA-immobilized bead was quantified the fluorescence reacted with Amplex Red substrate. The lab-on-a-Disk for EIA that had the calibration curve observed good linearity until 66 µg/ml and the limit of detection (LOD) was 2.5 µg/ml (6.4 nM) of sIgA in Fig.2. The reproducibility of assay was 8.5 % in RSD. Thus, sIgA-immobilized bead using E-PCD and sample dispenser system on the Lab-on-a-Disk could detect low level of sIgA in human saliva without troublesome-handling, and make it possible to perform high-reproducible assay.

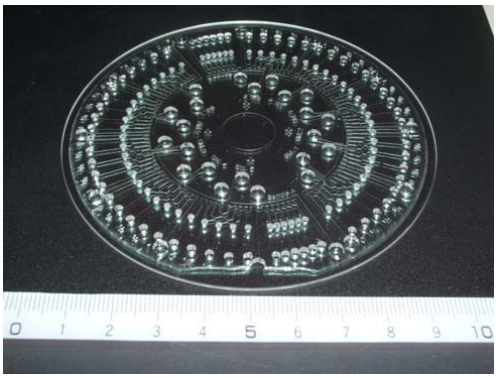

Fig. 1 Disk-shaped microfluidic device for EIA.

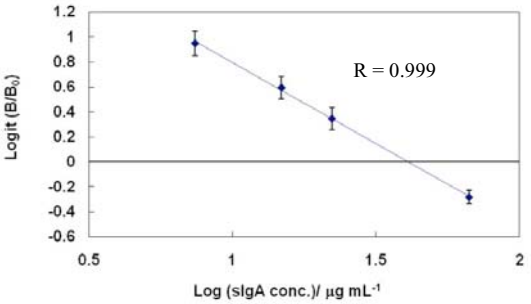

Fig. 2 Calibration curve for sIgA using the device.