Electronic Supplementary Material (ESI) for Lab on a Chip

Supporting Information

Ultrasensitive Microfluidic Solid-Phase ELISA Using an Actuatable Microwell-Patterned PDMS Chip

Tanyu Wang[1,2], Mohan Zhang[1], Dakota Dreher[1], and Yong Zeng[1,2,3,*]
1. Department of Chemistry, 2. Ralph N Adams Institute of Bioanalytical Chemistry, 3. Bioengineering Graduate Program, University of Kansas, Lawrence, KS 66045
* Corresponding author.
Tel: (785) 864-8105; Fax: (785) 864-5396; E-mail: yongz@ku.edu

Supporting Figure S1.

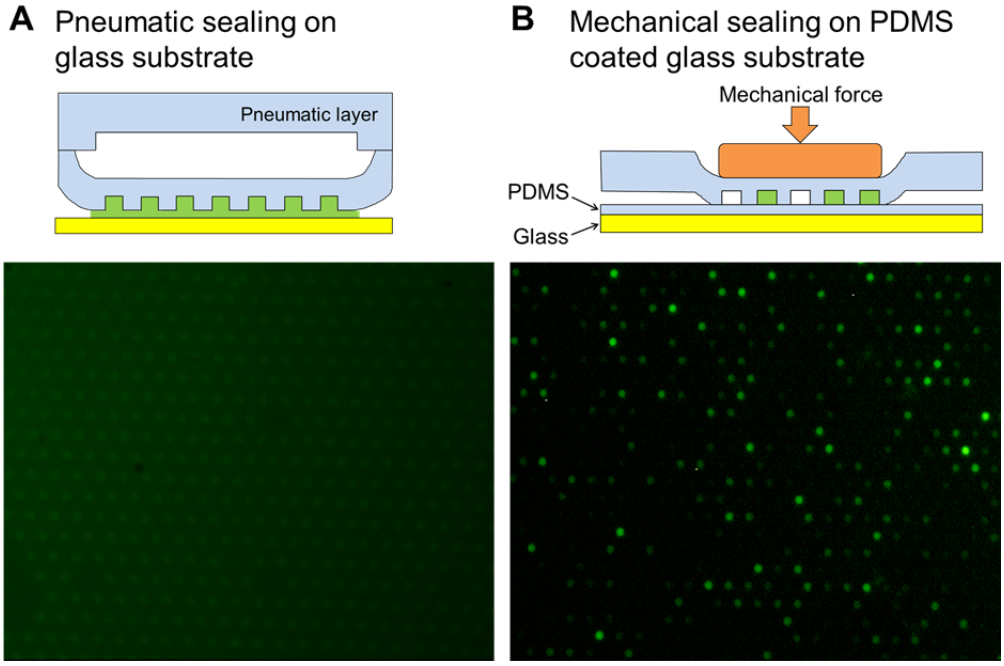

Figure S1. Chemifluorescence detection of β-galactosidase (β-gal) using the femtoliter microwell arrays (~83 fl). Here a final concentration of 1 ng/mL β-gal was used, which is equivalent to an effective concentration of 0.09 copies per well. (A) The PDMS chip was assembled directly onto a clean glass slide. The assembled device was first primed with 1% BSA to coat the channel surface for 30 mins. Then 2 ng/mL β-gal was mixed with 0.5 mM FDG substrate in PBS working buffer and injected into the assay chamber immediately. A relatively low pressure (60 kPa) was applied to press the microwell-patterned membrane against the glass surface. It was observed that all the wells became fluorescent after 20 min incubation. (B) The microwell-patterned PDMS chip was assembled onto a glass slide spin-coated with a thin layer of PDMS. The assay procedure was the same as in (A) except that the PDMS microwells were sealed tightly against the PDMS coated substrate by a strong mechanical force. In this case, digital counting of single β-gal molecules statistically partitioned into individual femtoliter wells was achieved at the same β-gal concentration as in (A). This comparison confirms the formation of a thin-layer of aqueous solution connecting the wells in the experiment of (A).

Supporting Figure S2.

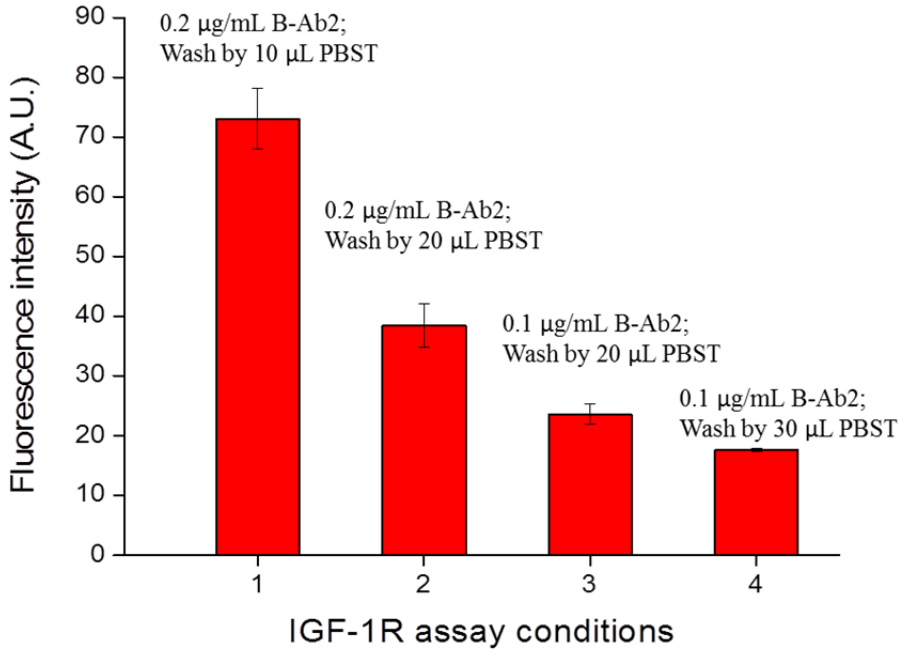

Figure S2. Effects of the concentration of biotinylated detection antibody (B-Ab2) and washing volume on the background for the microfluidic IGF-1R assay. The combination of 0.1 mg/mL B-Ab2 with 30 µL PBST washing yielded a dramatically reduced background level without excessively extending the assay time. Chemifluorescence detection conditions: [SβG] = 0.2 µg/mL, [FDG] = 0.5 mM, reaction time = 900 s and exposure time = 2.5 s. Error bars indicate standard deviations of fluorescence intensity in the microwells (n > 100) in multiple images obtained in one experiment.

Supporting Figure S3.

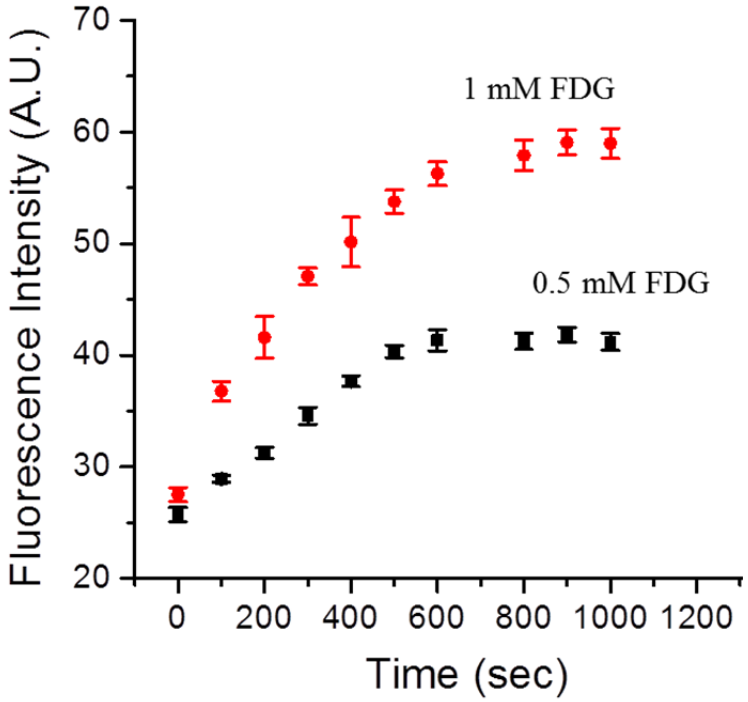

Figure S3. Time-lapse measurements of fluorescein products converted by β-gal captured by the sandwiched antibody-antigen complex on the surface at [FDG] of 0.5 and 1 mM. . Chemifluorescence detection conditions: [IGF-1F] = 0.1 ng/mL, [SβG] = 0.2 μg/mL, exposure time = 2.5 s. Error bars indicate standard deviations of fluorescence intensity in the microwells (n > 100) in multiple images obtained in one experiment.

Table 1S. Performance comparison between our microfluidic ELISA and the commercial ELISA kits for IGF-1R.

Human IGF-1R Assay	Manufacturer	LOD (pg/mL)	Detection Range (pg/mL)	Sample Consumption	Detection Antibody Consumption	Total Incubation Time[d]
Microfluidic ELISA	present work	0.0035[b]	0.01 - 1000	10 μL	10 μL	0.75 h
Commercial ELISA kits[a]	Uscn Life Science Inc.	105[c]	312 - 20000	100 μL	100 μL	3.5 h
	RayBio / abcam	6	8.23 - 6000	100 μL	100 μL	4.25 h
	Millipore	160	160 - 10000	100 μL	100 μL	3 h
	Assaybiotech / Abnova	250	250 - 16000	100 μL	100 μL	4.5 h

a Detection is based on absorption measurement using HRP/TMB
b LOD is calculated from the value of blank signal plus three standard deviations.
c LOD is calculated from the value of blank signal plus three standard deviations.
d Total incubation time excludes the time for immobilization of capture antibody and washing steps.