Preparation of MTB Oligonucletide and Albumine Microarray Platforms with Soft Lithography and Their Applications

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Introduction

Array technology is a new, fast developing technology branch and with this technology many species can be detected in one assay. In contrast, traditional laboratory diagnostic methods are demanding, long-term tests and they need expensive equipments that only give the possibility of 'one determination in one assay'. Generally, in assay systems the probe-target interactions are detected by means of optic/radioactive labels. However using label is very expensive, difficult and time consuming process [1]. Nowadays many researches are focused on label-free techniques like QCM, SPR, MALDI on array technologies. One of the most important parts in array technologies is the preparing of array platforms in order to immobilize different molecules in different areas with appropriate patterns. There are different lithography techniques such as soft lithography, photolithography, dip-pen lithography etc. This study aims to prepare human serum albumin (HSA) and M. tuberculosis Complex (MTB) probe oligonucleotide (5'–HS-(CH2)6–(T)15 GGG TGC ATG ACA ACA AAG TTG G–3') microarrays with micro-contact printing (μCP) that is used to capture anti-human serum albumine antibody (anti-HSA antibody) and MTB target oligonucleotide. Hybridization/interaction kinetics of target molecules on array platforms are determined by SPR and then hybridized/interacted MTB probe oligonucleotide/ albumine array platform surfaces are analyzed with MALDI-MS.

Experimental

Preparation of PDMS stamps for μCP
PDMS (Sylgard 184), which is purchased from Dow Corning, was used for preparing these stamps. PDMS was casted in an aluminium master which has patterns. Sylgard 184 elastomer prepared by mixing of base and curing agent at a ratio of 10:1 and followed by degassing to remove air bubbles. Mixed PDMS was poured in to templates and cured at 100^{o}C for 1 hour [2].

Preparation of Array Platforms and measurements
In order to obtain hydrophilic surface, substrates were cleaned by rinsing with ethanol and distilled water, then treated with plasma for 30 min before printing. Self assembled monolayer (SAM) of mercaptoundecanoic acid (MUA) and oligonucleotide probes were formed via micro-contact printing of probe solutions on to substrate with PDMS stamp.
To find optimum printing concentration, various concentrations of molecules were prepared and printed on substrate surface. Thickness of SAMs of MUA and MTB oligonucleotides were evaluated by ellipsometry and surface analysis were made by MALDI-MS. Sensor surfaces were then used as SPR sensor for anti-HSA antibody and MTB target oligonucleotide capturing to determine sensor response. Sensor linearity and detection limits of SPR and MALDI sensors were evaluated in array form.

Results and Discussion

In order to compare the sensor responses of SPR and MALDI techniques, MTB probe oligonucleotide based sensor and HSA based sensor were prepared. Probe density on the sensor surface was determined using ellipsometry and MALDI.
In order to obtain HSA array platforms first MUA was printed on gold coated glass slides by μCP method.

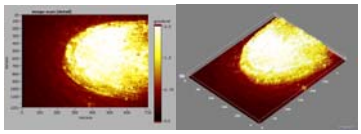

Fig. 1 Ellipsometric and 3D image of micro-contact printed MUA monolayer.

The optical thickness of SAMs of MUA in Figure 1 was determined by using ellipsometry as 1.42 nm and this value is consistent with theoretical value of the monolayer of MUA.

Table 1 Measured and Theoretical Thickness of MUA

Thicknesses	(nm)
Measured MUA Thickness	1.43
Theoretical MUA Thickness	1.53

HSA was chemically bonded to MUA printed regions ('spot') by carbodiimide reaction. According to the binding kinetics that were measured by Surface Plasmon Resonance device and thickness values that were measured by ellipsometer of HSA at different concentrations (Fig. 2), the optimum immobilization concentration of HSA was determined as 10nM.

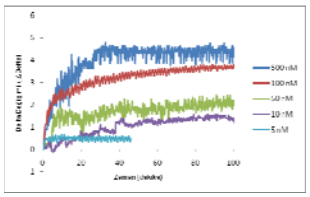

Fig. 2 Binding Kinetics of HSA to MUA printed regions

The MTB probe oligonucleotide array platforms were also prepared by using micro-contact printing method that prints 5 µM MTB probe oligonucleotide solution at pH 3.8 with PDMS stamps on gold coated glass slides. The average thickness values of the MTB probe oligonucleotide spots were measured as 2.78 by using ellipsometer and it is seen that this value is compatible with the theoretical values.

By examining the hybridization of MTB target oligonucleotides with MTB probe oligonucleotide spots and interaction anti-HSA antibodies with HSA spots at different concentrations with SPR, array platform performances were determined. According to obtained binding kinetics and ellipsometric thickness values, hybridization concentration of MTB target oligonucleotide and interaction concentration of anti-HSA antibody were optimized as 1 µM and 10 nM, respectively.

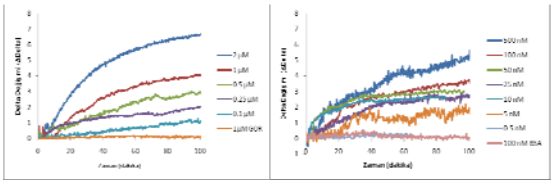

Fig. 3 Hybridization/interaction of MTB target oligonucleotide and anti-HSA antibody on MTB probe oligonucleotide/ HSA array platforms

The surface analysis of MTB target oligonucleotide and anti-HSA antibody spots done by Matrix-Assisted Laser Desorption/Ionization ('MALDI') as shown in Figure 3.

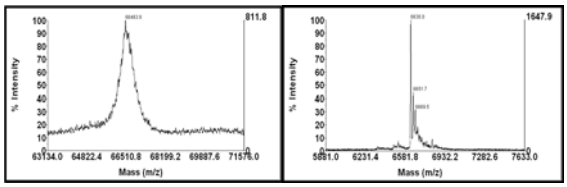

Fig. 4 MALDI-MS results for MTB target oligonucleotide and anti-HSA antibody on array platforms, respectively.

Conclusion

Performances of array platforms are determined by observation of binding behaviors of molecules on corresponding spots. By using imaging SPR, response difference between spot and spot free surfaces was determined in an array platform. Hybridization/interaction of target molecules on spots was determined by both MALDI-MS and SPR and the optimum concentrations and detection limits of molecules are observed for both SPR and MALDI-MS. It has been proved that HSA and MTB target oligonucleotides can

be defined with MALDI-MS at low concentrations by the formation SAMs on surfaces.

References

1. Iqbal S.S., Mayo M. W., Bruno J. G., Bronk B. V., Batt C.A. and Chambers J.P. A review of molecular recognition technologies for detection of biological threat agents. Biosensors and Bioelectronics, 2000, 15:549-578
2. Younan Xia and George M. Whitesides Soft Lithography Angew. Chem. Int. Ed. 1998, 37, 550 ± 57