DROPLET-TRAIN SPR MICROCHIP FOR LABEL-FREE DETECTION OF BIO-INTERACTION USING NANOLITERS OF DRUG SAMPLE

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ABSTRACT

We demonstrate a new *Droplet-Train SPR* technique for label-free detection and quantification of bio-interactions on fixed volumes of samples. Bio-samples are first discretized within ~100 nL plug droplets separated by an immiscible phase. The plugs are sequentially transported onto SPR sensing sites within a surface-modified PDMS microchip. The measured kinetic constants k_a and k_d for the model protein-drug interaction are 8.33×10^3 1/M-s and 4.58×10^{-2} 1/s, matching well with previous reports.

KEYWORDS

Teflon-coated PDMS, Surface Plasmon Resonance, Droplets

INTRODUCTION

Over the last few decades, pharmaceutical industry has employed Surface Plasmon Resonance (SPR) as a viable real-time label free technology for drug discovery. Most conventional SPR step response [1] and other detection schemes [2-4] utilize continuous flow schemes that consume a substantial volume of bio-samples ranging from few micro-liters to few milliliters resulting in high detection costs. While in some cases the continuous-flow of sample is needed to eliminate transport limited reactions, in many cases the sample consumption can be reduced by confinement of sample into plugs. Using short discrete solution plugs while employing conventional step-response method for extracting reaction constants is potentially a fast, inexpensive and more accurate technique for label-free high throughput drug screening. The dual-slope continuous flow SPR technique [4] is an example of such scheme where mixing of reaction plugs (buffer, analyte, regeneration) is minimized through a careful choice of channel dimensions, flow velocities and plug volumes. Recently Chen et al [5-7] demonstrated that singulated drug plugs separated by an immiscible oil phase can be manipulated to deliver the drug compound to surface immobilized cells. Therefore this suggests that a similar technique can be used to separate plugs while delivering different compounds to a reacting SPR surface. In this paper, we report the fabrication and implementation of a *Droplet-Train SPR Microchip* where discrete reactant droplets are isolated by an inert gas phase and transported to SPR sensing sites.

DROPLET-TRAIN SPR METHOD

Fig. 1 shows the basic principle of droplet-train technique. Unlike other SPR schemes, solution samples are first loaded as discrete droplet plugs separated by an immiscible inert phase. In our chip we use an inert gas as the separating phase as it was determined that the delicate chemistry of the functionalized SPR surface was adversely affected by oil exposure, but it remained intact when exposed to a gas. In order to assure complete droplet separation, the implementation of the chip requires both *hydrophobic surfaces* on the channel walls and *hydrophilic* SPR gold spots. When these plugs are transported to the Au sensing functionalized spot, brief association and dissociation reactions occur for analyte and buffer plugs respectively. The alternate air and solution plugs are transported sequentially over the gold spots to the output storage reservoir. The plug volume ranges between 80-200 nL. Kinetic constants are then measured using conventional step response curve fitting of observed droplet sensorgrams [1,2].
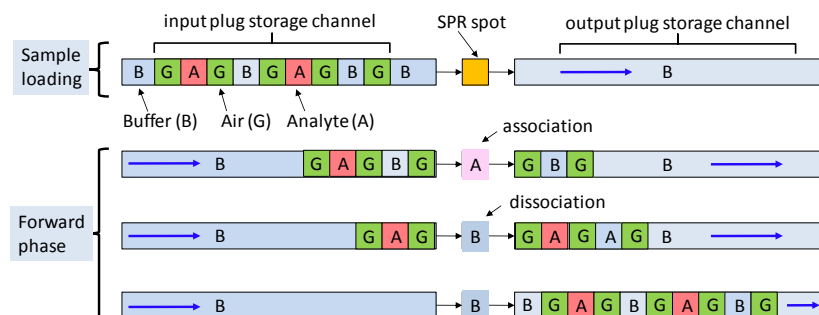

Fig. 1: Schematic of droplet-train SPR microchip scheme. The chip consists of two channel reservoirs where analyte and buffer are separated into plugs by an immiscible inert phase (air). First alternating plugs are loaded in the input reservoir. In the forward phase association and dissociation cycles are measured with all plugs ending at the output reservoir.

TEFLON-COATED PDMS MICROCHIP IMPLEMENTATION

Fig. 2 shows the schematic of a droplet-train SPR Microchip implementing a dual (sense + reference) chamber arrangement. Each of its chambers has four pressure driven load sources connected to buffer, analyte, regeneration and a heterogeneous separation phase (air) respectively and two outputs, all connected to flow channels. Flow of each of these solution sources is controlled by a set of microvalves. The chip microchannels are lined with functionalized SPR sensing and reference spots in order to record sensorgrams. Fig. 3 shows a photograph of the
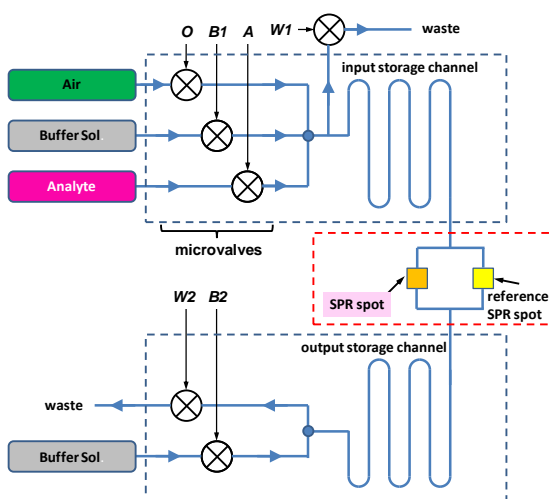
Fig. 2: Schematic of droplet-based SPR chip. Droplet plugs are first loaded in the input reservoir and flown through the SPR spots to the output storage channel. Valves W1 and W2 control the buffer flow.

droplet-train microchip. The microchip is fabricated as shown in Fig. 4 using a modified two-level PDMS technique that incorporates resist-protected Au spots. At the glass substrate Ti/W-Au spots are first patterned by conventional deposition, lithography and etching leaving the photoresist covering the spots. The Au-protected glass substrate is next bonded to a conventional two-level PDMS chip layer. Next, the microchannel walls are coated with a fluorination agent (Teflon-AF) that renders the channel walls permanently hydrophobic ($\theta \sim 110^{o}$). The Au protective resist is next removed by acetone exposure, rinsing and subsequent HCL cleaning. This is followed by the SPR target and reference spot functionalization as discussed in the experimental section below.

EXPERIMENTAL

Reagents and Setup: Polyethylene Glycol (PEG) compounds were purchased from Laysan Bio. These include 5 kDa carboxymethyl-PEG-thiol (cm-PEG) and 2 kDa methoxy-PEG-thiol (m-PEG). N-e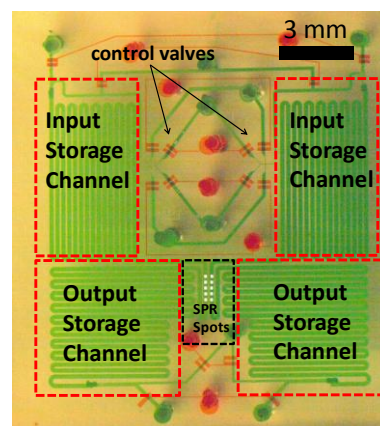thyl-N'-(3-dimethylaminopropyl) carbodiimide (EDC), Sulfo-N-hydroxysuccinimide (S-NHS) and Phosphate buffered saline (PBS) tablets were purchased from Thermo Scientific. Carbonic anhydrase II (CA-II, MW ~29 kDa), 10% Sodium dodecyl sulfate (SDS) and 4-(2-aminoethyl) benzenesulfonamide (ABS) were all purchased from Sigma-Aldrich. Sylgard 184 Polydimethylsiloxane (PDMS) kit was purchased from Dow Chemicals. Deionized 18 MΩ water (DI water) was provided by the University of Utah nanofab facility. SF10 Schott glass substrates (2 x 2 sq. inch) were custom ordered from Schott. Teflon-AF (6 %, C5-18) solution and Fluorinert (FC-3283) solvent were purchased from DuPont[TM] and 3M Fluorinert[TM] respectively. The sensorgram data is acquired using a manufacturer modified GWC Technologies SPRimager2 that accomodates our chip. Additional modifications are reported elsewhere [2].

Gold/Glass Substrate: SF10 glass substrates were cleaned with piranha (3:1, H2SO4:H2O2) solution for 10 mins, rinsed in DI water for 10 mins and blow dried with N2 gas followed by baking at 90° C in an oven for 10 mins. They are then transferred to a TM Vacuum Sputtering machine and a 3 nm adhesion layer of Ti/W is deposited followed by a 40 nm layer of gold. The metal is then patterned using photolithography. The array of Au spots patterned have dimensions of 200 x 200 μm^2 and thickness $\sim 43\pm2$ nm. The metals are then etched away, glass slides are cleaned with DI water, blow dried and finally stored in a vacuum desiccator until being used. The patterned gold spots have a layer of 1.5 µm thick photoresist to

Fig. 3: Chip photograph. The chip measures 1.8x2.2 cm.

protect them from fluorination.

Fluorination of channel walls: A solution of 0.2 % Teflon-AF in fluorinert solvent is prepared and flown inside the microchannel for 1 minute followed by removal of excess fluorinating solvent using pressure. The chip is baked at 90^0 C for about an hour to complete the hydrophobic coating followed by acetone rinse to remove photoresist from gold spots for functionalization.

Gold Spot Functionalization: The bare gold surface is first modified with PEGs similar to our previous paper and elsewhere [2-4]. The surface modification using PEGs is quite effective in reducing non-specific adsorption. Briefly, gold spots inside the microchannels are first rinsed with 0.01 N HCL solution followed by PEGylation in PBS buffer (50 mM Phosphate, 1 M NaCl, pH 7.4) using cm-PEG. A short
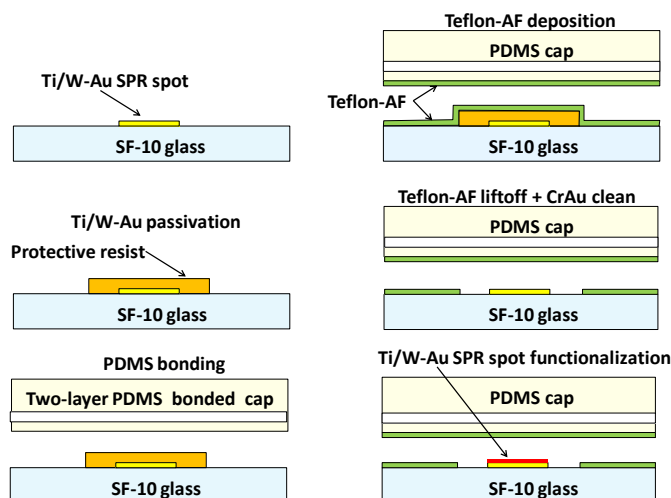

Fig. 4: Process for teflon-coated two-level SPR-PDMS chip

underbrushed layer of m-PEG further minimizes the non-specific adsorption. Excess PEG is removed by a short rinse of 50 mM NaOH. This is followed by immobilization of ABS ligand using standard amine-coupling procedure

to form a two-dimensional sensing surface. A solution of Sulfo-NHS (0.1 M) and EDC (0.4 M) is used to facilitate crosslinking of activated cm-PEG to ABS. The control surface is blocked with ethanolamine (50 mM) in PBS buffer (25 mM Phosphate, pH 8.4 with 0.01% SDS) after cm-PEG activation.

RESULTS AND DISCUSSION

The droplet based SPR technique was demonstrated using a model system of Carbonic Anhydrase-II (CAII) analyte and immobilized 4-(2-Aminoethyl) benzenesulfonamide (ABS) ligand [2-4] on amine reactive PEG modified gold surface. Short separate nano-liter droplets of buffer, analyte and regeneration plugs are first loaded in the long flow channels which are then sequentially transported to sensing site. Fig. 5(a) shows the time-domain fluorescent signal over a sample spot in the flow channel. Fluorescent plugs have an approximate volume of 90 nL corresponding to plug duration of 5 seconds and flow pressure of 10 PSI. Fig. 6 shows a time-domain SPR sensorgram obtained from the sense and reference Au spots under a droplet-train flow of alternating analyte and buffer droplet plugs separated by an air plug. The measured values of k_a and k_d are 8.33×10^3 1/M-s and 4.58×10^{-2} 1/s respectively for an analyte concentration of 1.2 µM, matching reasonably well with our previously reported step-response values [2].

CONCLUSION

Fig. 5: Snapshots of alternating fluorescein, buffer and air plugs moving forward and backward and corresponding fluorescent signal intensity.

We have demonstrated the realization of a droplet-train based SPR microchip. In this chip ~100 nL nanoliter plug samples of analyte, buffer and regeneration solutions are separated by an inert, immiscible gas phase thus dramatically reducing the consumption of analyte samples. Measurements of the association and dissociation constants were performed using slope-based fitting methods. The device is well suited for multi-analyte high throughput drug screening systems where the sample cost is critical, and the reaction rates are sufficiently slow to be independent of transport effects.
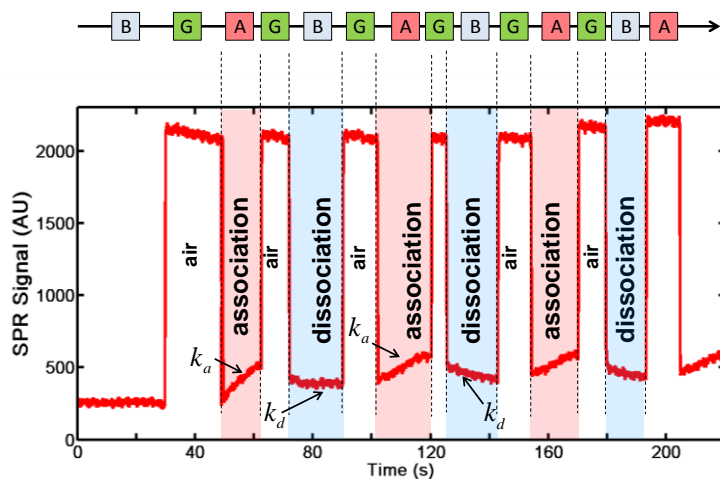

Fig. 6: Droplet-based SPR measurement of association and dissociation for the CAII-ABS system using alternating droplets of analyte, air and buffer. The flow pressure was 10 PSI.

REFERENCES

[1] U. Jönsson, L. Fägerstam, H. Östlin and M. Malmqvist, "Real-time biospecific interaction analysis using surface plasmon resonance and a sensor chip technology," *Biotechniques*, 11, 620 -627(1991).
[2] L. Williams, T. Ghosh and C. Mastrangelo, "Low noise detection of biomolecular interactions with signal-locking surface plasmon resonance," *Analytical Chemistry*, 82, 6025 (2010).
[3] T. Ghosh, L. Williams and C. Mastrangelo, "Label-free detection of protein binding with multisine SPR microchips," *Lab on a Chip*, 11, 4194-4199 (2011).
[4] T. Ghosh and C. Mastrangelo, "Fast measurement of binding kinetics with dual slope SPR microchips," *Analyst*, 137, 2381-2385(2012).
[5] D. Chen, W. Du, y. Liu, W. Liu, A. Kuznestov, F. E. Mendez, L. H. Philipson and R. F. Ismagilov, "The chemistrode: A droplet-based microfluidic device for stimulation and recording with high temporal, sptial and chemical resolution," *PNAS*, 105, 16843-16848 (2008).
[6] Y. Liu and R. F. Ismagilov, " Dynamics and coalescence of plugs with a hydrophobic wetting layer induced by flow in a microfluidic chemistrode," Langmuir, 25, 2854-2859 (2009).
[7] L. S. Roach, H. Song and R. F. Ismagilov, "Controlling nonspecific protein adsorption in a plug-based microfluidic system by controlling interfacial chemistry using fluorous-phase surfactants," *Analytical Chemistry*, 77, 785-796, (2005).

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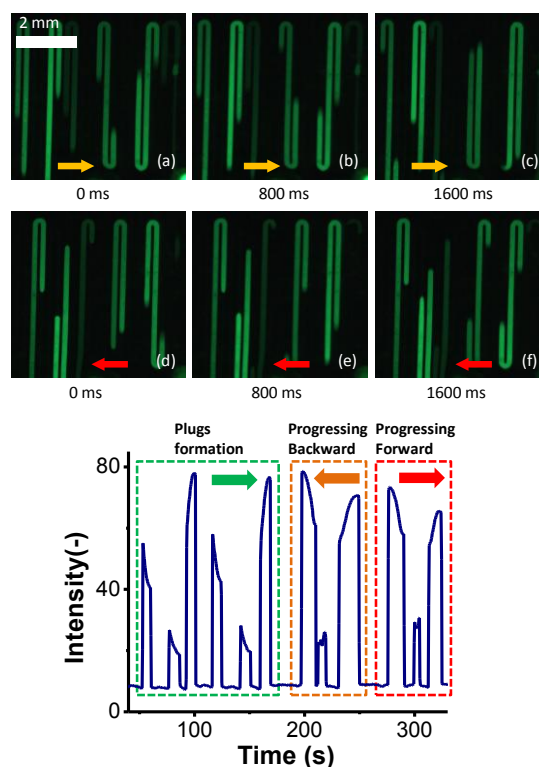[1]Tridib Ghosh, tel: +1-801-425-1565: t.ghosh@utah.edu