Extended Abstracts of the 2004 International Conference on Solid State Devices and Materials, Tokyo, 2004, pp. 368-369

I-4-3

Potentiometric Detection of Single Nucleotide Polymorphism Using Genetic Field Effect Transistor

Toshiya Sakata and Yuji Miyahara

Biomaterials Center, National Institute for Materials Science, 1-1 Namiki, Tsukuba, Ibaraki 305-0044, Japan
Phone: +81-29-860-4506 E-mail: MIYAHARA.Yuji@nims.go.jp

1. Introduction

We have been investigating a new approach to realize an electrochemical detection for DNA chips, although a number of fluorescent detection methods are widely used for SNP genotyping [1]. The novel concept of a genetic field effect transistor (FET) is proposed in the present study for improving precision, standardization and miniaturization of a DNA chip system. The genetic FET is composed of Si with Si_3N_4/SiO_2 as the gate insulator on which DNA probes are immobilized and subsequently hybridized with target DNA in sample solutions. The potentiometric detection method is based on the direct transduction of surface density change of charged biomolecules into electrical signal by the field effect and is effective for charged species such as DNA molecules. Here, we report the concept of genetic FET and the ability of SNP genotyping by controlling hybridization temperatures, and by the utilization of intercalator or primer extension reaction using the genetic FET.

2. Principle of genetic FET

The measurement diagram and the photograph of FET chip for direct detection of allele specific oligonucleotide hybridization are shown in **Fig. 1**. The genetic FET is immersed in a measurement solution together with a Ag/AgCl reference electrode with saturated KCl solution. The potential of a measurement solution is controlled by the reference electrode. Oligonucleotide probes are immobilized on the gate surface. When target DNA are contained in a sample solution, hybridization occurs at the surface of the gate area. Since DNA molecules are negatively charged in an aqueous solution, they electrostatically interact with electrons in Si crystal through the thin gate insulator. As a result of the interaction, hybridization reaction can be detected in principle by measuring the change in the electrical characteristics of the FET, such as the threshold voltage (V_T) shift at constant I_D.

We make use of ionized characteristic of intercalator in combination with the genetic FET, while it is used as a fluorescent reagent in the conventional methods [2]. Intercalator is ionized and positively charged in an aqueous solution, and reacts specifically with double-stranded DNA on the gate surface. Hence, an undesirable background noise such as non-specific adsorption of single-stranded DNA can be eliminated by the use of intercalator.

A primer extension can be controlled by a match or mismatch at the 3'-terminus of the probe. When the 3'-terminus of the oligonucleotide probe is matched to the target DNA, primer extension reaction occurs and oligonucleotide is synthesized and extended. Since oligonucleotide is negatively charged in an aqueous solution, increase of the negative charges at the gate surface can be detected with the genetic FETs (**Fig. 2a**). On the other hand, extension reaction does not occur and the charges at the gate surface dose not change, when the 3'-terminus of the oligonucleotide probe is not matched to the target DNA (**Fig. 2b**). Thus, the allele specific extension event on genetic FET will allow detection of SNP genotyping.

3. Results and Discussion

3.1. Control of hybridization temperature and utilization of intercalator for SNP genotyping

Based on the principle of genetic FET, allele specific oligonucleotide hybridization and reaction between double-stranded DNA and intercalator were successfully detected with genetic FETs. The V_T of the genetic FET shifted in the positive direction for hybridization and in the negative direction for intercalation, because of intrinsic charges of DNA and intercalator (**Fig. 3**).

Ability to discriminate SNP was examined using the genetic FET. We have prepared two types of the genetic FETs to detect single base change in the target DNA. We used the R353Q locus of factor VII gene [3] as a model sample. Normal oligonucleotide probes were immobilized on the gate surface of one of the genetic FETs (N-type genetic FET), while mutant oligonucleotide probes were immobilized on the gate surface of the other genetic FETs (M-type genetic FET). Both genetic FETs were hybridized with a normal sample and a mutant sample, respectively. One-base change of the target DNA could be detected with the N-type genetic FET and the M-type genetic FET. Our preliminary results show that control of hybridization temperature (**Fig. 4a**) and utilization of intercalator (**Fig. 4b**) lead to the significant improvement of the signal to noise ratio for SNP analysis using the genetic FET.

3.2. SNP genotyping based on primer extension

When thermostable DNA polymerase and dNTPs were introduced into the gate surface after hybridization with the complementally target DNA, the interface potential at the gate surface was monitored. The time course of the V_T change during primer extension reaction is shown in **Fig. 5**. The V_T shift during extension reaction increased drastically up to 10mV. This positive change in the V_T of genetic FET is due to negative charges of polynucleotide extended by primer extension reaction.

For SNP genotyping based on allele specific primer extension, we used the -122 locus of factor VII gene [3] as a model sample. We have prepared two types of N-type and M-type genetic FETs for the simple SNP typing analysis. The N-type genetic FET and M-type genetic FET were hybridized with a normal/normal (n/n) homozygote sample, a normal/mutant (n/m) heterozygote sample and a mutant/mutant (m/m) homozygote sample, respectively. When the DNA polymerase-assisted extension reaction occurred at the gate surface after hybridization, the ratios of the V_T shift of the N-type genetic FET and M-type genetic FET were 9.90, 0.90, 0.06 for a n/n homozygote sample, a n/m heterozygote sample and a m/m homozygote sample, respectively (**Fig. 6**). We found that the primer extension reaction after hybridization could be transduced directly into electrical signal using genetic FETs and was effective to distinguish genotypes of the target DNA.

4. Conclusions

A new method to detect DNA molecules has been proposed using a field effect transistor (FET). Using genetic FET, single nucleotide polymorphism (SNP) genotyping could be realized by controlling hybridization temperatures, and by the utilization of intercalator and primer extension reaction.

Acknowledgements

We would like to thank Drs. J. Tanaka and Y. Horiike, H. Otsuka, S. Maruyama and J.P. Hulme of National Institute for Materials Science, Mrs. Y. Nakajima and S. Matsumoto of Ryokusei M.E.S., Mr. M. Kamahori of Hitachi Ltd., and Dr. P. Fortina of Thomas Jefferson University for their help and useful discussions.

References

[1] D.J. Cutler et al., Genome Res. **11** (2001) 1913.
[2] D. L. Boger et al., *J. Am. Chem. Soc.* **123** (2001) 5878.
[3] T. Kajiyama et al., Genome Res. **13** (2003) 467.

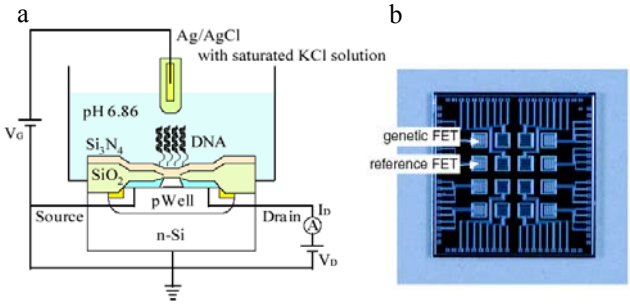

Figure 1 Schematic diagram for measurements of electrical characteristics of genetic FET (a) and photograph of the fabricated genetic FET chip, which is n-channel depletion type (b).

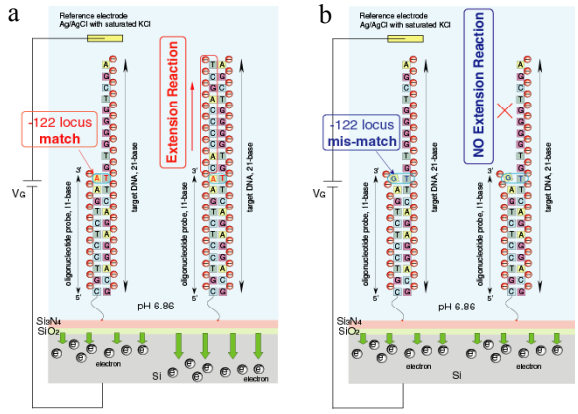

Figure 2 Scheme for potentiometric detection of allele specific extension using genetic FET. We used the -122 locus of factor VII gene as a model sample.

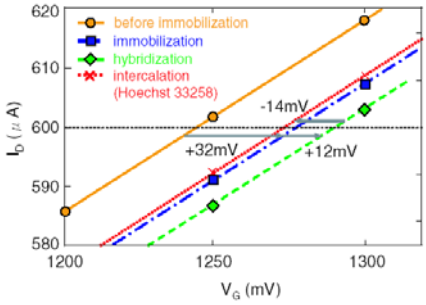

Figure 3 Electrical signals of molecular recognition events on the genetic field effect transistor (FET).

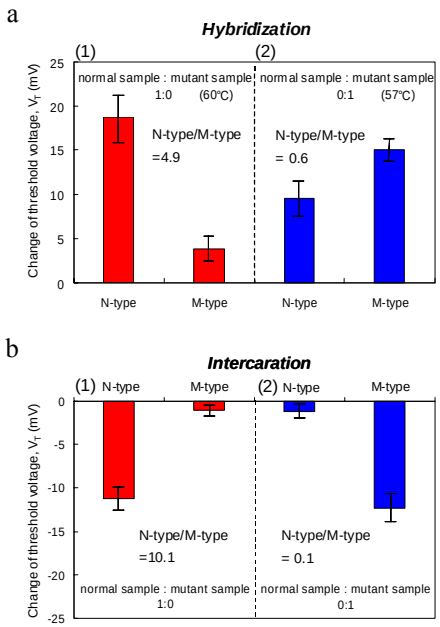

Figure 4 Discrimination of single nucleotide polymorphism (SNP) using genetic field effect transistor (FET). a, SNP detection by allele specific oligonucleotide hybridization at controlled temperatures. Hybridization was carried out at 60 ℃ for normal sample, and at 57 ℃ for mutant sample, respectively. b, SNP detection using genetic FETs in combination with intercalator.

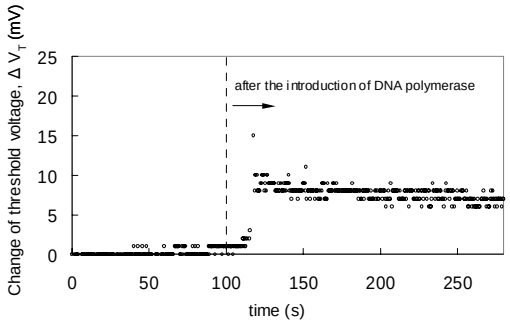

Figure 5 Time course of threshold voltage V_T shift during primer extension reaction. DNA polymerase was introduced into genetic FET at 100 s.

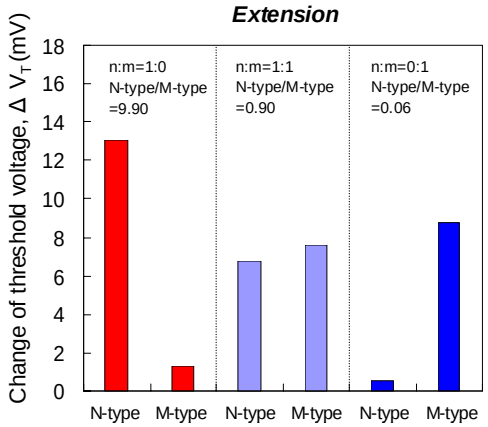

Figure 6 Genotyping analysis using genetic FET. The N-type and M-type genetic FETs were hybridized with a n/n homozygote sample, a n/m heterozygote sample and a m/m homozygote sample, respectively.