Development of DNA chip for SNP detection using oligonucleotide-arrayed on TFT photosensor

K. Hatakeyama[1], T. Tanaka[1], M. Sawaguchi[2], A. Iwadate[2], J. Ogura[2], N. Tateishi[2], H. Takeyama[1] and T. Matsunaga[1]
[1]Tokyo Univ. of Agric. & Technol, [2]Casio Computer Co. Ltd.
[1]2-24-16 Naka-cho, Koganei, Tokyo 184-8588, Japan, [2]1-6-2, Honmachi, Shibuya-ku, Tokyo 151-8543, Japan

A novel detection system of single nucleotide polymorphisms (SNPs) using the amorphous silicon-thin film transistor (TFT) arrays was proposed. The detection of SNP in aldehyde dehydrogenase 2 (ALDH2) gene, which encodes the principal enzyme for the oxidative metabolism of ethanol, was performed as a model system. The principle of SNP detection in ALDH2 gene was based on hybridization between oligonucleotide arrayed on the TFT photo-sensor and fluorescence dye-labeled single stranded DNA (ssDNA).

TFT photo-sensor consists of amorphous silicon TFT array coated with TiO_2 in order to cut off excitation wavelength of ultraviolet region. Oligonucleotide-arrayed on TiO_2 surface of TFT photo-sensor was performed as followings; TiO_2 surface of TFT photosensor was activated by oxygen plasma treatment, and immediately the sensor was immersed into γ-aminopropyltriethoxysilane (γ-APTES). The γ-APTES-coated TiO_2 surface was reacted with *N*-(γ-maleimidobutyloxy)sulfosuccinimide ester (Sulfo-GMBS), and subsequently thiolated oligonucleotide on the 5' end was immobilized on their surface. A fluorescent-based assay using Cy3 labeled *N*-hydroxysuccinimide (NHS) ester was adopted to quantify the number of amino groups on γ-APTES-coated TiO_2 surface. Cy3-NHS ester is a fluorescent dye that is specifically reactive towards amino groups. The number of amino groups on TiO_2 surface increased as increasing plasma treatment time. The amine number reached its maximum (16.9 pmol/cm^2) by plasma treatment for 60 s. To determine the contribution of thiol group to the immobilization of oligonucleotide on GMBS-coated substrates, 10 μM TAMRA-labeled oligonucleotides with or without thiol group on the 5'-end were spotted with SPBIO 2000 spotter (Hitachi Software, Japan). The fluorescence was quantified with a Scan Array Lite microarray scanner (GSI Lumonics, USA). TiO_2 coated TFT photosensor and glass slide (SiO_2) were used as substrates. The number of oligonucleotide on TiO_2-substrate was 13.5 pmol/cm^2, resulting in 2 times higher than that on SiO_2-surface (Fig. 1). This value is similar to the previous reports by the analysis using radio-isotope labeled oligonucleotide[(1)]. The oligonucleotide-arrayed on TiO_2 were used in the following experiments.

SNP detection was performed using ALDH2*1 or ALDH2*2 oligonucleotide-arrayed on TiO_2 surface. Quantum dot (QD; em: 565 nm)-labeled oligonucleotide (21-mer; complementary with ALDH2*1 or ALDH2*2 oligonucleotide) was used as model target ssDNA. Fluorescence signals were observed using microarray scanner when QD-labeled complementary ssDNA was used, while negligible fluorescence was obtained by hybridization with one-base mismatched ssDNA (Table). Furthermore, SNPs were detected in the presence of both target ssDNA, i.e. biotin-labeled ALDH2*1 and TAMRA-labeled ALDH2*2 oligonucleotides. Fluorescence signals from TAMRA were observed only on ALDH2*2-array (Fig.2). When streptavidin-QD was introduced to detect biotin-labeled oligonucleotide after hybridization, fluorescence signals from QD were observed only on ALDH2*1-array. These results indicate that the oligonucleotide-array allows selective detection of SNPs in the presence of both target ssDNA. Based on these results, SNP detection was performed on TFT photosensor. Specific detection of QD-labeled ALDH2*1 ssDNA was successfully achieved by and on TFT photosensor when an excitation by Spot Cure spot UV curing equipment (less than 300 nm, USHIO, Japan) was applied. Furthermore, SNP detection by and on TFT photosensor using ALDH2 fragments amplified from genomic DNA was investigated. The use of TFT photosensor will enable the further miniaturization and low cost of DNA chip-based detection system.

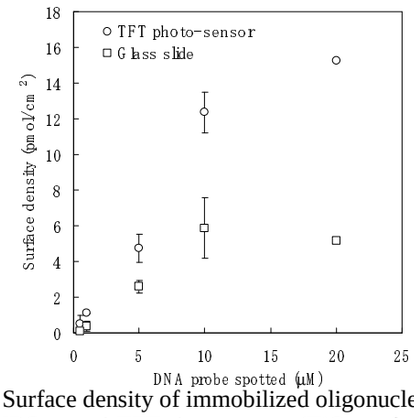

Fig. 1 Surface density of immobilized oligonucleotide on GMBS-coated TFT photo-sensor (TiO_2 surface) and glass slide. Sequence of oligonucleotide used was 5' SH- TTCACTTCAGTGTAT-TAMRA-3' (15 mer)

Table surface density of QD-labeled target ssDNA reacted with arrayed oligonucleotides

Targets	arrayed oligonucleotide*	Surface density of target ssDNA** (fmol/cm^2)
ALDH2*1	ALDH2*1	217.6
	ALDH2*2	0.5
ALDH2*2	ALDH2*1	8.8
	ALDH2*2	164.2

The detection oligonucleotides were spotted in 10 μM concentration.
* Sequence of arrayed oligonucleotides used were 5' SH-TTC ACTTCAGTGTAT-3' (ALDH2*1) and 5' SH-TTTCACTTT AGTGTAT-3' (ALDH2*2)
** Sequence of target ssDNA used were 5'-GGCATACACTG AAGTGAAAAC-3' (ALDH2*1) and 5'-GGCATACACTAA AGTGAAAACT-3' (ALDH2*2).

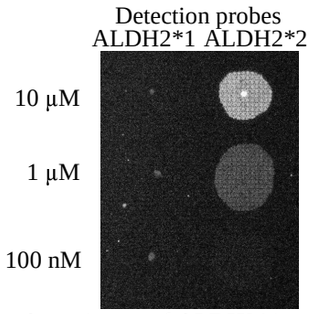

Fig. 2 Scan Array image of ALDH2*1 and ALDH2*2 oligonucleotide-arrayed on TFT photosensor. The oligonucleotides were reacted with TAMRA-labeled ALDH2*2 oligonucleotide. Diameter of spots: 400-500 μm.

REFERENCE

(1) Linda A. Chrisey, Gil U Lee and Elizabeth O'Ferrall (1996) *Nucleic Acids Res.* **24**, 3031-3039.