Liposome immunoassay based on bioluminescent detection and its application to on-chip analysis

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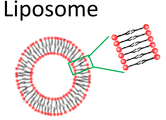
INTRODUCTION

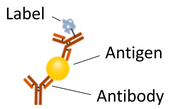
1) Background

a) Liposome



Offer an aqueous media surrounded by lipid bilayer membranes, in which many kinds of enzymes can be encapsulated.

b) Immunoassay



Widely used in clinical diagnoses, environmental analyses and biochemical studies because of its extremely high selectivity and sensitivity.

2) Luc-encapsulated liposome

Firefly Luciferase (Luc)

- ✓ High emission efficiency (Φ=0.41)
- × Luc activity loss by labeling
- × Small number of label

⇨

Luc-encapsulated liposome

- ✓ Label with many Lucs
- ✓ No need for direct modification
- ✓ Higher activity of Luc
- ✓ Higher stability of Luc

3) On-chip immunoassay

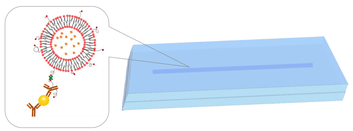
Microtiter plate

- × Large sample volume
- × Long measuring time

⇨

Microchip

- ✓ Small sample volume
- ✓ Short measuring time



Develop on-chip immunoassay using Luc-encapsulated liposome as a label

EXPERIMENT

1) Material

a) Luc-encapsulated liposome

Thermostable Luc : purified from *E.coli*.

Liposome : prepared by freeze-thaw methods with extrusion and gel-filtration chromatography.

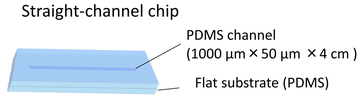
Composition of liposome	
DMPC (T_m=25 ℃)	30 % mol
DPPC(T_m=41 ℃)	29.9 % mol
Cholesterol	40 % mol
Biotinylated PE	0.1 % mol

Liposome size : 300 nm

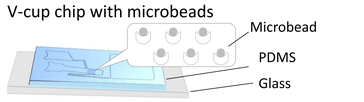
Estimate Luc molecule / liposome : 58000

b) Microchip

Straight-channel chip

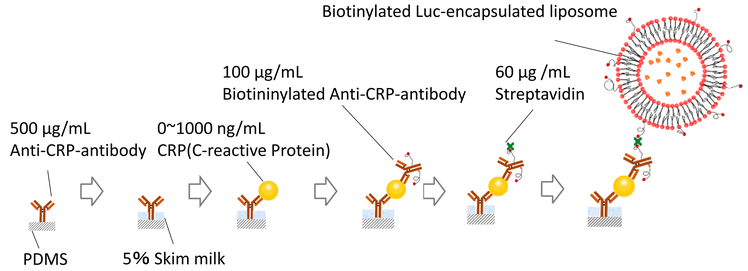


V-cup chip with microbeads



Burger, Robert, et al. *Lab on a Chip.* **2015**, 15(2), 378-381.

2) Immunoassay procedure



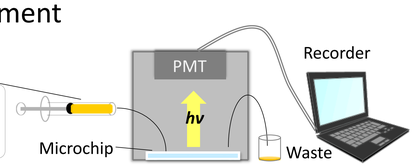
Incubation (5 min) and washing with TBS (2 times) are included in each step

3) Bioluminescence measurement

a) BL (bioluminescence) detector

Substrate 10 μl (300μl/min for 2 sec)

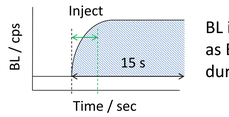
0.2 mM Luciferin	24 mM Mg^{2+}
1 mM ATP	2 mM DTT
60 μg/mL BSA	0.05M Tris-HCl buffer(pH 8.0)



b) BL reaction

$$\text{Luciferin} + \text{ATP} + O_2 \xrightarrow[Mg^{2+}]{\text{Luc}} \text{Oxi-luciferin} + \text{AMP} + CO_2 + \text{PPi} + h\nu$$

c) BL response



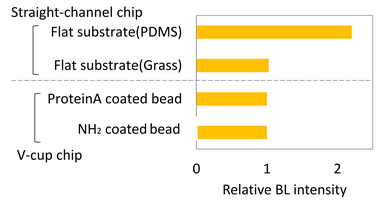
BL intensity was defined as BL integration during 15 sec.

RESULTS

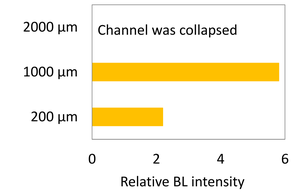
1) Optimization of microchip

Firstly, immunoassay for CRP was carried out on microbead and Straight-channel chip.
Relative BL intensity is defined as (BL intensity of 1000 ng/mL CRP)/(BL intensity of 0 ng/mL CRP).

(a) Bead and flat substrate



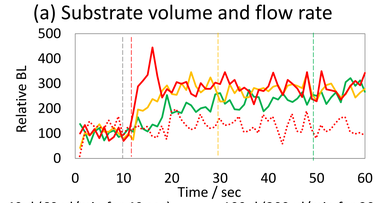
(b) Channel width (Straight-channel chip)



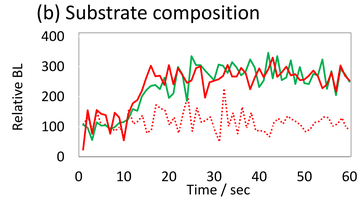
2) Optimization of substrate injection

Secondly, we study the effect of substrate volume, flow rate and substrate composition on BL response. Relative BL is defined as average of background during 10 sec = 100
Solid lines was BL of 1000 ng /mL CRP and dot lines was BL of 0 ng/mL CRP.

(a) Substrate volume and flow rate

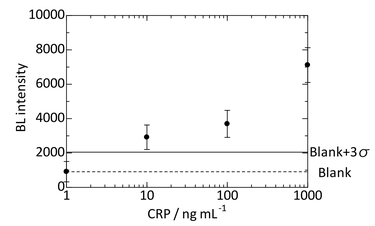


(b) Substrate composition



3) Calibration curve

Finally, immunoassay for CRP was carried out under optimum assay condition.
The limit of detection (LOD) defined as the concentration giving BL of blank + 3σ.
BL measurement was carried out 3 times at each concentration (n=3).



CONCLUSION

Straight-channel chip was better than microbead for V-cup chip to immobilize many antibody. Straight-channel chip that has PDMS flat substrate and wider channel (1000 μm) was optimum for this assay. To detect high BL intensity, fast flow rate (300 μl/min) was better than slow flow rate because channel was filled up with substrate quickly. However, substrate volume and lysis buffer didn't affect to detect high BL intensity. This means a sufficient amount of substrate was delivered to Luc in liposome. The LOD of CRP in this assay was 10 ng/mL. This result shows Luc-encapsulated liposome can be applied to on-chip immunoassay.