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Protein Interactions on Nano-Scale Controlled Surface

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Abstract

In this study, antibody microarrays on a nano-scale controlled surface were prepared. Aspects of performance such as signal intensity, dependence of signal intensity on target antigen concentration, etc. were evaluated and compared with those of microarrays on amine, aldehyde, and epoxy surfaces. The signal intensities of the anti-TNF-α antibody microarray fabricated on the nano-scale controlled surface were found to be 2-8 times higher than those prepared on the other surfaces. Additionally, the anti-TNF-α and anti-IL-1β antibody microarrays evidenced linear correlations between their signal intensities and concentrations of target antigen applied to the microarrays in a range between 3.0 nM-1.0 μM. Furthermore, the antibody microarrays detected two different antigens simultaneously with similar signal intensity to those achieved in single antigen detection experiments.

Keywords: Protein interaction, Protein microarray, Antibody, Antigen, Nano-scale controlled surface

Introduction

Recently, a new type of surface as a substrate for DNA microarrays has been prepared via the self-assembly of conical-shaped dendron molecules on glass slides[1-5]. It was determined that a conical-shaped dendron provided primary amino groups on a surface which are separated from each other by an average of 3 nm[1]. The DNA microarrays fabricated on this nano-scale controlled surface evidenced enhanced signal intensity and discrimination efficiency for a variety of single nucleotide polymorphism types[2,3]. Additionally, when subjected to the detection of single nucleotide variation of the p53 gene in genomic DNAs obtained from cancer cell lines, the DNA microarrays clearly discriminated single nucleotide variations in hotspot codons with high degrees of selectivity and sensitivity[4].

The outstanding performance of DNA microarrays fabricated on the nano-scale controlled surface is profoundly related to the novel properties of the dendron molecule, which features a conical structure that allows for mesospacing between the capture probes. Microarrays on the dendron-modified surface can reduce the steric hindrance not only between the solid surface and target DNA, but also among the immobilized capture probes, thereby rendering the hybridization process on the surface extremely effective.

Protein is a totally different molecule from DNA. Proteins are chemically and structurally substantially more complex and heterogeneous than are DNA molecules, and far more readily lose their conformation and biochemical activity as the result of denaturation, dehydration, or oxidation. Therefore, more sophisticated immobilization chemistries are required to immobilize proteins in active conformations and to maintain the biochemical properties or activities of proteins during experimentation using microarrays[6-32].

Proteins can be immobilized covalently onto glass surfaces modified with aldehyde[33,34], epoxide[35], or linker-modified amine[36] functional groups. The proteins are immobilized on aldehyde surfaces via the Schiff base reaction between the aldehydes on surface and the amino groups (lysine residues) on the proteins. In a similar fashion, the amino groups of proteins react with the epoxide group on the surface, thereby forming a covalent bond. Such covalent immobilization approaches result in stronger attachment, but lack defined orientations of the capture agents on the solid support. Therefore, maintaining the activity of the immobilized proteins and low signal intensity are ongoing problems for the protein microarrays fabricated on these surfaces[37-43].

In order to elucidate the characteristics of the nano-scale controlled surface as a substrate for protein microarrays, we fabricated antibody microarrays on the surface. Additionally, properties such as signal intensity, the relationship between signal intensity and target antigen concentration, etc. of the microarrays were compared with those of other surfaces, namely the amine, aldehyde, and epoxide-modified surfaces.

Results and Discussion

Preparation of Antibody Microarrays

The nano-scale controlled surface was treated with the homobifunctional linker, N,N-disuccinimidyl carbonate (DSC)[5], and capture anti-TNF-α and/or anti-IL-1β antibodies were subsequently microarrayed on the DSC-treated surface. The reaction of the amino group of the nano-scale controlled surface with DSC generates a succinimidyl surface which can form a covalent bond in aqueous solution with the amino group of the capture antibody to generate the antibody-modified surface. On the other hand, antibody microarrays on the amine, aldehyde, and epoxy surfaces were prepared via the direct spotting of capture antibodies onto the surfaces in accordance with the supplier's recommended protocols.

Detection Strategies for Protein Microarray

In an effort to detect the protein interaction between the capture antibody immobilized on the surface and the incoming target antigen, we employed a four-component sandwich assay, as is shown in Figure 2.

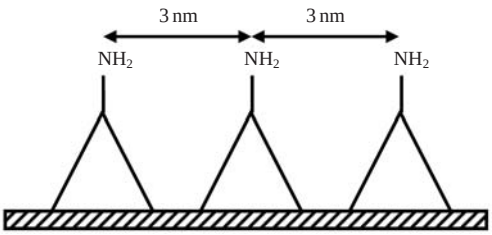

Figure 1. Schematic drawing of nano-scale controlled surface. The surface was generated via the self-assembly of conical-shaped dendron molecules on glass slides.

The sandwich assay has evolved directly from the radioimmunoassay or ELISA protocols in which they are extensively employed. The sandwich assay format relies on immobilized antibodies to capture the protein of interest (antigens), whereas a second labeled antibody directed against the captured protein and the reporter molecule (which can bind specifically to the detection antibody) are utilized for detection. In this approach, two distinct antibodies, each with affinity to separate epitopes on the protein of interest, are required. The binding events between the capture antibodies and antigens were detected via sequential incubation with the detection antibody labeled with biotin and with streptavidin labeled with Cy5 (streptavidin-Cy5, reporter molecule).

Properties of Antibody Microarrays

In order to evaluate the signal intensities of microarrays fabricated on four different kinds of surfaces, anti-TNF-α antibody was microarrayed twice in a 2 × 5 format. 300 nM of TNF-α antigen was applied to the microarrays. As is shown in Figure 3, the signal intensities of spots on the nano-scale controlled surfaces were 2-8 times higher than those on the other surfaces. At other concentrations of TNF-α antigen, similar results were observed. These findings indicate that substantially more TNF-α antigens were captured by the anti-TNF-α antibodies immobilized on the nano-scale controlled surface than on the other surfaces. This is an interesting result, considering that the density of the surface functional group of the nano-scale controlled surface is only 1/100 of those on the other surfaces[44,45]. As the number of functional groups on the surface decreases, the amount of captured antibodies immobilized also decreases. Therefore, the higher intensity of the microarray fabricated on the nano-scale controlled surface originates not from the

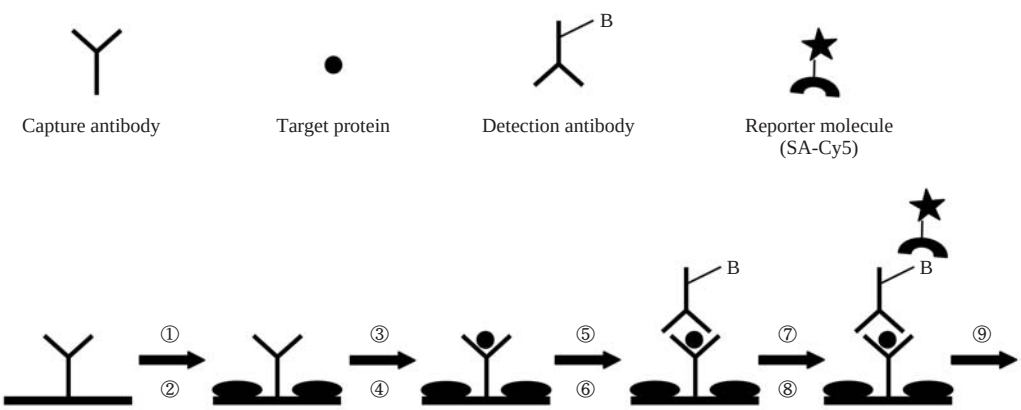

Figure 2. Scheme of the four-component sandwich assay used in antibody microarray detection. ① Blocking, ② Washing, ③ Target protein, ④ Washing, ⑤ Detection antibody, ⑥ Washing, ⑦ Reporter molecule, ⑧ Washing and drying, ⑨ Scanning

quantity of bound antibodies but rather is a consequence of the quantity of bound antibodies in active form. This result indicates that the nano-scale controlled surface is capable of maintaining the activity of proteins immobilized on the surface.

Correlation between Fluorescence Intensity and Concentration of the Target Antigen

In order to elucidate the correlation between fluorescence intensity and target antigen concentration, the solution of a capture antibody of anti-TNF-α (1.0×10^{-5} M) was microarrayed on DSC-modified dendron surfaces. The prepared antibody microarray was then permitted to react with TNF-α antigen at a variety of concentrations-for example, 3.0 nM, 10 nM, 100 nM, 300 nM, and 1.0 μM. After rinsing with washing buffer, the microarray was permitted to react with detection antibody sequentially labeled with biotin and streptavidin-Cy5. As is shown in Figure 4, the fluorescence intensity increased with the concentration of target antigen in a range of 3 nM-1.0 μM. This finding indicates that the antibody microarrays fabricated on the nano-scale controlled surface might be employed to detect nanomolar concentrations of target antigen.

Both the anti-TNF-α and anti-IL-1β antibodies were spotted on the same nano-scale controlled surface in a 2×5 format, respectively. The prepared microarrays were then treated with a mixture of TNF-α and IL-1β antigens. As is shown in Figure 5, the fluorescence intensities obtained from the simultaneous detection of two different antigens, TNF-α and IL-1β, were similar to those acquired from the single antigen detection experiment. This result indicates that the antibody microarrays fabricated on the nano-scale controlled surface can selectively detect the target antigen, without sacrificing its binding ability.

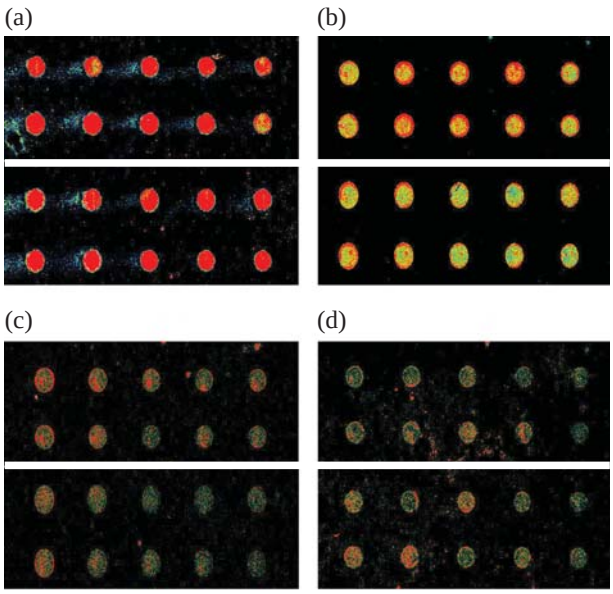

Figure 3. Fluorescence signals obtained from anti-TNF-α antibody microarrays fabricated on (a) nano-scale controlled (b) amine (c) aldehyde, and (d) epoxy glass slides. The concentration of TNF-α target antigen used was 300 nM.

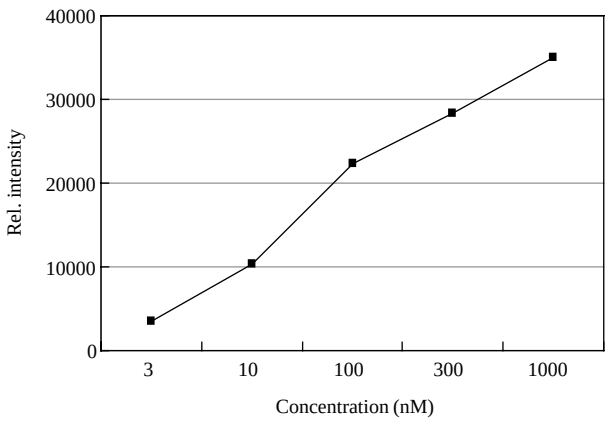

Figure 4. Dependence of signal intensity of antibody microarrays fabricated on the nano-scale controlled surface on the concentration of target antigen.

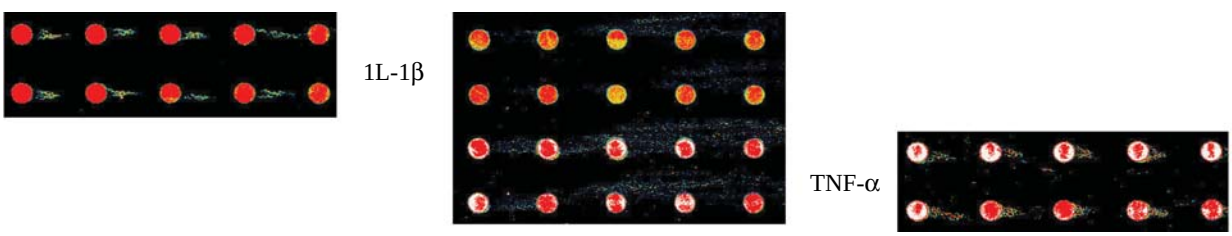

Figure 5. Fluorescence signals from the simultaneous detection of two different antigens, IL-1β and TNF-α, on the same microarray (middle). The signal intensities were similar to those obtained from single antigen detection experiments (left and right).

Conclusions

In this study, the performance of the nano-scale controlled surface as a substrate for protein microarray was assessed and compared with those of three other types of glass slides-namely, amine, aldehyde, or epoxy-modified surfaces. The protein microarrays fabricated on the nano-scale controlled surface evidenced higher signal intensities and more reliable data than those prepared on the other surfaces. In particular, the nano-scale controlled surface evidenced substantially better ability to maintain the activity of immobilized protein than was noted with the other surfaces. Therefore, we conclude that the nano-scale controlled surface assessed herein can be utilized as a promising surface for protein microarrays.

Materials and Methods

SuperAmine, SuperAldehyde, and SuperEpoxy glass slides were purchased from ArrayIt (USA) and NSB amine (dendron-modified) slides from NSBPOSTECH (Korea). The protein microarrays on the above slides were prepared in accordance with the supplier's recommendations. Streptavidin-Cy5, monoclonal anti-TNF-α, and anti-IL-1β antibodies were purchased from Sigma (St. Louis, MO). Solutions of TNF-α and IL-1β antigens were allowed to react with the antibody microarrays for 3 hours. After washing with washing buffer, the microarrays were sequentially treated with detection antibody labeled with biotin and washed again with streptavidin labeled with Cy5. The fluorescence signal of the microarray was measured using ScanArray Lite (GSI Lumonics).

Acknowledgements

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