Radioimmunoassay of Biomolecule Based on the Lab-On-a-Chip Platform

Jin-Hee Kim [a,b], So-Young Lee [a] and Seung-Kon Lee [a*]
[a]*Neutron and Radioisotope Application Research Division, Korea Atomic Energy Research Institute, 111, Daedeok-daero 989beon-gil, Yuseong-gu, Daejeon 34057, Republic of Korea*
[b]*School of Architectural, Civil, Environmental and Energy Engineering, Kyungpook National University, 80, Daehak-ro, Buk-gu, Daegu 41566, Republic of Korea*
[*]*Corresponding author: seungkonlee@kaeri.re.kr*

1. Introduction

RIA is an extremely specific and highly sensitive immunoassay, but the use of RIA is restrictive for the following reasons. RIA generally requires the long incubation time and this might result in the excessive radiation exposure of worker. The use of radioisotopes generates the radioactive wastes. Solid-phase RIA simplified the analysis procedures [1, 2], but it was not effective on the reduction of radioactive wastes and analysis time. The scintillation proximity assay (SPA) reduced the generation of radioactive wastes [3]. However, the reduction of analysis time remains an ongoing challenge.

Lab-On-a-Chip (LOC) platform is the miniaturized version of laboratory system. The LOC platform is composed of the microfluidic channel and it provides the advantages for bioassays such as short analysis time, less reagents consumption and automatic performance. The immunoassay based on the LOC platform reduces the reaction time, compared to the common immunoassay. It requires less reagents consumption, but it sometimes has the problem of low signal intensity.

We presented the RIA based on the microfluidic platform (μ-RIA) and optimized the reaction time for the analysis in previous study [4]. Here, we utilize the μ-RIA platform for the analysis of thyroxine (T4) antigen. The standard curve depending on the concentration of antigen is derived. The amount of antigen in QC sample of RIA kit is measured with the standard curve. Through the experimental results, we show the applicability of μ-RIA for the analysis of biomolecules.

2. Experiments

2.1. Fabrication of microfluidic chip

We applied the soft lithography to fabricate the microfluidic chip for RIA. Briefly, a 10:1 mixture of polydimethylsiloxane (PDMS) 184-A (base) and 184-B (curing agent) was poured on the wafer patterned with the microfluidic channel and cured it. The PDMS replica was peeled off 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.

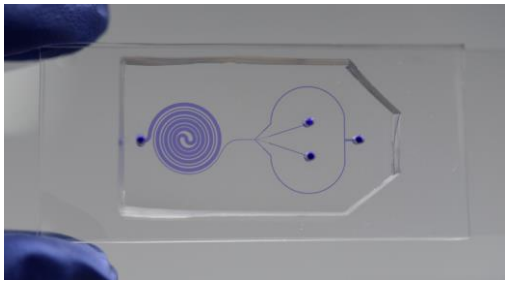

Fig. 1 Photograph of microfluidic chip for RIA

2.2. Surface modification of the channel

The surface of microfluidic channel was treated with (3-aminopropyl) trimethoxysilane (APTMS) and glutaraldehyde (GA) to bind the biomolecules. We injected the T4-specific antibody into the channel and incubate overnight. There might be the residual active sites where the T4 antibodies were not bound. This results in the non-specific binding of biomolecules, reducing the sensitivity and reliability of detection. We treated bovine serum albumin (BSA) blocking to prevent the non-specific binding. 1% BSA in phosphate buffered saline (PBS) was injected into the microfluidic channel for 1 hour, followed by washing with the PBS. Fig. 1 shows that the treatment of 1% BSA was effective on the prevention of non-specific binding, unlike the 0.4% BSA (Fig. 2).

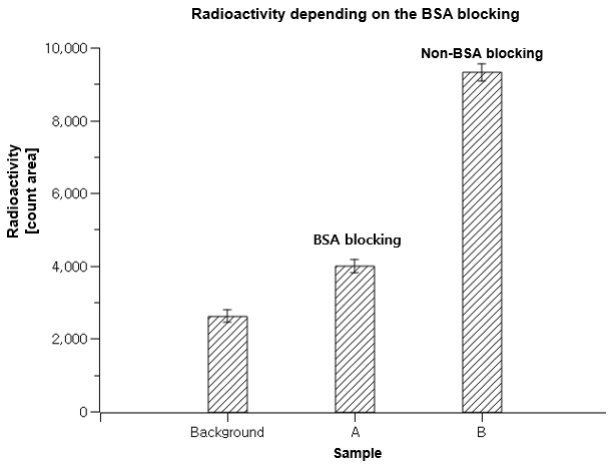

Fig. 2 Evaluation of prevention of non-specific antigen binding. Background is the result of measuring the radioactivity of PDMS chip without the radiolabeled antigen binding.

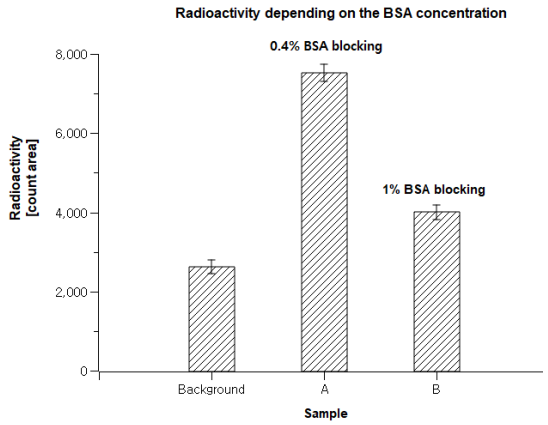

Fig. 3 Prevention of non-specific antigen binding depending on the concentration of BSA solution.

3. Results and discussion

3.1. Application of the μ-RIA for T4 antigen analysis

We used the commercial T4 RIA kit and microfluidic chips where the microfluidic channel was bound with T4 antibodies and BSA. 10 μL of standard solution in RIA kit was injected into the channel and incubated for 5 minutes. The concentrations of standard solutions were 2, 4 and 8 μg/dL. Then, 10 μL of ^{125}I tracer (radiolabeled T4 antigen) was injected into the channel and incubated for 5 minutes. After the reaction, the ^{125}I tracer in the channel was removed by 100 μL of PBS washing. We measured the radioactivity of chip using automatic gamma counter and the result of measured radioactivity was shown in Fig. 4. We derived the standard curve with R^2 = 0.9951 depending on the concentration of standard solution. The measured radioactivity in QC sample analysis was 433 CPM (blue triangle) and this corresponded to 2.6 μL/dL of antigen. This result showed that the μ-RIA was suitable for the analysis because the accredited criteria of QC sample was 2.5 to 4.5 μg/dL (red box)

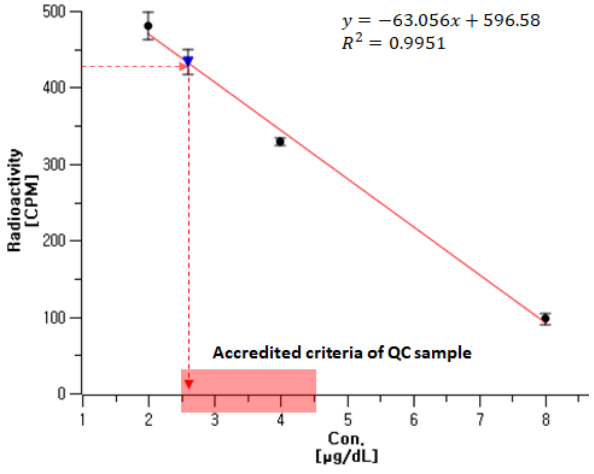

Fig. 4 The standard curve depending on the concentration of standard solution

3.2. Comparison of μ-RIA and RIA kit

We compared the protocol of μ-RIA and commercial RIA kit. As shown in Fig. 5, the μ-RIA based on the LOC platform can reduce the reaction time and the amount of reagent consumption than RIA kit. Therefore, the μ-RIA is expected to reduce the radiation exposure of workers and the generation of radioactive wastes.

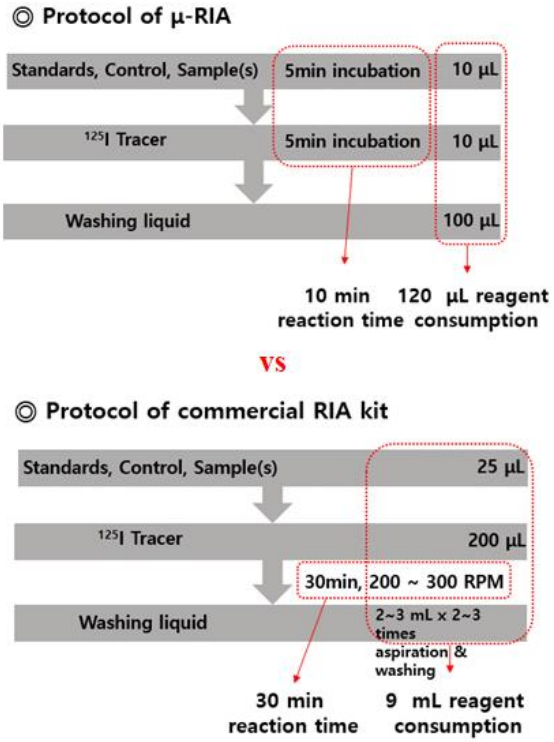

Fig. 5 Comparison of protocol of μ-RIA and RIA kit

4. Conclusion

In previous study, we presented the μ-RIA to complement the conventional RIA and optimized the procedure of μ-RIA. Here, we carried out the RIA of T4 antigen on the μ-RIA platform with the optimized conditions. We derived the standard curve with R^2 = 0.9951 from the analysis of standard solution. The result of QC sample test showed that the μ-RIA was suitable for the analysis of biomolecules and we could quantitatively evaluate the amount of antigen in unknown sample.

REFERENCES

[1] Sallam, K. M., El-Bayoumy, A., & Mehany, N. (2016). Development of solid phase immunoradiometric assay for determination of carcinoembryonic antigen as a tumor marker. Journal of Radioanalytical and Nuclear Chemistry, 307(2), 1375-1383.
[2] Mehany, N., El Kolaly, M., Ayyoub, S., & Hassan, S. (2005). Immunoradiometric assay for the in-vitro

determination of thyroid stimulating hormone in human serum and plasma using solid phase anti-TSH cellulose particles. Journal of Radioanalytical and Nuclear Chemistry, 265(1), 61-71.
[3] Lee, S., Lim, J., Cho, E., & Jung, S. (2016). A new scintillation proximity assay-based approach for the detection of KRAS mutations. Radiochimica Acta, 104(1), 59-65.
[4] Kim, J., Lee, S., & Lee, S. (2019) Microfluidic platform for advanced radioimmunoassay, 2019 Koean Nuclear Society Fall Meeting