INTEGRATION OF NEURAMINIDASE INHIBITOR ASSAY INTO SINGLE STEP OPERATION USING COMBINABLE PDMS CAPILLARY (CPC) SENSOR

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ABSTRACT

Neuraminidase inhibitor assay requiring complicated step-by-step operation was successfully integrated into ”Single-Step” by using CPC sensor. We developed a combinable PDMS capillary (CPC) sensor for enzyme inhibition assay using the concave and convex type PDMS substrates. In the conventional enzyme inhibitor assay, complicated analytical procedures, such as mixing and dissolution, are required. In contrast, this sensor allows single-step assay possible only by sample introduction via capillary action. Here, we applied this technique for neuraminidase inhibitor assay as application and found appropriate experimental condition allowing single-step assay of neuraminidase inhibitor.

KEYWORDS

Capillary array sensor, Combinable PDMS capillary, Neuraminidase inhibitor assay, Single step operation

INTRODUCTION

Neuraminidase is an enzyme which exists on the surface of an influenza virus. This releases the newly reproduced virus from a host cell and promotes the infection to a surrounding cell (Fig. 1).

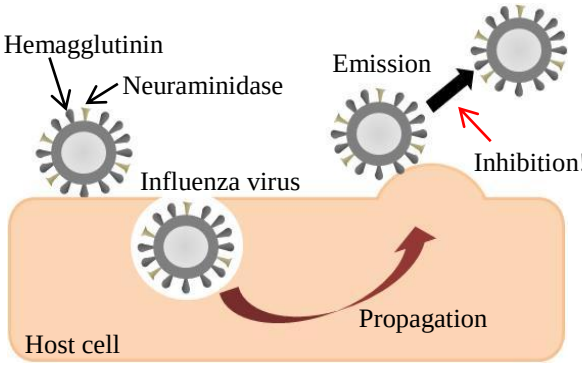

Fig.1: The principle of infection of influenza virus by neuraminidase

For this reason, neuraminidase inhibitor assay is very important for influenza drug development. However, in the conventional assay [1], complicated step-by-step operation is necessary, because optimal pH of the enzyme reaction differs from that of the fluorescence detection.

On the other hand, recently, we developed a combinable PDMS capillary (CPC) sensor for enzyme inhibitor assay using the concave and convex type PDMS substrates immobilizing enzyme and fluorescent substrate independently (Fig. 2).

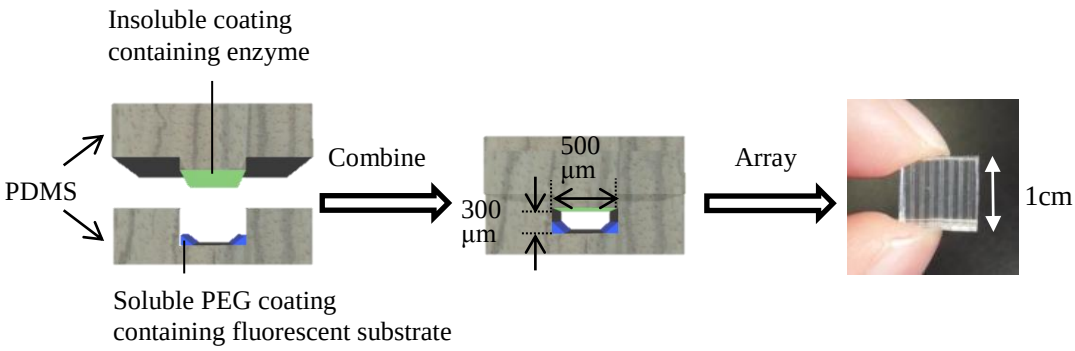

Fig. 2: Preparation of CPC sensor

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In the conventional enzyme inhibitor assay, complicated analytical procedures, such as mixing and dissolution, are required. However, this sensor allows single-step assay possible only by sample introduction via capillary action (Fig. 3).As a proof of concept, single-step trypsin inhibitor assay was successfully performed previously [2].
Thus, this work is aiming at the single step detection of the neuraminidase inhibitor using CPC sensor by controlling the pH of the sample inhibitor solution, and evaluation of the inhibition constant using typical inhibitor.

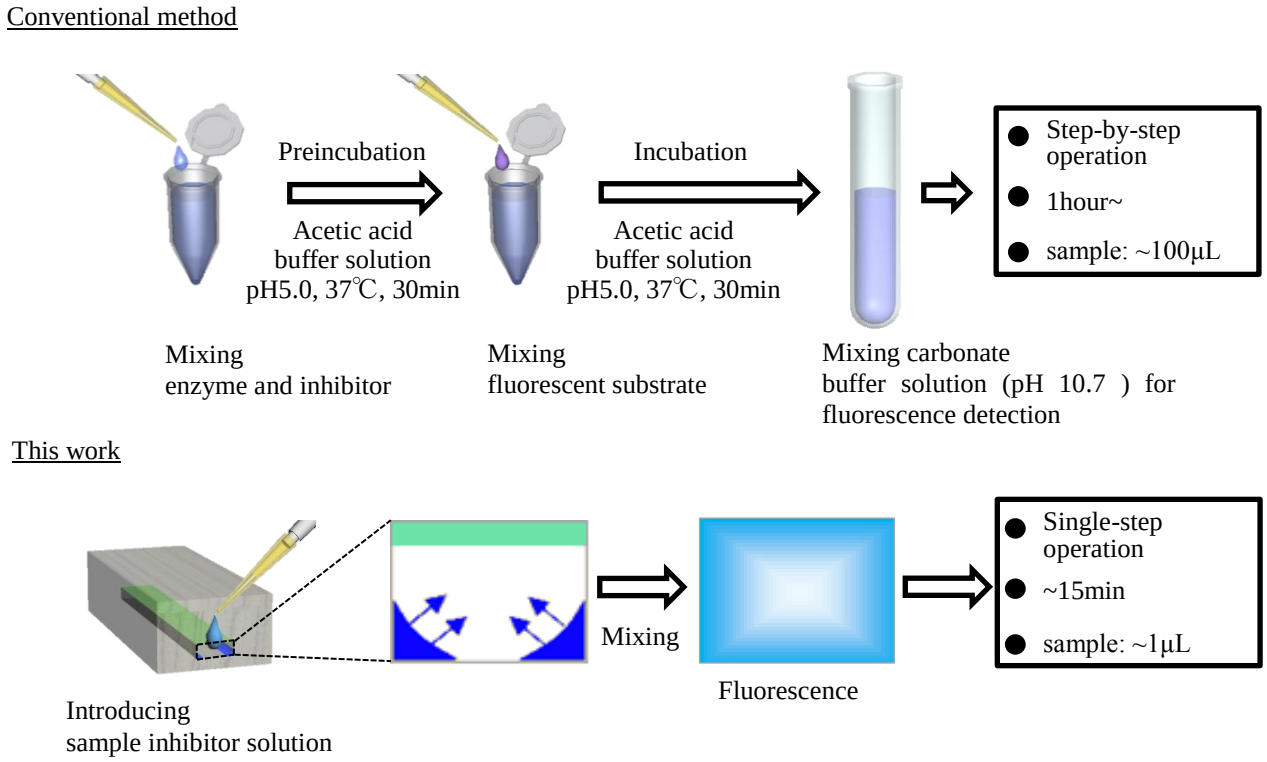

Fig. 3: Comparison of conventional inhibitor assay and single step inhibitor assay using CPC sensor

EXPERIMENT

”Concave”- and ”Convex”-shaped PDMS substrates were prepared by the conventional molding.
(1) The method of immobilizing the fluorescent substrate to the concave-shaped PDMS substrate
Fluorescent substrate solution containing PEG 20000 and PDMS-PEG was introduced into concave-shaped PDMS substrate, then, the soluble coating was immobilized by vacuum drying.
(2) The method of immobilizing neuraminidase to the convex-shaped PDMS substrate
After adsorbing BSA on the surface of the convex-shaped PDMS substrate, neuraminidase was covalently immobilized using glutaraldehyde.
Finally, the concave and convex-shaped PDMS substrates were combined, and then fluorescence intensity was measured with fluorescence microscope by introducing buffer solution or inhibitor solution.

RESULTS AND DISCUSSION

First, we investigated the optimal pH which allows neuraminidase inhibitor assay to be attained by single step using CPC sensor, and found that strong fluorescence response was observed at pH7.8. When the reaction profile was investigated under this condition, reaction time was shortened from 1 hour in the conventional assay to 15 minutes. The sample solution required in this assay was 1 μL or less by using CPC sensor, while it was 100μL in the conventional assay. Next, when neuraminidase inhibitor assay was performed using typical inhibitor, N-Acetyl-2,3-dehydro-2-deoxyneuraminic acid, inhibition constant Ki of 4.1×10^{-6}, which was very close to the literature value (8.6×10^{-6}) [3], was obtained (Fig. 4). Ki was calculated from obtained IC_{50} [4]. Finally, comparison of fluorescence responses for buffer solution and well-known influenza drug of Relenza solution, was demonstrated and found successful inhibition by Relenza (Fig. 5).

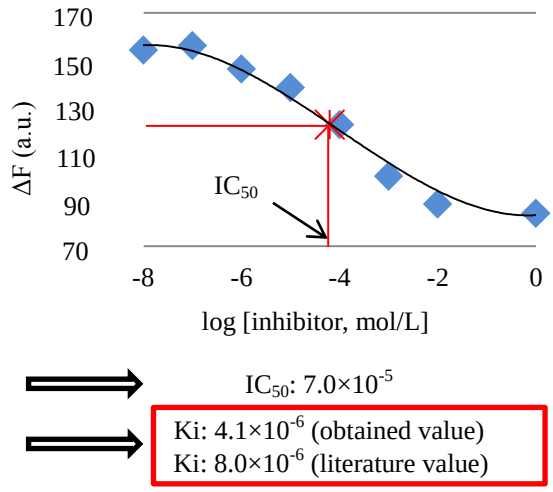

Fig. 4: Neuraminidase inhibitor assay using CPC sensor array

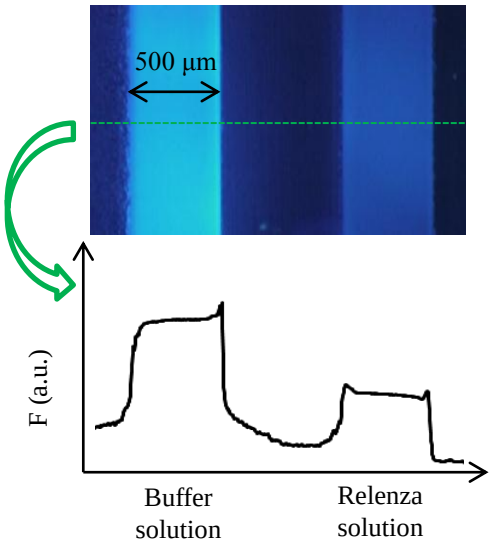

Fig. 5: Inhibition by Relenza

CONCLUSION

In conclusion, integration of neuraminidase inhibitor assay into single step operation was successfully performed by using CPC sensor. In addition, sample consumption was reduced 100-fold and reaction time was also reduced 4-fold. Furthermore, this concept could be applied to Relenza which was well-known as an influenza drug. An arraying various CPC sensors for different inhibitor assays is expected to develop a useful drug screening device.

ACKNOLEDGEMENTS

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