NANOFLUIDIC PRECONCENTRATION DEVICE FOR SENSITIVE AND WIDE DYNAMIC RANGE IMMUNO-SENSING

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ABSTRACT

This paper describes a microfluidic immunoassay device with integrated preconcentration system, enabling the detection of various analytes (proteins) at low target concentrations with faster binding kinetics. It not only complements recent advances of novel immuno-biosensors, but also can be used to further enhance the most novel and advanced immuno-biosensor systems developed. The work has demonstrated significant sensitivity/kinetics enhancement by the preconcentration of antigens before the binding.

Keywords: Nanofluidics, preconcentration, immuno-sensing

1. INTRODUCTION

One of the key technological challenges in immuno-biosensing is its sensitivity and selectivity is inherently dependent on the properties of antibody-antigen pairs. Reliable detection becomes progressively harder and requires longer reaction time with lower target analyte concentrations, which are often the case in disease biomarker detection. While much progress has been made in the area of immuno-detection of proteins and other biomolecules, most of the existing techniques rely on the post-binding amplification (either physical or chemical) to enhance the signal created by the primary binding events. Therefore, the sensing would be still ultimately limited by the binding characteristics of primary antibodies. In this study, we have developed a novel strategy using electrokinetic pre-binding concentration. Instead of amplifying the signal after the primary immuno-reaction, we seek to enhance the concentration of the target molecule before the reaction using a unique molecular preconcentration device. The uniqueness of this novel method comes from the fact that the sensitivity, selectivity and kinetics of binding between the target analyte and the (primary) antibody are significantly enhanced, via an efficient preconcentration process before the binding. Because the number of target analytes near the binding site is increased via preconcentration, binding characteristics of primary antibody-antigen is directly improved, therefore leading to faster and more sensitive detection. We have demonstrated 500~1000 fold enhancements in sensitivity and much faster binding using a standard, bead-based immunoassay device over a molecular background.

2. EXPERIMENTAL

The idea was implemented by integrating nanofluidic preconcentrator and standard bead-based immunoassay in a microchip, therefore, the target concentration can be continuously increased at the site of primary immuno-binding by the concentrator. The nanofluidic preconcentrator applies the unique ion depletion force from charge-selective sub-50 nm nanofluidic channel to trap biomolecules in the adjacent microchannel (12x50 μm). To integrate the concentrator with immuno-sensors, capture (primary) antibodies were immobilized on streptavidin-labeled polystyrene beads and delivered to a predefined sample collection area of the preconcentrator. While the major limiting factor in low

concentration analyte detection is posed by the quality of the antibody (binding constant, K_D), prolonged incubation time is unavoidable to reach sufficient primary binding. The presented integrated device can be used to address these issues by adjusting the analyte concentration and hence the reaction kinetics and sensitivity [1].
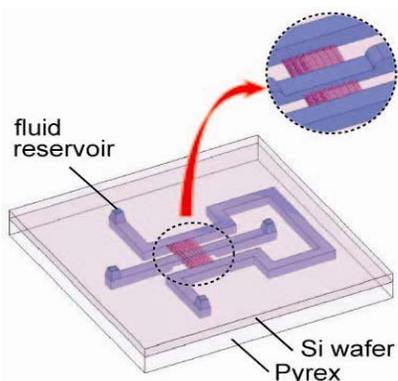

Figure 1. Schematics of the nanofluidic preconcentrator. The center sample channel is connected to the U shaped buffer channel by nanochannel arrays. The close view shows the nanochannels and bead trapping structure from the back side.
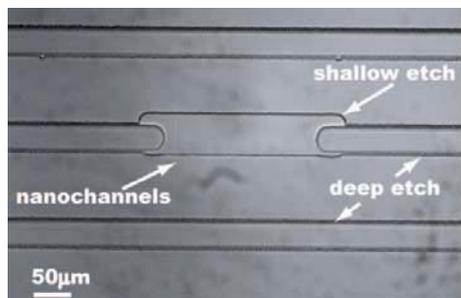

Figure 2. Bright field image of the nanofluidic channel array and bead trapping dam structure. The 40 nm deep nanochannel arrays have a width of 200 µm and are bridging microchannels 100 µm away from each other. As for the microchannels, except of the 5.5 µm shallow etches for bead trapping, all regions were etched to a 12 µm depth.
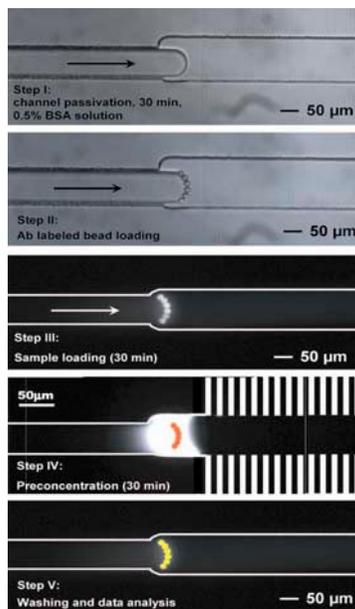

Figure 3. Bead loading, immunosensing and preconcentration procedure (target molecule R-phycoerythrin, false color images).
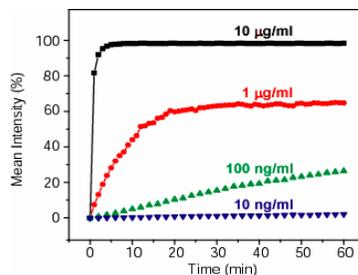

Figure 4 Binding kinetics of R-PE samples with various concentrations, the intensity represents the fluorescent signal from R-PE molecule captured on polystyrene beads.

3. RESULTS AND DISCUSSION

The novel electrokinetic preconcentration device was first published in 2006 [2], where we reported a million-fold biomolecule pre-concentration in a silicon-based nanofluidic system. Because the preconcentrator can increase the local biomolecule concentration by many orders of magnitude without complex buffer arrangement or in-line porous material, it allows direct coupling between the preconcentrator and the immuno-sensor.

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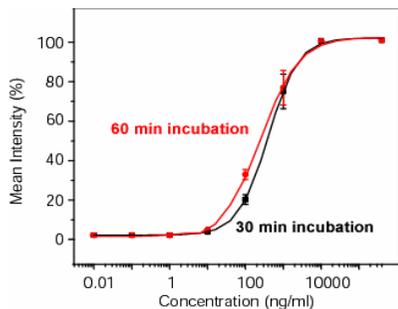7 – 11 October 2007, Paris, FRANCE

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Figure 5 Dose response curves of R-PE molecule on anti-R-PE beads. As shown in the plot, longer incubation time can only slightly increase the signal intensity. Compares the responses between 30 min and 1 hr incubation, the detection limited (10 ng/ml) is not improved by 30 min longer incubations.
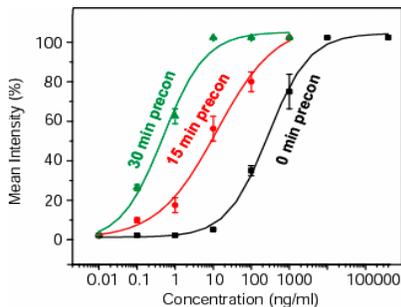
Figure 6 Plots shown the dose response of the immunoassay without preconcentration, with 15 min and 30 min preconcentration. Through maintaining a 30 min on-site preconcentration, this approach can lower the sensitivity limit by about 500 fold from 50 pM to sub 100 fM range. (with 10 µg/ml GFP as simulated molecular background)

Figure 1 is the layout of the device, which has nanochannel arrays on both side of the trapping region. **Figure 2** shows the image of the preconcentrator and the bead trapping area defined by two-step wetting etching process. The shallow microchannel was etched to a depth of 5.5 µm to hinder the 7.5 µm polystyrene beads (with anti-phycoerythrin labeled) and the deep microchannels were etched to a depth of 12 µm. The experimental procedure is shown in **Figure 3**. After channel passivation with BSA, beads and sample buffers where introduced before we started the preconcentration. In **Figure 4**, analytes with lower initial target concentration takes longer to reach binding equilibrium due to the diffusion-limited transport. **Figure 5** shows a 3 orders of magnitude dose response of the assay. In **Figure 6**, with a 30 min preconcentration we were able to enhance the immunoassay sensitivity by more than 500 fold from 50 pM to the sub 100 fM range. This was achieved with simulated molecular background in the sample solution, demonstrating possibility of detecting directly from complicated protein mixtures. Moreover, by adjusting the preconcentration time, we can tune the immunoassay detection range to cover 6 orders of magnitude concentration variation, which is critical for multiplexed detection of multiple target molecules.

4. CONCLUSIONS

As the system can be coupled to existing sensing and post-amplification techniques, it could be used to enhance the dynamic range of detection, binding kinetics, and sensitivity. We believe the integrated device can effectively address the most challenging detection issues in immuno-sensing. Moreover, the preconcentration system has been tested successfully for detection from serum samples with albumin and IgG depletion. Since the preconcentrator can concentrate target enzymes rapidly, it can also be used to probe various biochemical activities associated with human physiology.

REFERENCES

[1] P. R. Nair and M. A. Alam, *Applied Physics Letters* 88, 233120 (2006).
[2] Y.-C. Wang, A. L. Stevens, and J. Han, *Analytical Chemistry* 77, 4293 (2005).

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