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Development of PNA-Array Platforms for Detection of Genetic Polymorphism of Cytochrome P450 2C19†

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The selective and sensitive detection of target DNA is an important issue for clinical diagnostics, including identification of single nucleotide polymorphism (SNP), as well as for fundamental studies in molecular biology.[1-3] The commonly used DNA-detection techniques are mainly based on the DNA microarrays, which present probe-DNA molecules immobilized onto a solid surface in an array format for the bio-specific hybridization with the unknown target-DNA fragments.[4] Although the DNA microarrays have been utilized as a basic platform for the clinical detection, selectivity and sensitivity are still limited toward the single-nucleotide discrimination, which precludes the accurate analysis of SNP.[5] In addition, duplexes between the target and probe DNAs exist in a stable form only in the presence of salt because of the electrostatic repulsion between negatively charged ribose-phosphate backbones of DNA;[6] these salt conditions also induce the secondary and tertiary structures of DNA oligomers themselves, which could disturb the hybridization between the target and probe DNAs.[7] On the other hand, peptide nucleic acid (PNA), an artificial nucleic acid analog, has been introduced to overcome the drawbacks of natural DNA in the aspects of specificity, sensitivity, and stability,[8-10] and been as a molecular tool for the identification of SNP.[11-13] The neutral backbone of PNA enhances the stability of PNA-DNA duplexes due to the absence of interstrand repulsion between the negatively charged phosphate groups of DNA.[10,14] In addition, the unnatural structure makes PNA not degraded by enzymatic reactions.

In this work, we generated a PNA-array platform to detect the SNP of cytochrome P450 2C19 (CYP2C19) gene. The CYP2C19 gene is known to play an important role in the metabolism of therapeutic drugs, including diazepam, proguanil, lansoprazole, and omeprazole.[15] It is noteworthy that 15-20% of the Asians are genetically deficient in CYP2C19, failing to produce enzymes for the metabolism, compared with the occurrence of 3-5% of Caucasian populations.[16] Specifically, there exist two variant alleles leading to the mutant types. The first varient is CYP2C19*2, which has a G → A nucleotide substitution at position 681, resulting in an aberrant splicing site; the second one, CYP2C19*3, which does a G → A substitution at position 636, resulting in a premature termination codon. According to the previous report, the frequency of CYP2C19*2 and CYP2C19*3 for Koreans is 0.21 and 0.12, respectively.[17]

Onto the silicon oxide surface were aldehyde groups introduced via imine formation between amine-terminated self-assembled monolayers (SAMs) and glutaldehyde. Another imine linkage was used for immobilizing the PNA probes, and unreacted aldehyde groups were passivated with 2- (2-aminoethoxy)ethanol. We designed four probe-PNAs based on the clinically important SNPs of CYP2C19, and denoted them 636W and 636M for the wild and mutant types of CYP2C19*3, and 681W and 681M for CYP2C19*2, respectively (Fig. 1). The linker of the PNA probes contained repetitive ethylene glycol groups and ended with several lysine groups. The immobilization of the probe PNAs was confirmed by hybridization of target DNAs. The target DNAs (T-636W, T-636M, T-681W, and T-681M), complementary to each of the PNA probes, were tagged with Cy3 (green) for wild type and Cy5 (red) for mutant type, respectively. The hybridization was performed for 1 h at 40 °C with the DNA solution containing both T-636W and T-636M (100 nM), and the same conditions were used for hybridization of T-681W/M. As shown in Fig. 2a, each DNA was selectively hybridized with its complementary probe-

Probe sequence (N terminal ⟶ C terminal)	
636W	NH_2-Linker-TCTTGAT**C**CAGGGGG
636M	NH_2-Linker-ACCCCCTG**A**ATCCAG
681W	NH_2-Linker-TTATTTCCC**G**GGAACCC
681M	NH_2-Linker-TATTTCCC**A**GGAACC

Figure 1. PNA probes studied in this work. The underline represents the single-point mutation site.

†This paper is dedicated to Professor Eun Lee on the occasion of his honourable retirement.

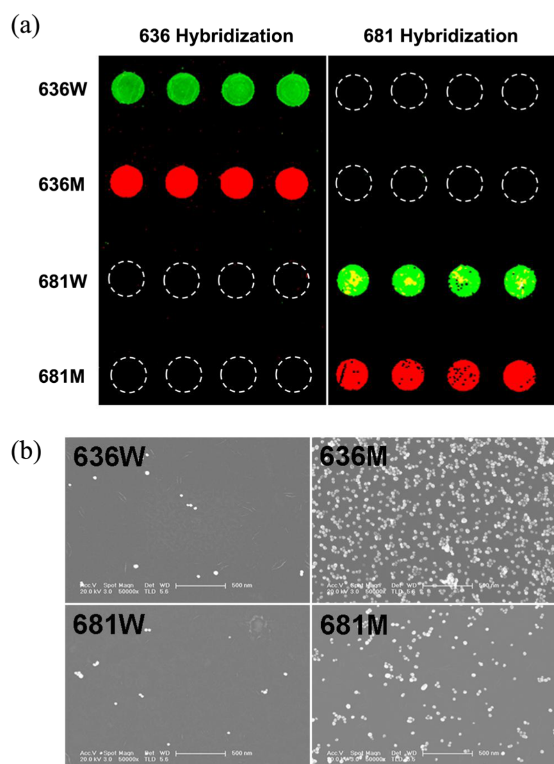

Figure 2. (a) Fluorescence micrographs of the PNA array after hybridization with target DNAs. Each PNAs were spotted four times and hybridized with target DNAs. The left figure shows the PNA array hybridized with T-636W/636M, and the right figure with T-636W/T681M, respectively. (b) SEM images of the four different probe surfaces after hybridization with biotinylated DNA, complementary to 636M probe, and AuNP-SA deposition.

PNA, demonstrating the SNP-detection ability of the PNA platform developed in this study.

The selective hybridization was also confirmed with streptavidin-conjugated gold nanoparticles (AuNP-SA). The AuNP-assisted detection of prostate-specific antigen has recently been demonstrated by us.[18] Based on the previous results, we envisioned that the AuNP-based nanogap device would be applied to the CYP2C19 system seamlessly. In this work, we investigated the selective deposition of AuNP-SA, because it was a prerequisite for the successful implementation of the nanogap device to achieve the probe-specific conjugation of AuNPs. The target DNA, complementary to 636M, was designed to present biotin, which was subsequently coupled with AuNP-SA after PNA-DNA hybridization. The incubation was performed for 4 h with the 50-mM HEPES buffer (pH 7.4) solution of AuNP-SA. Figure 2b shows the scanning electron microscopy (SEM) images of the four different surfaces for each PNA probes. The numbers of immobilized AuNPs for 636W, 681W, and 681M were found to be significantly low compared with that for 636M. The results clearly indicated that the AuNP-assisted detection of the target DNA was possible with high selectivity. Of more importance, the significant difference in the number of the deposited AuNPs would make it possible to apply the nanogap device to the CYP2C19 system. The combination of PNA-based surface treatments and nanogap fabrication would be optimized for high-sensitive detection of SNPs in CYP2C19 via electric signals, which is our next research thrust.

In summary, we generated a PNA array for the sensitive detection of SNPs found in CYP2C19, by using the formation of self-assembled monolayers and subsequent surface-based organic reactions. The four different PNA probes, 681W/M and 636W/M, were confirmed to be differentiated at surfaces by fluorescent measurements. As a proof-of-concept, AuNPs were introduced to detect the target DNA, demonstrating the accessibility toward the electronic devices. We also believe that our PNA-array platform would be further adjustable toward microarray technology for clinical diagnostics.

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