INTEGRATED ELECTROKINETIC LAB-ON-A-CHIP BASED BIOSENSOR - A TOOL FOR DRUG SCREENING APPLICATIONS

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ABSTRACT

We report a new integrated Lab-on-a-Chip (LOC) and surface plasmon resonance imaging (iSPR) system that uses electrokinetic (EK) sample transport simultaneously into 10 individual microchannels and 100 sensing locations, where all sensing locations can be measured simultaneously and kinetics and affinity parameters extracted from all the interactant pairs. This new chip can be used for high-throughput drug screening applications and the simultaneous extraction of kinetics and affinity parameters from interactant-protein complex formation.

KEYWORDS: Lab-on-a-Chip, Biosensor, Surface plasmon resonance imaging, Drug screening, Electrokinetics

INTRODUCTION

iSPR [1] is playing an increasingly more important role in high-throughput screening applications, such as drug discovery for the ranking of potential drug candidates [2]. The integration of LOC with iSPR adds the important capability of multiplexing, which is important for high-throughput assays. Integrated high-throughput multiplex systems are important for drug discovery applications as they can drastically reduce assay times and costs. Potential drug candidates can be screened by evaluating the binding affinity and kinetic behavior, which can be directly extracted from the measured iSPR data in a highly parallel manner. Conventional systems that use pressure driven microchannel flow require multiple pumps and valves operating in parallel, which can be complicated when the number of independent microchannels is greater than about ten. To overcome this problem, EK fluid transport can be used for sample transport in multiple microchannels simultaneously using a single voltage source [3,4]. We have recently reported [3,4,5] an integrated LOC-iSPR system with EK sample transport for biomolecular interaction measurements as shown in Fig. 1.

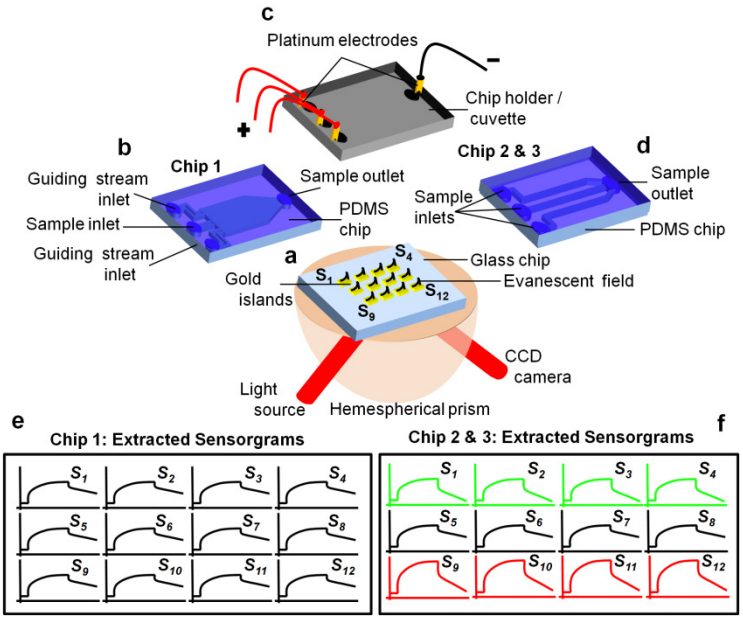

Figure 1: a) iSPR configuration: glass chip with gold islands for microarray index matched with hemispherical prism together with light source and CCD camera for iSPR measurements. b) Chip 1: Electrokinetic focusing chip for 1 sample inlet. The sample is focused to specific locations of the chip using two guiding streams. c) Custom-made chip holder and cuvette integrated with platinum electrodes. d) Chip 2 & 3: Electrokinetic multi-ligand/multi-analyte chip for multiple samples. e) Illustration of iSPR results extracted from chip 1. f) Illustration of multiplexed iSPR results extracted from chip 2 & 3.

We report here a simple integrated system with 10 parallel microchannels and each microchannel has 10 gold sensing islands. The distribution of buffers and samples throughout the chip is performed with EK transport. A two-layer hybrid chip (Glass-PDMS) was used and the fabrication procedure is similar to that previously described [4,5]. The illustration of the two layer chip together with chip holder integrated with platinum electrodes, as well as the electrical connection as shown in Fig. 2.

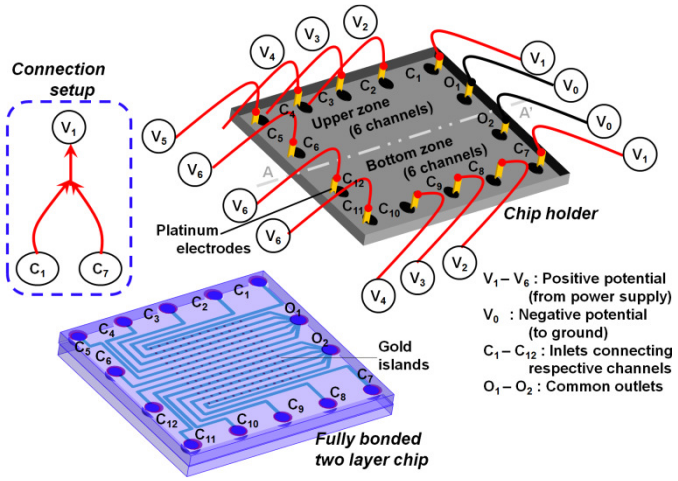

Figure 2: Biochip (top layer: PDMS and bottom layer: glass with gold islands) with chip holder (integrated with platinum electrodes as well as reservoir holes for extra sample volume). A UV curable glue (NOA-81) was used for chip bonding. Top left side image represents the electrical connections. A-A' (Grey line) represents the splitting of two groups of channels structures.

EXPERIMENTS & RESULTS

Well-known biomolecular interactant pairs have been used and are listed in Table 1. Prior to bonding the PDMS chip to the glass chip, the chip surface was activated with mixture of 0.4 M EDC and 0.1 M NHS for 20 minutes [3-5]. After the activation step, the chip surface was washed with acetic acid (pH 4.5) to maintain the acidic property for the covalent immobilization of ligands. The processed chip was then dried with dry nitrogen. A 10 mM sodium acetate (Sigma, the Netherlands) immobilization buffer with pH 5.2 was used and the running buffer was 10 mM HEPES (Sigma, the Netherlands) with pH 7.2 and conductivity of 296 µS/cm. All ligands used in this study were diluted 2 times from stock solutions using 10 mM sodium acetate buffer with pH 5.2 and their final concentrations are listed in Table 1. 4 µl ligand samples were immobilized in each channel. The chip was stored in a humidity chamber (1 hour).

The reproducibility of the iSPR (IBIS Technologies B.V., Hengelo, the Netherlands) measurement could be checked directly without repeating the experiment as each channel has 10 gold sensing islands for the measurement of the same interactant pairs. The microchannels were washed and filled with a running buffer, and the chip has been placed over the iSPR interface module with a drop of refractive index matching oil. The iSPR interface module together with the chip is docked into the iSPR system followed by fixing of chip holder on the top of the chip. Various analyte samples were filled into their respective cuvettes of the chip holder. The electrodes were connected to the voltage supply (IBIS Technologies B.V., Hengelo, the Netherlands). The analyte sample and ligand concentrations used in this study are listed in Table 1. The results are shown in Fig. 3 for all the channels and gold sensing islands. The response observed in the association phase (t = 500 sec) with its standard error over the entire channel (n=9) are also listed in last column of Table 1. The extracted affinity parameters are listed in Table 1.

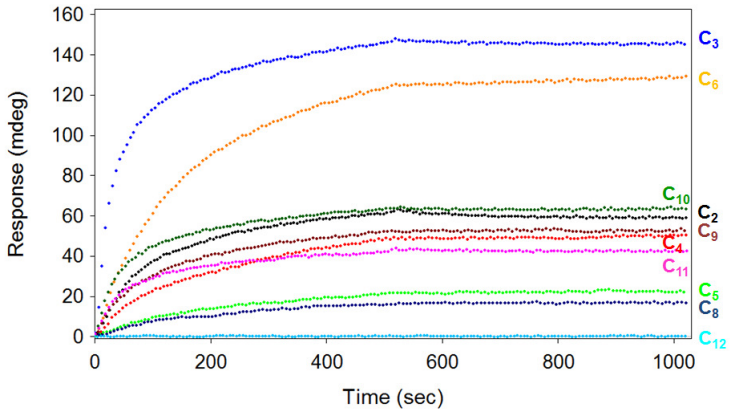

Figure 3. Measured sensorgram from 10 microchannels (single measurement in each microchannel). The interactant pairs concentrations are listed in Table 1.

Deviations in the response (angle shift) were observed in the iSPR responses of the channels C_2 and C_4 (result not shown). This could be due to uncontrolled immobilization or due to the fact that the molecules are multivalent. The responses observed in the channels: C_2, C_3, C_6, C_8 and C_9 can be influenced by steric hindrance due to the fact that the analyte sample used is larger (150 KDa) than the immobilized molecules (e.g. the immobilized ligands, such as BSA or HSA (66 KDa), neomycin (615 Da), gentamycin (576 Da) and Fab fragments of HIgG (50 KDa)

and F(ab')$_2$ fragments of HIgG (100 KDa)). Highly oriented or high order spaced immobilization could be helpful in these cases or immobilizing a very small amount of samples (low ligand density) could avoid this problem and should not affect the binding kinetics. Another observation is slow dissociation rates, indicating a strong complex formation (C_3, C_4, C_6 and C_{10}). In the following cases, Fab HIgG (C_8), b2M (C_{11}) and neomycin (C_5), the dissociation phase starts slightly after 500 seconds, and could be an indication of a flow rate change during the experiment which might be due to the adsorption of molecules on the PDMS walls.

Table 1: Summary of various channel dimensions, fluid transport voltages, as well as ligands, analytes and concentrations used.

Channels	Ligands	Analytes	Ligand Conc. (mg/ml)	Analyte Conc. (nM)	Voltage (V)	Response (mdeg)	K_D (nM)
C_2	Bovine Serum Albumin (BSA)	Anti-BSA	5.0	533	185	54±7	6.42
C_3	Human Serum Albumin (HSA)	Anti-HSA	5.0	533	171	142±5	0.67
C_4	Human Immuno-globilin G (HIgG)	Anti-HIgG	0.5	133	159	51±4	1.15
C_5	Neomycin	Anti-Neomycin	17.0	440	159	20±1	8.90
C_6	Gentamycin	Anti-Genta-mycin	7.5	80	130	124±3	0.04
C_8	Fab HIgG	Fab Specific AHIgG	2.2	520	185	16±1	4.23
C_9	$(Fab)_2$ HIgG	$(Fab)_2$ Specific AHIgG	2.2	460	171	49±2	1.63
C_{10}	Fc HIgG	Fc Specific AHIgG	1.2	260	159	63±2	0.03
C_{11}	β2-Micro-globulin	Anti-β2M	0.13	1430	159	42±1	4.26
C_{12}	Buffer	Buffer	0	0	130	0±0.3	0

CONCLUSION

The successful implementation of a newly designed chip was demonstrated for the multiple ligands - analytes (up to 10) interactions. Multiple kinetics and affinity parameters can be extracted from the recorded iSPR data. This device is also useful in many biological application areas apart from drug discovery, where both the qualitative and quantitative approaches are necessary.

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