DETECTION OF DNA HYBRIDIZATION ON A CONFIGURABLE DIGITAL MICROFLUIDIC BIO-CHIP USING SPR IMAGING

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ABSTRACT

This paper presents a configurable digital microfluidic-based surface plasmon resonance (SPR) biochip platform comprising an electrowetting-on-dielectric (EWOD) microfluidic device coupled to SPR imaging (SPRi). We demonstrate its application for dynamic on-chip simultaneous immobilization of different DNA probes in combination with multichannel label-free real-time detection of subsequent hybridization reactions. The integrated EWOD-SPRi system would enable the development of high-throughput, rapid and ultrasensitive biomolecular detection strategies beyond DNA microarray applications.

KEYWORDS: Digital microfluidics, EWOD, SPR imaging, DNA hybridization

INTRODUCTION

EWOD microfluidics has attracted considerable attention in the past decade and the most recent efforts are directed towards its application in biomedical research [1-3]. While these studies demonstrate the versatility of EWOD devices, the reported applications involve homogeneous phase reactions [4] and detection methods that require labeled biomolecules [2] or sample extraction from the chip [1]. This increases both the time and complexity of the assay. To introduce new applications relying on label-free, real-time surface sensitive detection techniques such as SPRi, it would be advantageous to use droplet-based EWOD actuation for surface specific biomolecule immobilization. However, the need of hydrophobic properties for EWOD actuation renders immobilization of biomolecules such as DNA on the surface of the chip impossible [3, 4]. In this paper, we demonstrate for the first time the use of an EWOD microfluidic chip to dynamically immobilize DNA probes in a two-dimensional array, followed by SPRi detection of bioaffinity interactions.

EXPERIMENTAL

The EWOD digital microfluidic chip for DNA immobilization and SPRi detection of DNA hybridization is shown in Fig.1a and consists of two parallel glass plates separated by 150 μm spacers. The top plate serves as the SPRi-biochip and is comprised of a 50 nm thick gold ground electrode coated with a 30 nm thick hydrophobic Teflon film. To enable biomolecule immobilization on the surface of the top plate, local functional zones that consist of four 300 μm diameter gold detection spots (Fig.1b) are defined by patterning the Teflon film using a thick photoresist lift-off technique. The bottom plate, containing the reservoir and the path electrodes, also houses the dedicated actuation electrodes which are aligned with the detection

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spots (Fig.1a). To immobilize the probe DNA on the detection spots, the 180 nL droplets containing 1 μM DNA probes in 1M K_2HPO_4 were created on-chip from the reservoirs, displaced along the path electrodes and finally positioned on the control electrodes underneath the detection spots. The droplets were then left stationary for two hours in humid environment to allow for the thiolated DNA probes to covalently attach to the gold surface with or without an applied electric field. Finally, the functionalized top plate was mounted onto the prism of SPRi-Lab+ apparatus (Gen-Optics, France) to monitor DNA hybridization (Fig.1b).

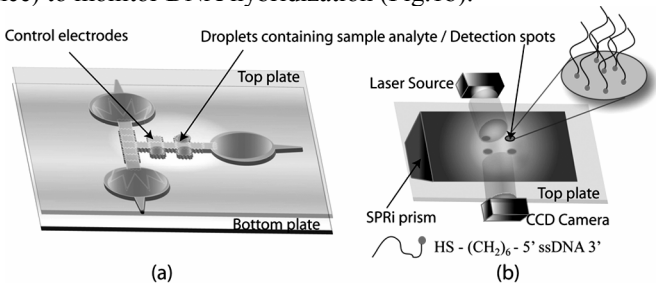

Figure 1. Schematic of EWOD-based SPRi bio-chip design (a) 3D View with the bottom and top plates aligned; (b) View of the top plate mounted on the SPRi prism.

RESULTS AND DISCUSSION

The specificity of DNA target binding on the surface of the biochip containing spots with three different immobilized DNA probes was monitored using SPRi. One spot was left untreated to serve as a control. The DNA hybridization for each spot was carried out by injecting sequentially different 250 nM DNA target sequences in 1 M NaCl in TE buffer, allowing them to hybridize with the probes. The SPRi difference images obtained during DNA hybridization show that the hybridization was specific and that only complementary sequences hybridized (Fig.2).

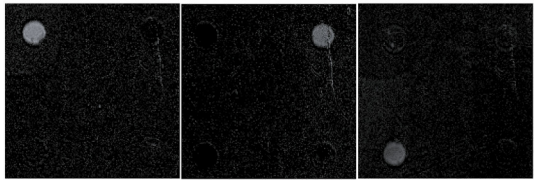

Figure 2. SPRi difference images of sequential DNA hybridization. Bright spot indicates complementary sequence binding to the specific DNA probe immobilized on one of the detection spots.

In addition to passive DNA immobilization, EWOD was employed to dynamically control the immobilized probe density using its inherent electric interface. Droplets containing DNA probes were left in contact with the detection spots for two hours with the applied potential of 0V (control) or +/- 90V at the dedicated control electrodes. The resulting e-field created a negative or positive charge buildup at the top electrode thus repulsing or attracting the negatively charged DNA probe to the surface, respectively. This resulted in different DNA probe density on the surface which is seen from different plasmon curves characteristics obtained using SPRi (Fig.3a). Consequently, different probe density affected the kinetics of hybridization, shown in Fig.3b. The hybridization signal during target injection stead-

ily increased for the probes immobilized under -90V and 0V, while it decreased after 3 min of hybridization for probes immobilized under +90V due to less efficient probe orientation. The inset of Fig.3b shows the hybridization signal represented as the difference in reflected intensity (%ΔR) between the initial and final buffer signals. While %ΔR for probe immobilized under +90V was similar to control, for the negatively applied voltage, the immobilized probes exhibited a two-fold increase in hybridization efficiency. This enhancement was due to the increased probe density and the more efficient probe orientation at the surface.

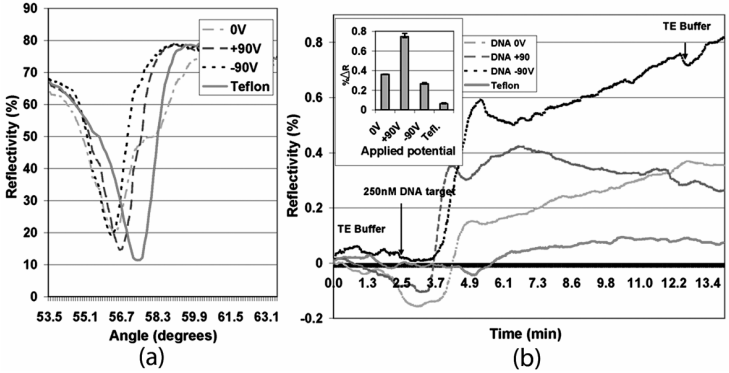

Figure 3. (a) SPR plasmon curves and (b) Kinetic curves of DNA hybridization for samples with probe immobilized at 0 and +/- 90 V.

CONCLUSIONS

In this paper, we employed the EWOD microfluidic technology to create a dynamically configurable SPRi biochip for dynamic DNA immobilization and detection of DNA hybridization. Similar to the dynamic probe immobilization, EWOD can also be used to dynamically regulate DNA target transport and concentration at the detection site, further enhancing DNA hybridization. Coupled with SPRi, EWOD biochip platform promises to dramatically increase the speed of DNA hybridization detection, finding its application in both research and clinical settings.

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