Supplementary Material (ESI) for Lab on a Chip

Supplementary Information

for

Immuno-pillar chip: a new platform for rapid and easy-to-use immunoassay

Mai Ikami,[a] Ayako Kawakami,[a] Masaya Kakuta,‡[b] Yukihiro Okamoto,[a,c]

Noritada Kaji,[a,c] Manabu Tokeshi*[a,b,c] and Yoshinobu Baba[a,c,d]

[a]*Department of Applied Chemistry, Graduate School of Engineering, Nagoya University, Furo-cho, Chikusa-ku, Nagoya 464-8603, Japan. E-mail: tokeshi@apchem.nagoya-u.ac.jp; Fax: +81-52-789-4666; Tel: +81-52-789-4498*

[b]*Institute of Microchemical Technology, Co., Ltd., 212 KSP East, Sakado, Takatsu-ku, Kawasaki 213-0012, Japan*

[c]*FIRST Research Center for Innovative Nanobiodevice, Nagoya University, Furo-cho, Chikusa-ku, Nagoya 464-8603, Japan*

[d]*Health Technology Research Center, National Institute of Advanced Industrial Science and Technology (AIST), 2217-14, Hayashi-cho, Takamatsu 761-0395, Japan*

‡Current Address: Sony Corporation, 4-14-1 Asahi-machi, Atsugi 243-0014, Japan

Pore size of the immuno-pillar

In order to estimate the pore size of the immuno-pillar, we prepared three kinds of immuno-pillars containing 100 nm, 200 nm or 1 μm diameter fluorescence particles (Fluoresbrite® YG Carboxylate Microspheres, Polyscience, Inc., Warrington, PA) instead of antibody-immobilized polystyrene beads. We observed the fluorescence particles in the pillars using a fluorescent microscope (IX-71, Olympus, Tokyo, Japan) equipped with a CCD camera (EB-CCD, Hamamatsu Photonics, Hamamatsu, Japan) and a laser (λ=488 nm, 35LAL415, Melles Griot, Carlsbad, CA). The 200-nm and 1-μm diameter fluorescence particles did not leak from the pillars but the 100-nm diameter fluorescence particles did. From these results, we judged the pore size of the immuno-pillar was likely 100-200 nm.

Diffusion of antigen and antibody in the immuno-pillar

Diffusion of antigen and antibody in the pillar is one of the important factors that influence the assay time. We investigated the diffusion of FITC-labeled CRP antibody in the pillar. The immuno-pillar chip for CRP assay was used for this experiment. 0.25 μL of CRP solution (100 ng/mL) were introduced into the microchannel through the inlet with a pipette. After incubation for 5 min, the unreacted CRP solution was sucked from the outlet with the pipette, and then the microchannel was washed three times with PBS. After sucking out the PBS solution, the FITC-labeled CRP antibody solution (200 μg/mL) was introduced into the microchannel. The incubation time of FITC-labeled CRP antibody was changed from 10 to 180 s, and the fluorescence image was measured every 10 s. A typical example of the obtained fluorescence images is shown in Fig.S1. We observed diffusion of the FITC-labeled CRP antibody in the

pillar with increasing time. From the fluorescence images and the diameter of the pillar (200 μm), we roughly estimated the diffusion rate of FITC-labeled CRP antibody in the pillar. The results are shown in Fig.S2. The diffusion rate decreased with time, and became constant after 40 s. We thought that the water absorptivity of the hydrogel pillar was the reason why the diffusion was faster during the initial 40 s. Since the fluorescent-labeled secondary antibody solution was introduced into the microchannel after sucking out the PBS solution, the pillar was aerated as it came in contact with the antibody solution. Then, the pillar rapidly absorbed the solution when the two came in contact. In fact, when the solution was introduced into an empty channel, swelling of the pillar was observed. At 180 s, the fluorescence of the pillar was uniform. From the fluorescence image at 180 s, we estimated the diffusion rate of FITC-labeled CRP antibody to be about 0.4 μm/s. This value was comparable with the value, 0.63 μm/s, calculated from the diffusion constant of IgG in solution[1]. Moreover, the diffusion of the antigen in the pillar might be faster than that of the fluorescent-labeled secondary antibody because the molecular weight of the antigen is smaller. We concluded the advantageous rapid assay of the immuno-pillar chip was derived from the rapid diffusion of the antigen and the fluorescent-labeled secondary antibody in the pillar.

Surface area of the beads per unit volume (*S*/*V* ratio) of the immuno-pillar

Specific interface (*S*/*V*) is defined as the *S*-to-*V* ratio, where *S* and *V* are the surface area of the reaction solid and the solution volume, respectively. An increase in *S*/*V* means an increase in the number of reaction sites. In the assay using the immuno-pillar chip, since the sample and secondary antibody solutions introduced into the microchannel did not flow, the sample and the antibody that could take part in the

antigen-antibody reaction were limited to only the amounts which reached the pillar by diffusion within the incubation time (Fig.S3). Then, we could roughly consider the solution volume as follows:

$$V=(r_R+l)^2\times\pi\times h-(4/3\pi r_B^3\times N)$$

where r_R is the distance to which the sample and secondary antibody diffuse during the incubation time, l is the radius of the immuno-pillar (100 μm), h is the height of the immuno-pillar (50 μm), r_B is the radius of a bead (0.5 μm), and N is the number of beads inside the immuno-pillar (32700). Under our experimental conditions, r_Rs at incubation times of 1 min, 3 min and 5 min were estimated to be 85 μm, 147 μm and 190 μm, respectively. Then, the S/Vs at incubation times of 1 min, 3 min and 5 min were calculated to be 192 cm^{-1}, 107 cm^{-1} and 78 cm^{-1}, respectively. Because the volume of the hydrogel skeleton structure was not considered in this calculation, actual S/Vs were a little larger than the calculated values. Since the S/V of 50 μL solution in the microtiter plate well (0.65 mm in diameter) was estimated to be 13 cm^{-1}, the S/V of the immuno-pillar was 6-15 times larger than that of the microtiter plate, and the reaction rate may be increased by this larger S/V.

References

1. D. A. Berk, F. Yuan, M. Leunig and R. K. Jain, *Biophys. J.*, 1993, **65**, 2428-2436.

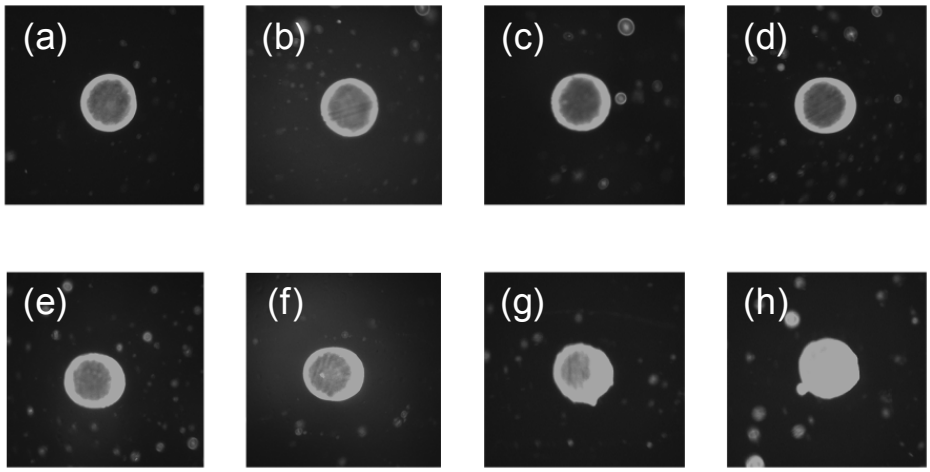

Fig.S1 Fluorescence images of the immuno-pillar at the incubation time of (a) 40, (b) 60, (c) 80, (d) 100, (e) 120, (f) 140, (g) 160 and (h) 180 s.

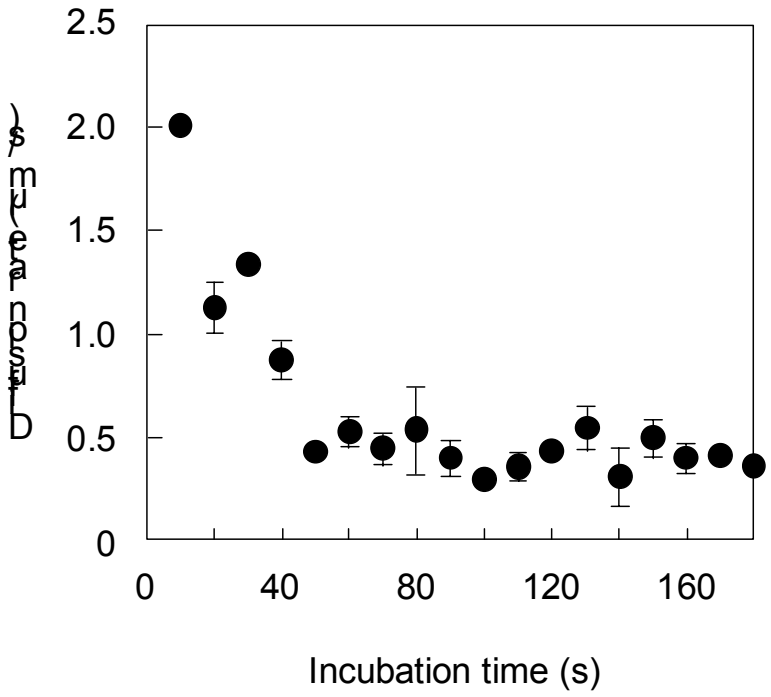

Fig.S2 Dependence of the diffusion rate on the incubation time.

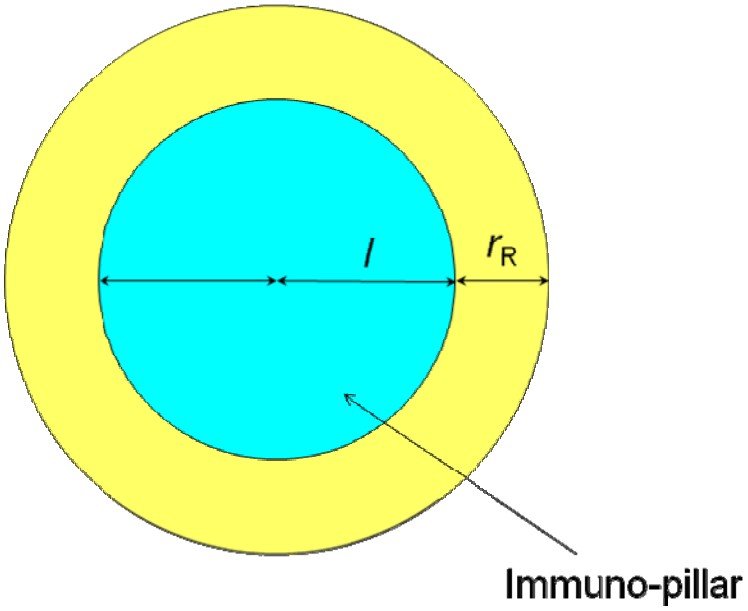

Fig.S3 Schematic illustration of the immuno-pillar and the diffusion area of the antigen and secondary antibody during the incubation time. r_R is the distance to which the sample and secondary antibody diffuse during the incubation time and l is the radius of the immuno-pillar (100 μm).