RAPID IMMUNOASSAY USING STEADY-STATE DISPERSION EFFECTS IN NANOCHANNELS

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ABSTRACT

We present a novel method for on-chip competitive dispersion immunoassay (DISIA) which aims at measuring the specificity between fluorescently-labeled antibodies (Ab*) and label-free antigens (Ag). We have fabricated a nanofluidic device that allows fast detection of interaction of antibodies and antigens (< 30s) based on lateral dispersion of fluorescent species in a nanoslit, using small amounts of solution (< 1 μl). We measured that doubling the weight of streptavidin protein results in an increase of 30% of the lateral dispersion of fluorescent coupled molecules in the nanoslit, which can be read easily.

KEYWORDS: Dispersion Immunoassay, Lateral dispersion, Nanofluidics

INTRODUCTION

Immunoassays are tests that identify a substance (for instance a protein) by its capacity to act as an antigen. Compared to classical methods, micro- and nano-immunoassays present several advantages as limited reagent consumption, faster analysis time due to a larger surface-to-volume ratio and improved mass transport efficiency.

As presented in figure 1, the nanofluidic device consists of two microchannels connected together by a nanochannel which ends in a lateral nanoslit area, parallel to the output microchannel.

Figure 1. (a) 3D representation of our nanofluidic device. In the bottom Pyrex wafer two wet-etched microchannels and a nanoslit defined by the deposited 50nm layer of amorphous silicon are closed by an anodically bonded pyrex cover. Solutions of antibodies and antigens are driven in the input microchannel and PBS in the output one. (b) SEM Picture of a nanochannel defined by the thickness of the amorphous silicon (top view). The white arrows represent the flow direction in the nanochannel, in the nanoslit area and finally ending in the microchannel.
EXPERIMENTAL

The microchannels are wet-etched in HF solution to a depth of 4 μm. Using standard photolithography and plasma etching (DRIE) on a 50nm layer of amorphous silicon (aSi) we obtain the nanochannel and nanoslit structures. Finally, the device is sealed by anodic bonding to a second Pyrex wafer containing the powderblasted access holes.

In our previous work [1], we have demonstrated that the lateral dispersion of species in the nanoslit is inversely proportional to their diffusion coefficient. Based on these findings, a specific antibody-antigen complex, having a lower diffusion coefficient than the single antibodies and antigens, results in an increase of the dispersion in the nanoslit area (figure 2).

RESULTS AND DISCUSSION

In the first experiments we added 2μM fluorescently-labeled Wheat Germ Agglutinin (WGA) in a solution of 10mM KCl in DI water (pH=7.7) and applied a fluid velocity in the nanochannel of 130nm/s. A flux in the range of several nl/min was also imposed in the output microchannel in order to avoid the filling of the nanoslit by fluorescent molecules from the microchannel. Figure 3 shows that the measured lateral distance of dispersion of fluorescently-labeled WGA is in good agreement with the simulated one.

Figure 2. (a) Finite Element simulation of the fluorescently-labeled antibodies (Ab*) concentration repartition in the nanoslit area after exiting the nanochannel. Colored areas correspond to relative intensity higher than 50% for dispersion of Ab* alone (dark grey) and Ab*-Ag complex (light grey). (b) Plotlines representing the simulated lateral distances of dispersion in both cases.

Figure 3. (a) Video image showing the dispersion of fluorescently-labeled WGA in the nanoslit in the conditions described above. (b) Plotline representing the experimental normalized intensity of conditions a), measured by the camera at y=1μm and compared with the simulated value (fitted with \(D_{\text{eff}}=8\cdot10^{-15} \text{m}^2/\text{s}\)).
We reproduce the experiment with 2μM fluorescent streptavidin (53kDa, from Invitrogen) before and after adding 6μM biotin-dextran (10kDa, from Sigma-Aldrich). Four biotin-dextran can link to each streptavidin and increase its molecular weight by a factor 2.

![Figure 4](image)

**Figure 4.** Measured lateral distance of dispersion as a function of the input pressures (i.e. flow velocities) for 2μM streptavidin and after adding 6μM of the complex biotin-dextran.

For a constant flow speed in the nanochannel, an increase of 30% of the lateral distance of dispersion is obtained when the molecular weight of our fluorescently-labeled streptavidin is doubled. This sensibility can also be improved when the primary fluorescent antibody is much smaller than the added antigen.

CONCLUSIONS

We have developed a rapid competitive assay based on the reading of lateral dispersion effects in a nanoslit. The fact that labeled antigens are not required, and the short time needed to achieve steady-state dispersion (few seconds), are unique advantages that our nanofluidic device offers compared to Diffusive Immunoassays (DIA) [2]. Our method therefore provides a powerful tool for fast detection and characterization of a wide range of analytes present in immunology and immuno-chemistry.

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REFERENCES
