ONE-STEP IMMUNOASSAY ON CAPILLARY DRIVEN MICROFLUIDICS

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ABSTRACT

In this paper we report a novel concept and its implementation for performing a sandwich fluorescence surface immunoassay using only 2 µL of sample, 4 nL of detection antibodies (dAbs), 2 min of assay time and one pipetting step. In particular, we use an inkjet printer to deposit minute quantities of dAbs in microchannels which economizes reagent and enables quick reconstitution of dAbs for fast assays.

KEYWORDS: Capillary, Microfluidic, Immunoassay

INTRODUCTION

Autonomous microfluidic capillary systems (CSs) are excellent to perform fast and sensitive immunoassays [1]. Sample and reagents move using capillary forces, alleviating the need for external pumps. CSs demonstrate efficient and uniform filling and use only a few microlitres of sample. Earlier immunoassay CSs required several pipetting steps for the sample, dAb and rinsing [1]. This work simplifies immunoassays by requiring only one analyte pipetting step.

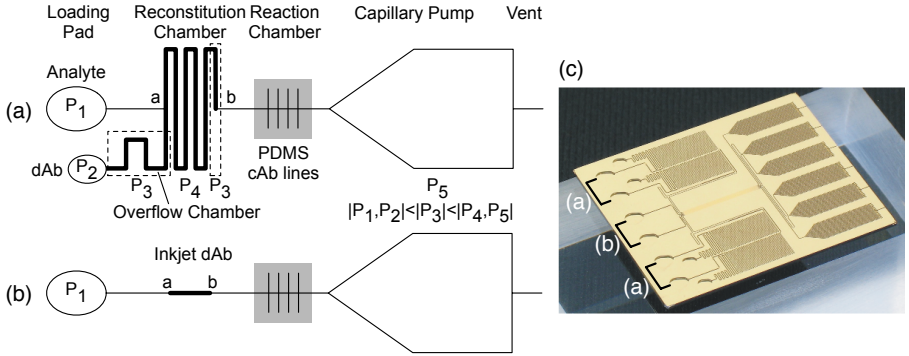

Figure 1. Schemes of CSs for one-step immunoassays. (a) micropipetting (b) inkjet depositing dAbs in the flow path of analyte. (c) image of a chip having 6 CSs.

One-handling step capillary systems are shown in Figure 1. They are prepared by depositing dAbs by pipette or inkjet. Capillary pressures (P_i) and geometries of the elements of the CS trigger spontaneous filling of liquid from low to high capillary pressure regions (absolute values). A solution of dAbs is pipetted in pad P_2 from where it flows through the reconstitution chamber, but not into pad P_1 since $|P_1, P_2| < |P_3, P_4|$. The reconstitution chamber has the highest capillary pressure and thus fills preferentially to other channels. The overflow chamber contains dAb solution that

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exceeds the volume of the reconstitution chamber. The sample placed in pad P_1 efficiently reconstitutes freeze-dried dabs and continues to the reaction chamber where the immunoassay is performed.

RESULTS AND DISCUSSION

Figure 2 shows a sample placed in pad P_1 efficiently reconstituting freeze-dried dAbs and exhibiting high fluorescence at the filling front. Little undisolved dAbs remain in the redissolution chamber or leaches into the reaction chamber.

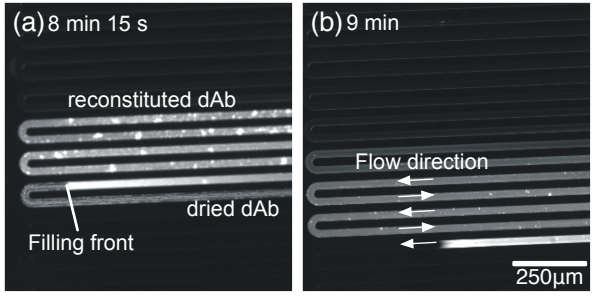

Figure 2. Optical fluorescence micrographs of dAbs in human serum in a reconstitution chamber. 0.5 µL of dAb solution (125 µg mL^{-1} of anti-CRP-Alexa647, 20 mg mL^{-1} L-phenylalanine, 60 mM trehalose) was pipetted in pad P_2, frozen at -196 °C and freeze-dried. dAbs reconstituted within ~9 min of injecting human serum.

Figure 3 shows an immunoassay for C-reactive protein (CRP) performed by crossing microchannels with stencil-patterned capture antibodies (cAbs) on poly(dimethylsiloxane) (PDMS) [2] with a sensitivity of 3 µg mL^{-1} after 10 min and 1 µg mL^{-1} after 25 min.

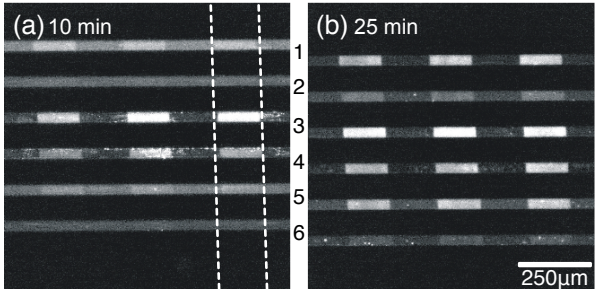

Figure 3. Optical fluorescence micrographs of CRP-Alexa647-dAb complexes in 6 CSs (horizontal microchannels) sealed with PDMS patterned with vertical lines of anti-CRP Abs. 2 µL of human serum with alternating CRP concentration of 3 and 1 µg mL^{-1} was injected to each CS.

Figure 4 shows dAbs deposited at room temperature using an inkjet and allowed to dry. The dAbs concentrate in the regions of high capillary pressure along the walls and corners of the microchannel.

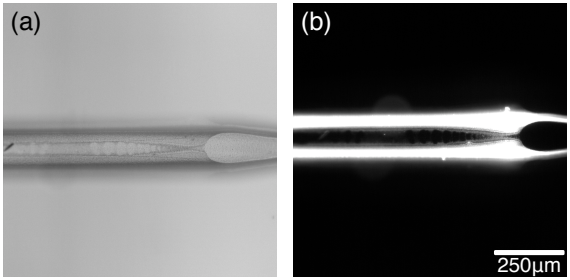

Figure 4. (a) Optical micrograph (b) fluorescence micrograph of 20 inkjet-deposited drops (3.6 nL) of dAb solution (100 µg mL^{-1} of anti-CRP-Alexa647, 1.2 mg mL^{-1} L-phenylalanine, 200 mM trehalose) in a channel before the reaction chamber

The trehalose and protein matrix efficiently reconstitutes in human serum enabling a one-step CRP assay to be performed at a high sensitivity of 100 ng mL^{-1} in only 2 min. The dAbs completely reconstitute within 1 min with low residual background signal. Alternatively, multiplexed assays using multiple dAbs may be performed on one chip [2]. To increase sensitivity, larger volumes of dAb can be inkjeted and flow rates can be reduced to increase incubation times.

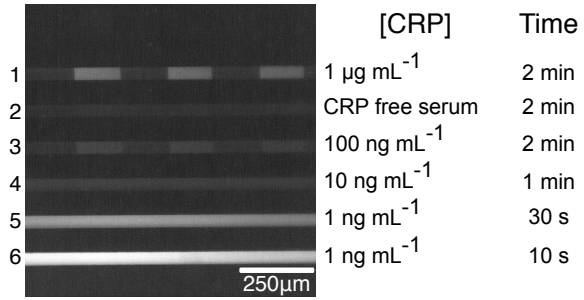

Figure 5. CRP assay inkjet-deposited dAbs. Channels 1–3: detection regions 2 min after injection of 2 µL of CRP in serum at 1 µg mL^{-1}, CRP free serum, and 100 ng mL^{-1}. Channels 4–6: particle free redisolution of dAb after 1 min, 30 s and 10 s.

CONCLUSIONS

Altogether, these possibilities suggest that rapid point of care immunoassays can be implemented on capillary driven microfluidic chips at least for clinical analytes such as CRP, which only need detection at microgram per milliliter concentrations.

REFERENCES

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