ENHANCEMENT OF IMMUNOREACTION ON MICROARRAY-INTEGRATED OPTOELECTROFLUIDIC ASSAY SYSTEM
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ABSTRACT
We present a novel microarray-integrated immunoassay platform using optically-induced electrophoresis from dynamic light patterns, which can provide programable binding on desired spot areas, overall reduced reaction time, and signal enhancement due to local concentration of molecules. In practical applications, the platform has also advantage such as perfect compatibility with conventional glass slides when the photoconductive device is integrated with planar-type microarray.

KEYWORDS: Optoelectrofluidics, Microarray, Immunoassay, Optically-induced electrophoresis

INTRODUCTION
Conventional microarray technology such as protein and DNA microarrays suffers from tedious sample preparation and long incubation time due to static reaction environment, leading to low sensitivity. Although various kinds of microarray-based assay systems have been proposed, they still depend on the passive and random diffusion of the target sample for immunoreaction or DNA hybridization. Recently, optoelectrofluidics, which is based on the electrophoretic motions of particles or fluids under a light-induced electric field, has become a fascinating technology for manipulating biological materials such as proteins and chemicals [1,2]. Because biomolecules can be concentrated on the local area which is activated by optically-induced AC electrophoresis (ACEO), an optoelectrofluidic manipulation technology has been applied to improve assay performances in conventional immunoassay. In this paper, we propose a new microarray-integrated optoelectrofluidic immunoassay platform which can enhance immunoreaction by optically-induced electrophoretic phenomena.

THEORY
In this system, a non-uniform electric field is formed in the liquid chamber when a dynamic light pattern is projected onto the optoelectrofluidic device. This nonuniform electric field generates ACEO flows. The ACEO is caused by the motion of ions, which locate the surface of electrode resulting in the electric double layers, and the fluidic motions can be explained by equation (1) which defines the slip velocity of the ions:

\[ \nu_{\text{Slip}} = -\frac{\zeta E_t}{\eta} \]  

(1)

where \( \varepsilon \) is the liquid permittivity, \( \zeta \) is the zeta potential, \( E_t \) is the tangential electric field, and \( \eta \) is the fluid viscosity.

EXPERIMENTAL
Figure 1a shows schematic illustrations of immunoreaction process in a microarray-integrated optoelectrofluidic system. First, an antibody microarray is constructed on a glass slide coated with indium tin oxide, which is used as upper electrode of optoelectrofluidic system. A 500-nL sample droplet, including target analytes and detection antibodies, is placed an 8 \( \mu \)m-height liquid chamber between upper antibody-coated electrode and bottom photoconductive electrode. After sample loading, ACEO is generated at each spot by optical regime to enhance immunoreaction. Finally, assay result can be analyzed by measuring intensity of fluorescence of probe molecules. When a light pattern is irradiated on an array spot under AC voltage, molecules are highly concentrated by induced ACEO flow, which helps immunoreaction enhanced by increasing local molecular concentration (Figure 1b).

Figure 1. Schematic illustrations of (a) whole process and (b) enhancing mechanism for immunoreaction of microarray-integrated optoelectrofluidic system.
RESULTS AND DISCUSSION

Figure 2. (a) Schematic illustration of microarray construction by microstamping method and microscopic image of protein microarray (scale bar: 50 μm). (b) Results of 3×3 microarray construction with five trials (n = 9).

First, a polydimethylsiloxane (PDMS)-based microstamping method was employed to construct a protein microarray on glass substrate, where average spot size was 41.61 μm in diameter (Figure 2a). Homogeneous micropatterned images from fluorescence-labeled antibodies were shown on the fabricated microarray (Figure 2b).

Figure 3. Microscopic images of optoelectrofluidic concentration of 50-nm colloidal nanoparticles in the illuminated region with an applied voltage of 14 V_{pp} at 10 kHz and temporal changes in local concentration of nanoparticles according to the applied voltage at 10 kHz (n = 3, scale bar: 50 μm).

Immunoreaction on microarray surface is enhanced by optically-generated strong vortex flow which provides rapid mass transport and high local concentration of target molecules. To confirm presence of vortex flow and its ability under the optoelectrofluidic system, light-induced concentration experiments were performed. Figure 3 shows the results of light-induced concentration of nano-sized materials such as macromolecules within the optoelectrofluidic system according to the applied voltage at 10 kHz. When a circular-shaped light pattern was irradiated on the system, the concentration of 50 nm-sized fluorescent colloidal nanoparticles increased at the center of the light-irradiated region with respect to time.

Figure 4. Microscopic images of optoelectrofluidic concentration of colloidal nanoparticles (green) with 5×5 microarray-patterned lights (red) with an applied voltage of 14 V_{pp} at 10 kHz for 30 s (scale bar: 100 μm).
To confirm multiplexed concentration capability in the optoelectrofluidic system, we applied microarray-patterned lights to a droplet of colloidal nanoparticles in the system with application of a 14 peak-to-peak voltage (Vpp) at 10 kHz for 30 s. The microscopic images show simultaneous local concentration of nanoparticles on each spot of irradiated regions with a 5×5 microarray format, which guaranteed the optoelectrofluidic system can control multiple regions on the microarray simultaneously to enhance immunoreaction (Figure 4).

![Microscopic images and fluorescence intensity of immunoreaction results on 3×3 microarray spots with respect to optoelectrofluidic enhancing (n = 3, scale bar: 100 μm).](image)

Finally, anti-goat immunoglobulin G (IgG) and goat IgG conjugated with fluorescein isothiocyanate were used for investigating enhancement of immunoreaction on a 3×3 antibody microarray. After constructing the antibody microarray and blocking the surface with 1 mg/ml bovine serum albumin for 30 min, antigen solution was loaded and the voltage (14 V_{pp} at 10 kHz) was applied for 30 s, followed by incubation for 10 min within the optoelectrofluidic system. The fluorescence results show that two-fold signal enhancement was observed in an optical enhancing scheme (Figure 5).

CONCLUSION

We have presented a novel optoelectrofluidic system integrated with the microarray technology to enhance immunoreaction in the optical regime. This technology provides a solution to overcome the limitation of incubation time of the conventional microarray technologies. Besides, this optoelectrofluidic system has high compatibility to be integrated with any type of microarray regardless of the shape or size of the array patterns because actuating regions can be controlled easily by modifying light pattern. These advantages are expected to improve performance of not only protein-based immunoassays but also other biochemical assays with various biomaterials such as nucleic acids and cells.

ACKNOWLEDGEMENTS

This research was supported by a national Leading Research Laboratory Program (Grant 2011-0018607), a Nano/Bio Science and Technology Program (Grant 2011-0002188), and a Converging Research Center Program (Grant. 2011K000864) through the national Research Foundation of Korea funded by the Ministry of Science, ICT and Future Planning.

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1299