Microfluidic Platform for Advanced Radioimmunoassay

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1. Introduction

Radioimmunoassay (RIA), developed by R. S. Yalow and S. A. Berson [1], uses radioactive isotopes such as ^{125}I, ^{14}C, ^{3}H and ^{35}S for quantification of analyte; RIA has been used to measure low concentration of substances such as drug, hormones and vitamins [2, 3]. Although RIA is extremely specific and highly sensitive immunoassay method, it has crucial disadvantages due to the use of radioisotopes. The use of radioisotopes requires special handlings and generates radioactive wastes inevitably. Also, long incubation time in RIA makes the workers excessively exposed to the radiation.

The microfluidic systems, composed of the micro-scale channels, provide various advantages for bioassays such as less samples/reagents consumption, short reaction time, low cost and automatic performance. With these advantages, the microfluidic system has been used as a powerful platform for bioassays and biological procedures, including DNA separation, cell counting and immunoassays [4, 5].

In this study, therefore, we suggested RIA based on the microfluidic system (μ-RIA) to complement the mentioned disadvantages of conventional RIA. Here, we fabricated the microfluidic chips for μ-RIA. And then, performance of μ-RIA was investigated using proper surface treatments and labeling techniques.

2. Experimental scheme

Fig. 1 shows our experimental scheme. Various reagents such as radiolabeled proteins and washing solution are injected into the micro-scale channel through three inlet holes and drained outside through the outlet hole. In the spiral section where antibodies were bound, cold (no radioisotope labeling) antigens of sample and radiolabeled antigens bind to the specific antibodies and compete with each other for a fixed number of antibodies. A standard curve depending on the amount of cold antigens can be derived and this allows us to evaluate quantitatively the amount of antigen present in unknown sample.

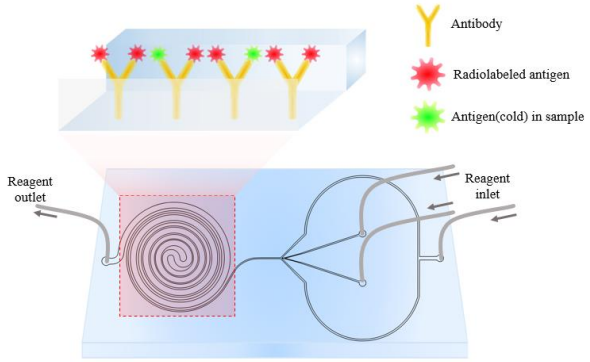

Fig. 1 Schematic diagram of RIA on microfluidic platform

3. Results and discussion

3.1. Fabrication of microfluidic chip

As shown in Fig. 2a, to fabricate the microfluidic chip for RIA we designed microfluidic pattern with computer-aided design (CAD) software program (Autodesk Auto CAD). A 10:1 mixture of polydimethylsiloxane (PDMS) 184-A (base) and 184-B (curing agent) was poured on the master patterned with the design and cured it for 1 hour at 80 ºC. The PDMS replica was peeled off from the master and 1 mm diameter holes were drilled using a puncher to connect tubing. The surface of resulting replica and a thin PDMS substrate were treated with oxygen plasma (Harrick Plasma) and bonded with each other (Fig. 3).

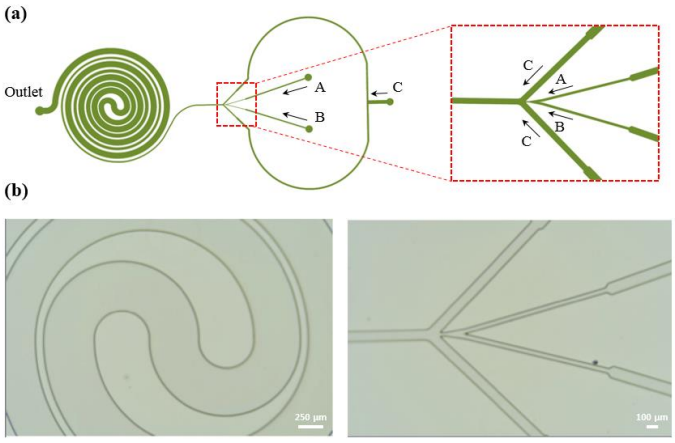

Fig. 2 Design of microfluidic channel (a) and microscope image of PDMS replica channel (b)

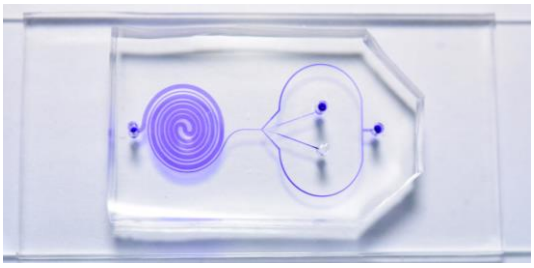

Fig. 3 Image of microfluidic chip for RIA

The spiral structure of microfluidic channel could provide amplification effect of radiation signal and the gradually increasing width allows the proteins to have longer residence time in the channel.

3.2. Evaluation of optimum reaction time

To determine the reaction time required for RIA analysis, we evaluated the radiation intensity depending on the reaction time of radiolabeled antigen and antibody adsorbed on the channel. To bind the antigen-specific antibody on the microfluidic channel, the surface of channel was treated with (3-aminopropyl) trimethoxysilane (APTMS) and glutaraldehyde (GA), followed by injection of the antibody into the channel. To block the residual active sites where the antibody was not bound, 1% bovine serum albumin (BSA) was introduced into the antibody bound channel.

To radiolabel 50 μg of the antigens with 20 μCi of ^{125}I, we applied the IODO-GEN method with Pierce Pre-Coated Iodination Tubes (Thermo Fisher Scientific). The radiochemical purity of radiolabeled antigen was evaluated using instant thin-layer chromatography (ITLC) and the radiolabeling result (Fig. 4) showed a radiochemical purity above 99%.

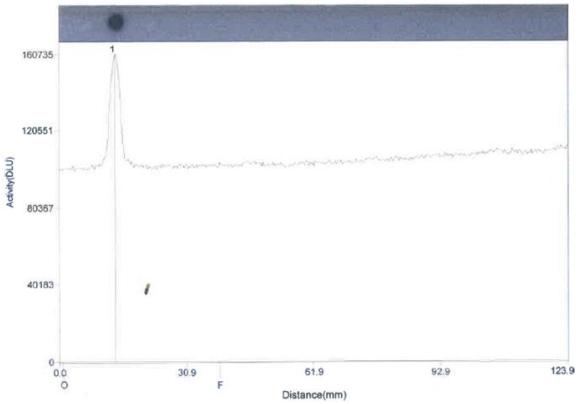

Fig. 4 Radiochemical purity of radiolabeled antigen

The radiolabeled antigen was injected into the antibody adsorbed channel and the reaction time was changed through the controlling of the injection flow rate. As shown in Fig. 5, the experimental results showed that 5 minutes is enough to determine the presence of antigens. Compared to the conventional RIA requiring incubation time more than 12 hours, total analysis time required for the μ-RIA was significantly shorter.

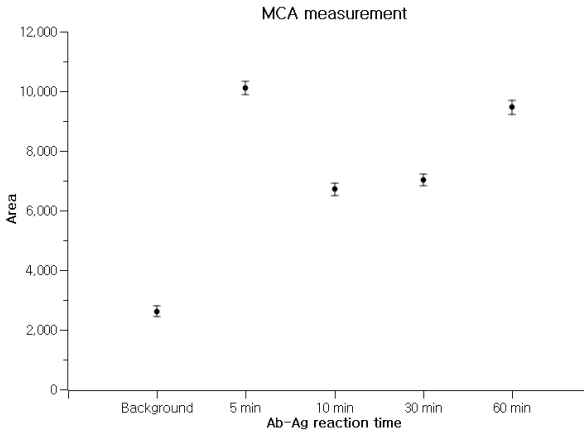

Fig. 5 Radioactivity measurement of chips depending on the antigen-antibody reaction time

4. Conclusions

In this study, we presented advanced RIA technology based on the microfluidic platform. The advantages of microfluidics enabled us to have better performance of RIA; RIA with microfluidic system of this study (μ-RIA) spent less reagents and shorter analysis time than conventional RIA. This means that the μ-RIA can reduce the generation of radioactive wastes and the radiation exposure of worker. The experimental results showed the applicability of microfluidic technology to complement the weakness of conventional RIA. Through further studies to optimize the analysis, the μ-RIA is expected to analyze the antigens in sample quantitatively and to be a promising platform detecting various antigens.

References

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