MICROFABRICATED QLISA BIOSENSORS WITH AN EMBEDDED MIXING ELEMENT

Peter Clark, Chengjie Yu, Elisabeth Papazoglou, and Hongseok (Moses) Noh
Drexel University, USA

ABSTRACT:

Optically transduced microfluidic immunoassays have proven to be a highly sensitive and rapid method to assess the concentrations of analytes within biological samples. Although microfluidic immunoassays facilitate higher throughput and automation than standard microtiter plates, the immunoreaction within such devices remains diffusion limited unless there is a way to achieve recursive or continuous sample flow. We aim to circumvent this issue and accelerate the immunoreaction by developing a microfluidic immunosensor with an integrated set of electrodes to facilitate electrothermal flow resulting from joule heating. We have demonstrated superior mixing and sensitivity with our novel quantum dot linked immunoassay biosensor platform, effectively reducing sample incubation times and reagent consumption compared to the standard microtiter plate immunoassay format.

KEYWORDS: Microfluidic Immunoassay, Quantum Dot, Electrothermal effect, Biosensor

INTRODUCTION

Microtiter plates have long been used to determine the concentration of various analytes present within biological samples with excellent sensitivity and specificity. The aim of next generation microfluidic immunosensors is to enhance the performance of immunoassays by facilitating high throughput sample analysis on microfluidic devices integrated with various components including micromixers, valves, micropumps, electrical and optical components to follow the design and development of highly automated, precise and portable micro total analysis systems capable of multianalyte detection as a point-of-care diagnostic platforms. The advantages that microfluidic immunosensor platforms have over standard microtiter plate based assays arise from their higher surface area to volume ratio, allowing smaller working volumes, decreased reagent consumption and shorter characteristic diffusion lengths for biomolecules.

The developed microfluidic immunoassay platform presented here is based upon a predicate, poly(methyl methacrylate) (PMMA) capillary immunoassay device coupled to an optical excitation and detection system for analyte detection. The heterogeneous immunoassay utilizes a sandwich immunoassay format with quantum dot conjugated detection antibodies to generate an optical signal and subsequently calculate analyte concentration. We have improved upon our initial design by fabricating microchannels on a PMMA substrate hermetically sealed onto a silicon substrate patterned with electrodes for parallel sample analysis and electrothermal mixing to enhance reaction kinetics and decrease incubation time. Our studies have shown comparable limits of detection and sensitivity to standard microtiter based immunoassays while requiring less than 1ul of sample and significantly reduced reagent consumption over conventional immunoassay methods.

THEORY

Static heterogeneous immunoassays may be modeled as a diffusion reaction system and have been well defined within the literature. Our system utilizes integrated electrodes to produce a thermal gradient within the microchannel, inducing electrothermal fluid mixing. The model used to calculate the theoretical particle velocity under electrothermal flow (ETF) is based upon previous models within the literature as well as our previously developed models. Briefly, the ETF effect was simulated using COMSOL by simultaneously solving for the electric potential of the system (1), the Navier-Stokes equation that includes the electrothermal force term (2) and the convection-conduction equation (3).

$$\nabla\left(\varepsilon\nabla V\right)=0 \tag{1}$$

$$\rho\frac{D\vec{V}}{Dt}=-\nabla p+\eta\nabla^2\vec{V}+\vec{f}_{ETF} \tag{2}$$

$$k\nabla^2T+\sigma E^2=\rho c_p\vec{V}\cdot\nabla T \tag{3}$$

EXPERIMENTAL

Two biosensor prototypes were microfabricated, one with integrated electrodes and one without electrodes in order to assess the effect of electrothermal mixing upon immunoassay kinetics. Both designs used a micropatterned piece of PMMA containing the microchannel design, which was then sealed to either a silicon substrate patterned with electrodes or another

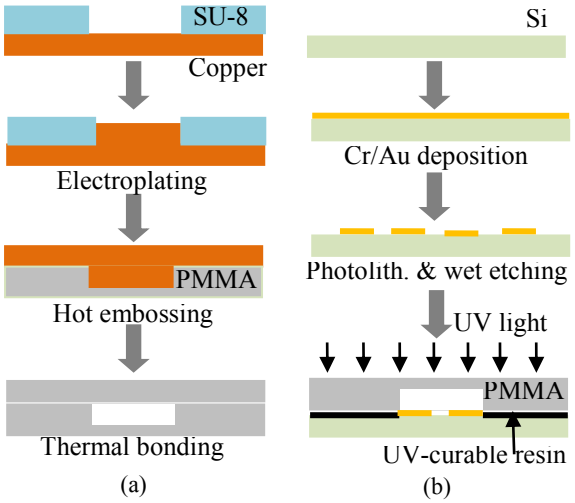

Figure 1: (a)Fabrication flow for all PMMA micro-channel and (b) PMMA-Si microchannel with an embedded mixing element.

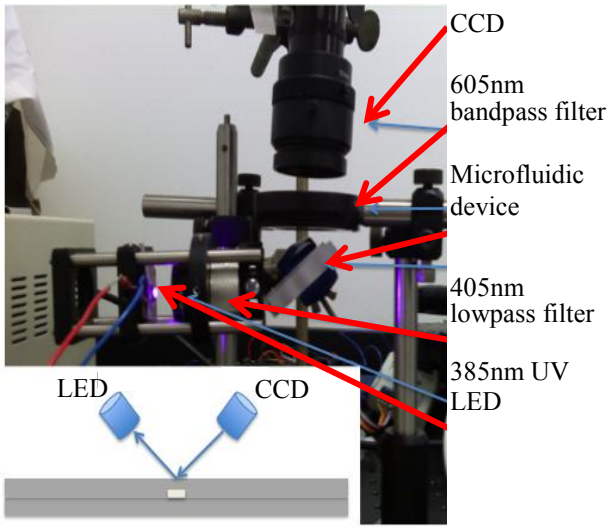

Figure 2: Microchannel excitation and imaging setup

piece of PMMA. An overview of the fabrication process for both the micropatterned PMMA piece (Figure 1a) and the integrated electrode design (Figure 1b) are presented above in Figure 1. The micropatterned PMMA piece containing the microchannels was fabricated by first patterning SU-8 2150 onto a 2.5" square piece of mirror finished copper (McMaster Carr). SU-8 2150 was spin coated onto the mirrored side of the copper substrate to a final thickness of 250um and patterned using standard techniques of photolithography. The SU-8 patterned copper plate was then electroplated under stirring with an applied current of 0.2A for 90 minutes using an electroplating bath. The SU-8 was then removed form the copper substrate using a novel thermal delamination technique taking advantage of the differences in the coefficients of thermal expansion between the copper and SU-8. The copper master was then used to transfer the microchannel design onto PMMA using a Carver hydraulic thermal press set to 120 ℃ under an applied force of 1000lb for 5 minutes. The final dimensions of the microchannels on the PMMA were 220um in width and 110um high with a length of 3cm. The micropatterned piece of PMMA was then sealed onto another piece of PMMA using solvent assisted thermal bonding (95% ethanol water mixture) and using the Carver thermal press (1000lb of force at 75 ℃ for 5 minutes).

Alternatively, the micropaterned PMMA piece was hermetically sealed onto a silicon substrate patterned with electrodes using a UV curable resin. Single channel pieces were cut from the imprinted pieces above. Then the microchannel was vertically aligned with fabricated electrodes, and a clamp was used to hold the assembly together. A UV-curable adhesive resin was introduced between the contact area of PMMA channels and silicon substrate. The resin filled up the gap due to capillary action but the microchannel was kept open (the resin did not continue flowing into the microchannel) due to the capillary pressure drop. 3 min UV exposure was applied to complete the bonding process.

An immunoassay for myeloperoxidase (MPO) was carried out as previously described. Briefly, capture antibody was covalently immobilized onto the lumen of the PMMA microchannel using EDC and sulfo-NHS. Antigen was then introduced into the microchannel and allowed to react for 60 minutes followed by several washes using 0.05% Tween 20 (1X PBS pH 7.4). Quantum dot conjugated capture antibody is then incubated for 60 minutes and allowed to react followed by several washes using 0.05% Tween 20 (1X PBS pH 7.4). the device is then imaged using the optical setup shown in Figure 2.

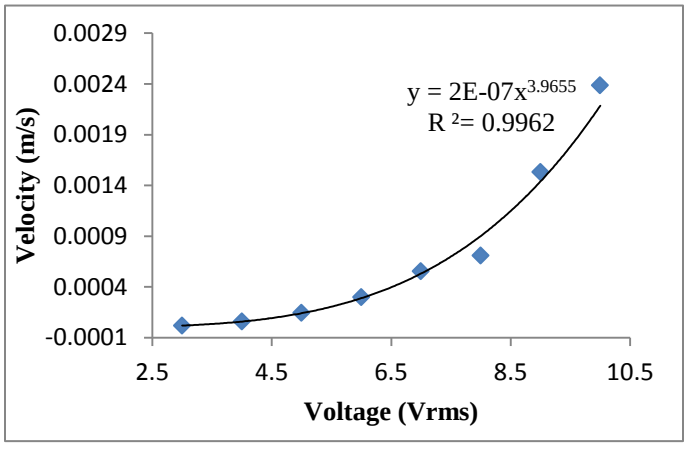

Figure 3: Numerical simulation result of fluid velocity as a function of the applied voltage. Frequency was fixed at 200kHz. At 6 Vrms, the velocity is 296 μm/s

RESULTS AND DISCUSSION

The particle movement due to ETF was observed using 2 μm polystyrene microbeads in 0.1M PBS buffer solution. The particle movement due to ETF was observed as the amplitude and frequency of the applied voltage was varied. Below 3Vrms no particle motion was observed. As the voltage was increased, particles became more and more active until the electrodes were damaged at 10Vrms. The experimental results had a good agreement with the numerical simulation shown in Figure 3. A set of parameters (6Vrms, 200kHz) was chosen for immunoassay experiments.

Immunassay performance between out predicate capillary based immunosensor and our developed microfluidic immunosensor platform was compared (Figure 4) in the absence of ETF mixing. As evident from Figure 4a, it is shown that the rectangular microchannel resulted in an increased sensitivity as well as a smaller standard deviation of measurement. A similar microfluidic assay was developed based upon previous work for myeloperoxidase (MPO), which was the basis for subsequent ETF enhancement experiments. Electric field was applied during MPO and QD-mAb incubation steps. Figure 4b shows significant improvement with ETF mixing. Without ETF mixing, the fluorescent signal was very low, indicating that there is limited binding between pAb and MPO, and between MPO and QD-mAb within 5 minutes. However, with ETF mixing, the binding efficiency was greatly improved. Signal intensity reaches the saturation at 50 μs of shutter speed.

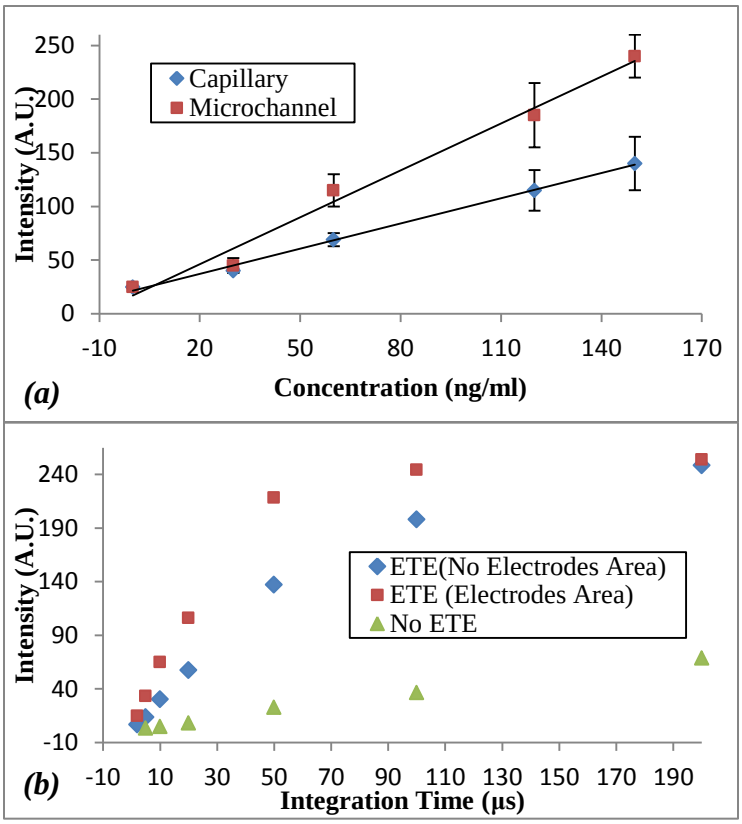

Figure 4: Comparison of capillary-based and microfabricated QLISA. (a) Lactoferrin calibration curve comparison between capillary and rectangular microchannel. (b) QLISA assay intensity with and without ETF mixing. Significant increase in the intensity is observed with ETF mixing. Electrode area gives the higher signal than non-electrode area in the case of ETF mixing.

CONCLUSION

Two types of quantum dots based PMMA microchannel immunosensor devices have been developed in this study. The high yield of QDs enables the devices the capability of detecting nanomolar quantities of analytes. With the enhancement of electrothermal effect, the reaction and response time could be significantly shortened. It reduced the time need for the whole assay by nearly 50%, while the signal intensity was comparable to normal assay.

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