Homogeneous Immobilization of Probe DNAs on DNA Chip Using Polyurea Thin Film

Atsushi Kira
Research & Development Division, ULVAC Inc, 2500 Hagizono, Chigasaki, Kanagawa 253-8543, Japan and Graduate School of Engineering, Yokohama National University, 79-5 Tokiwadai, Hodogaya-ku, Yokohama 240-8501, Japan

Hyonchol Kim
Kanagawa Academy of Science and Technology, KSP East 310, 3-2-1, Sakado, Takatsu-ku, Kawasaki, Kanagawa 213-0012, Japan

Kenji Yasuda
Kanagawa Academy of Science and Technology, KSP East 310, 3-2-1, Sakado, Takatsu-ku, Kawasaki, Kanagawa 213-0012, Japan and Department of Biomedical Information, Division of Biosystems, Institute of Biomaterials and Bioengineering, Tokyo Medical and Dental University, 2-5-10, Kanda-Surugadai, Chiyoda-ku, Tokyo 101-0062, Japan

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We propose a method to attach probe DNAs with a homogeneous population for quantitative measurement of a minimum number of target DNA/RNA for the next generation of DNA chip. To control the homogeneous population of DNA probes, polymer films having neighboring reactive groups at the same distance, i.e., aromatic polyurea film formed with 3,5-diaminobenzonic acid (DBA) and methylenedi(p-phenylene) diisocyanate (MDI) and aliphatic polyurea film formed with DBA and hexamethylene diisocyanate (HDI), were polymerized directly on the surface of a chip substrate by vapor deposition polymerization. The spatial arrangement and homogeneity of the reactive groups on the surfaces of the substrates were examined by observing the immobilization of DNA fragments bound to 20-nm gold nano-particles on the polymer films. The results showed that physical adsorption of the DNA was effectively inhibited on the DBA/HDI film in contrast to the DBA/MDI film and the conventional aminosilane surface. Moreover, the distribution of the particles was homogeneous on the DBA/HDI film, unlike on the other surfaces. These results indicate that the aliphatic polyurea film could be used to control the selectivity and uniformity of reactive groups on the surface of substrates for more precise quantitative measurement of DNA chip technology. [DOI: 10.1380/ejssnt.2009.728]

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I. INTRODUCTION

The DNA microarray is a powerful tool for various applications regarding nucleic acid analysis, such as the comprehensive analysis of both gene expression profiles and single nucleotide polymorphisms (SNPs), because of its simple procedure and structure [1, 2]. There are two major fabrication methods of DNA microarrays: the photolithographic synthesis of probes on a chip using light-sensitive masking agents and nucleoside phosphoramidites-based chemistry [3] and the covalent immobilization of prefabricated oligonucleotide probes onto chemically activated surfaces using spotting devices such as ink-jets [4]. Silica (glass or quartz) is commonly used as a microarray substrate due to its chemical resistance, low intrinsic fluorescence, and suitability for silane coupling formation. The reactive group of organosilane in probe oligonucleotides is easily immobilized covalently on such a substrate to form a matrix of probe arrays [5, 6].

However, conventional silane coupling has problems. Due to the probabilistic chemical reaction of the attachment between the probe oligonucleotide and the substrate, the intervals of the attached DNA probes are random and the spatial distribution of the probe DNA is not uniform in principle. To overcome the inhomogeneity of this organosilane-based surface modification, we apply a polymer surface having a homogeneous interval of reactive groups on the substrate to maintain the homogeneity of the spatial intervals of the probe DNAs.

We report here a new method to immobilize probe DNAs on a solid surface with a desired interval and homogeneous population. The polyurea polymer thin films were formed by vapor deposition polymerization (VDP), which is a simple and useful dry process to form various polyimide [7], polyamide [8], or polyurea [9] thin films with a desired molecular population. We prepared two kinds of films: an aromatic polyurea film formed with 3,5-diaminobenzonic acid (DBA) and methylenedi(p-phenylene) diisocyanate (MDI) and an aliphatic polyurea film formed with DBA and hexamethylene diisocyanate (HDI). Both the homogeneity of the introduced functional group and the control of the target...
immobilization were evaluated.

II. EXPERIMENTAL

A. Preparation of polyurea thin films

Polyurea thin films were formed from diamine and diisocyanate monomers on silicon substrates using the VDP system described previously [9]. A reaction scheme to derive polyurea thin films from diamine and diisocyanate monomers is shown in Fig. 1. DBA, MDI, and HDI monomers (Tokyo Chemical Industry Co. Ltd., Tokyo, Japan) were set into the VDP system and evaporated monomers (Tokyo Chemical Industry Co. Ltd., Tokyo, Japan) [5] was used as a control. (This substrate will be described as NCS, hereafter).

B. Evaluation of spatial arrangement and homogeneity of prepared films

We used gold nano-particle labels to visualize the positions of functional groups on the substrates. A solution with 20-nm diameter gold nano-particles (British BioCell International Inc., Cardiff, UK) was mixed with thiolated DNA fragments with the same condition as previously reported [10, 11]. In a typical condition, the DNA was adjusted as 12 μM and mixed with 12 nM 20-nm gold nanoparticle solutions. After incubation for 4 h, the mixture was diluted ten times with a 500-mM NaCl and 10-mM phosphate buffer (pH 7.4) and then incubated for 12 h. Excess reagents were removed by two rounds of centrifugation and re-suspension. Following this procedure, the DNA fragments were bound to the surface of gold nanoparticle with its number density of about 1.1 × 10^13 cm^-2 [11]. The DNA was commercially synthesized (Tsukuba Oligo Service, Co., Ltd., Ibaraki, Japan) with the following sequences and functional groups: 5’-SH-(CH2)5-GCAACAAGTGAGCATCATTC-NH2-3’.

The DBA/MDI, DBA/HDI, and NCS substrates reacted with the prepared gold nano-particles, and the positions of functional groups on the films were visualized through the particles. For both DBA/MDI and DBA/HDI, the carboxyl groups in DBA were pre-activated with 10 mg/mL of N-hydroxysuccinimide (NHS) and 10 mg/mL of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) in water for 20 min at room temperature. The substrates were then reacted with 6 nM of the prepared gold nano-particles in phosphate buffered saline (PBS) for 2 h at room temperature. For control experiments, the same films whose functional groups were not pre-activated were also reacted with gold nano-particles. For the NCS substrate, the prepared gold nano-particles were directly reacted in the same condition as the DBA/MDI and DBA/HDI polyurea films. For a control experiment, NCS was reacted with 1-mM 2-aminoethanol in PBS for 2 h at room temperature before being reacted with gold nano-particles in order to inactivate the NCS groups on the substrate.

III. RESULTS AND DISCUSSIONS

To evaluate the quality of the prepared films (DBA/MDI and DBA/HDI polyurea films and NCS), gold nano-particles bound to DNA fragments were attached to the films through DNA immobilization to visualize the positions of the functional groups, as described in the above experimental section. The functional group on each film was pre-activated to be able to react with amino group in 3’-terminal of DNA and was thus labeled with the gold nano-particles. Scanning electron microscope (SEM) images of the films with labeled functional groups are shown in Fig. 2. The same films whose functional groups were not pre-activated were also reacted with gold nano-particles as controls (Fig. 2 (B, D, and F)). The number densities of particles on both the pre-activated and non-activated films were calculated and compared to evaluate the controlled immobilization of DNA through the functional groups on surfaces (Table I). The number densities of particles for the pre-activated films were 286 ± 11 μm^-2 for NCS, 275 ± 16 μm^-2 for DBA/MDI, and 255 ± 8 μm^-2 for DBA/HDI, indicating a little difference of ability in immobilizing DNA with high density for each film. On the other hand, physical adsorptions evaluated using non-activated films were 129 ± 53 μm^-2 for NCS, 114 ± 50 μm^-2 for DBA/MDI, and 0.6 ± 0.4 μm^-2 for DBA/HDI. This result clearly indicates that DBA/HDI
FIG. 2: SEM photographs of NCS, DBA/MDI, and DBA/HDI films reacted with 20-nm gold nano-particles bound to reactive DNA. Functional groups on the films were labeled and visualized with the particles through DNA immobilization. In control experiments, functional groups on films were not activated before particles were reacted. (A) NCS, (B) control of NCS, (C) DBA/MDI, (D) control of DBA/MDI, (E) DBA/HDI, and (F) control of DBA/HDI.

TABLE I: Number densities of particles on pre-activated and non-activated films.

<table>
<thead>
<tr>
<th></th>
<th>Activated density of particles [µm$^{-2}$]</th>
<th>Non-activated (control) density of particles [µm$^{-2}$]</th>
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<tbody>
<tr>
<td>NCS</td>
<td>286 ± 11</td>
<td>129 ± 53</td>
</tr>
<tr>
<td>DBA/MDI</td>
<td>275 ± 16</td>
<td>114 ± 50</td>
</tr>
<tr>
<td>DBA/HDI</td>
<td>255 ± 8</td>
<td>0.6 ± 0.4</td>
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</tbody>
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The above results indicate that the DBA/HDI film has two advantages over the other two surfaces, that is, a high S/N ratio and homogeneous interval of reactive groups.

IV. CONCLUSION

To improve both the sensitivity and reproducibility of DNA microarrays, we prepared chemically active polyurea thin films using VDP technology. Fully controlled immobilization of DNA was evaluated by labeling functional groups on the films with gold nano-particles. The results showed that DNA could be immobilized on DBA/HDI films with both a high S/N ratio and homogeneous population, better than that for DBA/MDI and NCS films. These results indicated that DBA/HDI polyurea films have advantages for more precise quantitative measurement for the next generation of DNA chip technology.

TABLE II: Averages and variances of distances between adjacent particles on films.

<table>
<thead>
<tr>
<th></th>
<th>NCS</th>
<th>DBA/MDI</th>
<th>DBA/HDI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average</td>
<td>55.33</td>
<td>45.16</td>
<td>60.70</td>
</tr>
<tr>
<td>Variance</td>
<td>7962.5</td>
<td>358.9</td>
<td>184.2</td>
</tr>
</tbody>
</table>

$a$ Variance $S$ is given by $S = \frac{1}{N-1} \sum_{i=1}^{N} (x_i - \overline{x})^2$, where $x$ is distance between adjacent particles and $N$ is number of samples.