Electronic Supplementary Material (ESI) for Analytical Methods.
This journal is © The Royal Society of Chemistry 2014

Electronic Supplementary Information

Quantitative-nanoliter immunoassay in capillary immune microreactor adopted inkjet technology

Jianmin Yang, Hulie Zeng, Shuhua Xue, Fengming Chen, Hizuru Nakajima, Katsumi Uchiyama*

Department of Applied Chemistry, Graduate School of Urban Environmental Sciences, Tokyo Metropolitan University, Minamiohsawa, Hachioji, Tokyo 192-0397, Japan

*** Corresponding author**. *E-mail: uchiyama-katsumi@tmu.ac.jp; Tel: +81-42-6772835, Fax: +81-42-6772821*

Part A. Reagents and solutions

Purified human immunoglobulin A (IgA, 1 mg mL^{-1}), goat anti-human IgA (1 mg mL^{-1}), fluorescein-5-isothiocyanate (FITC)-conjugated goat anti-human IgA (FITC-anti-hIgA, 1 mg mL^{-1}), and bovine serum albumin (BSA) were purchased from Bethyl Laboratories (Montgomery, Texas, USA). 3-Aminopropyltriethoxysilane (3-APTES) was obtained from ShinEtsu (Tokyo, Japan). 1-Ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC) was obtained from Thermo Scientific (Rockford, Illinois, USA). N-hydroxysulfosuccinimide (SNHS) and fluorescein sodium salt was acquired from Sigma-Aldrich (Milwaukee, Wisconsin, USA). 2-Morpholinoethanesulfonic acid (MES), glycine, H_2O_2, and H_2SO_4 were supplied by Wako Pure Chemical (Osaka, Japan). Tween 20, toluene, acetone, methanol, KH_2PO_4, Na_2HPO_4, KCl, NaCl, and HCl were purchased from Kanto Chemical (Tokyo, Japan). Extran MA 01 used to clean the inkjet microchip was from Merck (Darmstadt, Germany). Deionized water obtained from a Direct-Q™5 Nihon Millipore ultrapure water system (Tokyo, Japan) and used for preparation of all aqueous solutions. Unless otherwise stated, all chemicals and reagents used in this study were analytical grade quality and used as received.

MES solution used in this experiment contains 0.1 M MES and 0.9% (w/w) NaCl and pH adjust to 4.7. PBS contains 0.01 M phosphate and 0.15 M NaCl and at pH 7.4. Blocking buffer was PBS containing 1% (w/v) BSA. To minimize unspecific adsorption, 0.05% (v/v) Tween 20 was spiked into PBS as wash buffer. Dissociation solution also used as regeneration solution was 0.1 M glycine-HCl at pH 2.2. A HM-25G pH meter (DKK-TOA, Tokyo, Japan) was used to measure the pH of the solutions.

Part B. LIF detection system

A solid blue laser (20 mW, 473 nm, Sanctity Laser, Shanghai, China) was used as the excitation light source and the light was focused by an objective lens (M10/0.25, Olympus, Tokyo, Japan). The fluorescent signal emitted from the sample in the capillary was filtered via a 530 ± 4 nm bandpass filter (A10033-01, Hamamatsu Photonics, Shizuoka, Japan) which was placed orthogonal to the laser beam. Subsequently, the fluorescent signal was collected by the photomultiplier tube (PMT, H10722-01, Hamamatsu Photonics, Shizuoka, Japan) and the signal was amplified by an in-house amplifier. A chromatographic data processing system (CDS-plus, LAS5 Soft, Chiba, Japan) was employed to record the fluorescence signal.

Part C. Capillary internal surface modification

The inner surface of capillary have modified by 3-aminopropyltriethoxysilane (APTES) which was used to covalently immobilize antibody on solid surface[1] to improve the sensitivity of immunoassays.[2-4]

Activation

A new capillary (L = 50.0 cm) was cleaned with acetone and deionized water by syringe pump at a flow rate of 10 μL min^{-1} for 10 min, respectively (Fig. S1a). Then, the capillary was treated with freshly prepared piranha solution (H_2SO_4:H_2O_2 = 3:1, v/v) by syringe pump at room temperature for 10 min. Subsequently, the capillary was cleaned with deionized water and dried under vacuum. Treated capillary was enriched with surface hydroxyl groups and therefore easy to silanization (Fig. S1b).

Amine functionalization

Amine functionalization was performed with aminosilane solution (2% v/v APTES diluted in anhydrous toluene) (Fig. S1c). Initially, the capillary was rinsed with anhydrous methanol, anhydrous toluene and aminosilane solution by syringe pump at a flow rate of 10 μL min^{-1} for 10 min, respectively. Then, capillary filled with aminosilane solution was incubated inside a vacuum desiccator at 75°C for 1 h. After the desiccator was equilibrated to room temperature, the amine functionalized capillary was subsequently washed with anhydrous toluene, anhydrous methanol, and deionized water in order to remove excess unbound APTES from the surface. Afterward, the capillary was dried in vacuum desiccator. Finally, the both ends of modified capillary were sealed with rubbers and stored at room temperature for further use.

Part D. Real sample collection and pretreatment

Whole unstimulated saliva samples were collected from five healthy adult volunteers according to the standard method.[5,6] Two milliliters saliva sample was loaded directly into 15 mL sterilized polypropylene centrifuge tubes (Asahi Glass Co., LTD, Tokyo, Japan) after washing oral cavity with appropriate amount of pure water. Subsequently, all saliva specimens were centrifuged (DSC-154T, Digisystem Laboratory Instruments Inc., Taiwan) at 3200 r min^{-1} for 20 min and the supernatants were collected and stored in -20 °C until assay. The thawed supernatant of saliva samples were diluted with 0.01 M PBS (pH 7.4) before analyzed.

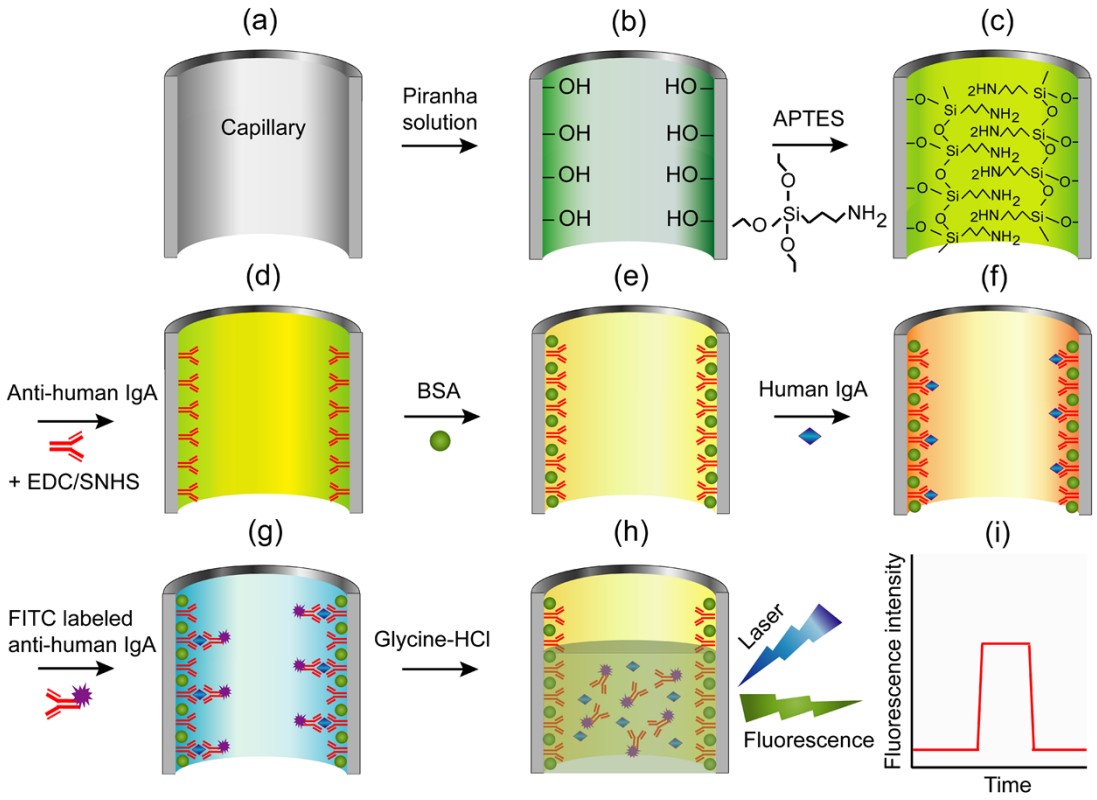

Fig. S1 Illustration of the experimental procedures. (a)-(c): capillary inner surface modification; (d)-(g): immunoassay procedures; (h): the FITC anti-human IgAs were eluted by the glycine-HCl, and (i): LIF detection.

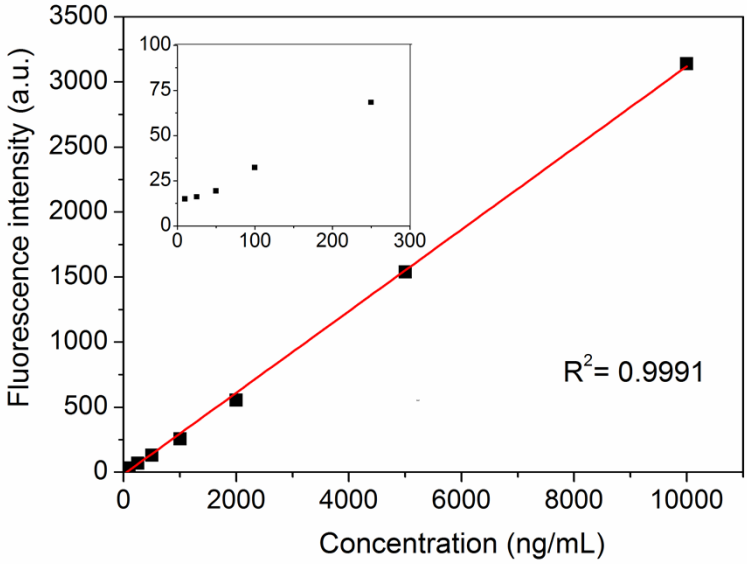

Fig. S2 Calibration curve for fluorescein sodium salt. The concentration were include 10, 25, 50, 100, 250, 500, 1000, 2000, 5000, and 10000 ng mL^{-1}. The RSDs obtained from all the concentrations were below 1.2%.

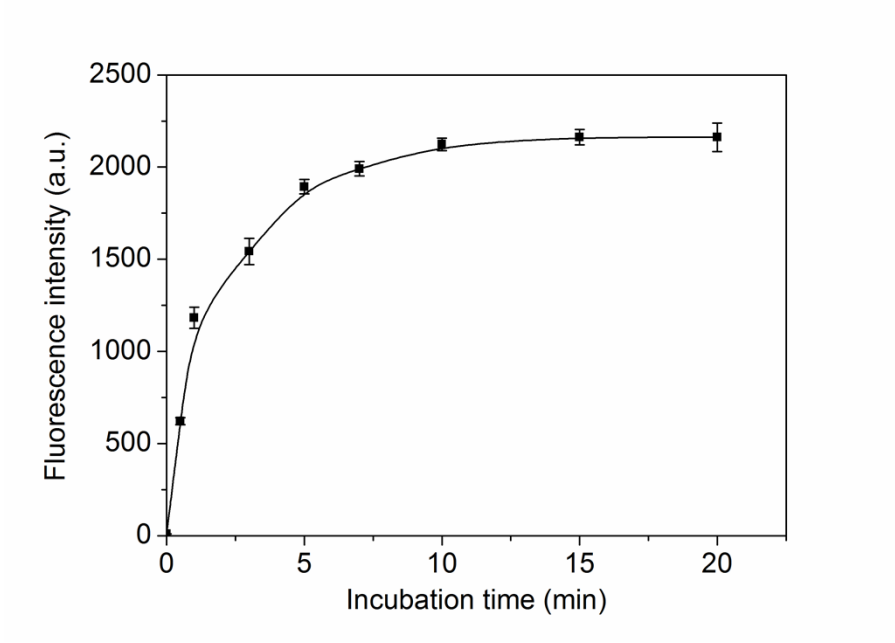

Fig. S3 Optimization of the incubation time between antibody and amine functionalization capillary internal surface wall. The concentration of FITC-anti-hIgA was 1.0 μg mL^{-1}. The error bars showed the standard deviation (SD) based on fluorescence intensity measurements of at least three independent experiments. The RSDs obtained from all the concentrations were below 4.9%.

Table S1 Optimization of the ejection conditions for each solution.

Channel number	Solution	Driving voltage (V)	Duration time (μs)											
			10	15	20	25	30	40	50	60	70	80	90	100
1	FITC-anti-hIgA (5 μg mL^{-1})	40			—	—	×	○	○	○	○	○	×	×
2	Tween 20-PBS (0.05% v/v)	40			—	—	×	○	○	×	×			
3	Human IgA (0.1 μg mL^{-1})	40	—	—	×	×	○	○	○	×	×			
4	Glycine-HCl (0.1 M, pH 2.2)	40	×	○	○	○	×	×	×					

— no ejection, ○ single and straight droplet, × not straight and containing satellite droplet.

Table S2 Determination of human IgA in saliva samples with the proposed system and conventional ELISA on 96-well plate.

Sample	Proposed system		96-well plate	
	Concentration (mean ± SD, μg mL^{-1})	RSD (%)	Concentration (mean ± SD, μg mL^{-1})	RSD (%)
1	204.9 ± 9.3	4.54	198.7 ± 14.4	6.69
2	221.6 ± 7.9	3.56	212.4 ± 8.0	3.48
3	210.7 ± 4.4	2.09	206.6 ± 11.8	5.28
4	239.2 ± 4.8	2.02	231.3 ± 21.2	8.55
5	201.8 ± 6.9	3.42	205.8 ± 17.3	7.78

References

1 J. Doh and D. J. Irvine, *Proc. Natl. Acad. Sci. U. S. A.*, 2006, **103**, 5700-5705.

2 C. K. Dixit, S. K. Vashist, F. T. O'Neill, B. O'Reilly, B. D. MacCraith and R. O'Kennedy, *Anal. Chem.*, 2010, **82**, 7049-7052.

3 C. K. Dixit, S. K. Vashist, B. D. MacCraith and R. O'Kennedy, *Nat. Protoc.*, 2011, **6**, 439-445.

4 X. Zhang, C. Song, L. Chen, K. Zhang, A. Fu, B. Jin, Z. Zhang and K. Yang, *Biosens. Bioelectron.*, 2011, **26**, 3958-3961.

5 S. M. Van Anders, *Am. J Hum. Biol.*, 2010, **22**, 348-352.

6 G. Bachrach, Z. Muster, I. Raz, G. Chaushu, A. Stabholz, G. Nussbaum, M. Gutner and S. Chaushu, *Oral Dis.*, 2008, **14**, 51-59.