Fabrication of protein microarray for high throughput immunoassay by electrospray deposition method and application to the detection of biomolecules

이 범환[a], Yutaka Yamagata[b], and Teruyuki Nagamune[a]

[a]Department of Chemistry and Biotechnology, School of Engineering, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8656, Japan
Tel: +81-48-467-9315, FAX: +81-48-462-4657

[b]The Institute of Physical and Chemical Research (RIKEN), Materials Fabrication Laboratory, 2-1 Hirosawa, Wako, Saitama, Japan
Tel: +81-48-467-9315, FAX: +81-48-462-4657

It was successfully deposited, and immobilized covalently onto a conductive substrate like ITO glass or surface modified ITO glass. The high-density arrays having uniform spots as 150 (m or smaller in diameter was successfully fabricated. The arrays were applied to detection of IgGs from six kinds of animals and eight kinds of human cytokines based on enzyme-linked immunosorbent assays (ELISA) with Horseradish Peroxidase (HRP) or Alkaline phosphotase (AP) conjugated detection antibodies, and the arrays were collectively detected with chemiluminescence and fluorescence. We succeeded to detect eight kinds of human cytokines and the signals of the microarrays were visualized with X-ray film by chemiluminescence. No cross-activity between deposited spots was occurred on detecting antigens with the microarray and quantitatively visualized with high-resolution charge-coupled device (CCD) detector by fluorescence. Sensitive and simultaneous detection of human cytokine under antigen concentration of 100 pg/ml could be performed by the microarray. Though application of the microarray was performed exclusively to the detection of IgG and human cytokines in this study, the microarray fabricated with ESD method promise further extensive application to new biomarker discovery, protein expression profiling, and disease diagnostics

References

1. Chee M.; Yang R.; Hubbell E.; Berno A.; Huang XC.; Stern D.; et al., "Accessing genetic information with high-density DNA arrays"(1996), Science 274, 610-614.
2. Mooney JF, Hunt AJ, McIntosh JR, Liberko CA, Walba DM, Rogers CT., "Patterning of functional antibodies and other proteins by photolithography of silane monolayers"(1996), Proc Natl Acad Sci U.S.A, 93, 12287-12291.
3. Hoyer B.; Sorensen G,; Jensen N,; Nielsen DB,; Larsen B., "Electrostatic spraying: A novel technique for preparation of polymer coatings on electrodes"(1996), Anal Chem. 68, 3840-3844.
4. Thundat T.; Warmack RJ.; Allison DP.; Ferrel TL., "Electrostatic spraying of DNA-molecules for investigation by scanning tunneling microscopy"(1992), Ultramicroscopy 42, 1083-1087.
5. Lee, B.; Kim, J.; Ishimoto, K.; Yamagata, Y.; Tanioka, A.; Nagamune, T., "Fabrication of Protein Microarrays for Immunoassay using the Electrospray Deposition (ESD) Method"(2003), *Journal of Chemical Engineering of Japan*, in press.