Identification of PCR Product by Non-surface-binding Method using Label-free Field-effect Transistor

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ABSTRACT

We demonstrate an innovative method of PCR product identification that is based on thermal denaturation of PCR product-captured bead using integrated BioFET. Laborious purification and concentration of PCR product were easily carried by capturing the samples on the solid surface like microbeads. When temperature raised upto the denaturation point of PCR product, potential of BioFET is changed by mobile DNA in solution from PCR product-captured beads. By differentiating potential change with respect to temperature change, melting temperature can be determined. In this way, we successfully discriminated three kinds of PCR product using their different melting temperature and improved sensitivity of detection. We expect that proposed method can potentially be used for the identification of multiplex PCR products.

Keywords: PCR product, Identification, Field-effect Transistor, Melting temperature (*Tm*)

1 INTRODUCTION

Ion sensitive field effect transistor (ISFET) based deoxyribonucleic acids (DNA) hybridization detection scheme, which measures the intrinsic molecular charge of hybridized DNA by field effect, has been widely investigated by many research groups [1-11]. It is considered to be one of the most promising approach to fast, simple, inexpensive and miniaturized gene detection application such as point of care testing (POCT) [12]. Up until now, however, the reported experimental results are not sufficient enough to vindicate its full potential as a hybridization sensor. Moreover, the lack of theoretical modeling and inconsistency among the obtained data make harder to evaluate its possibilities [10]. As often discussed by the authors, the optimal ionic strength of the buffer for hybridization contradicts with that for the detection due to the counter-ion screening effect [3], [6], [10]. This seems to impose a physical limit on sensitivity and detection time for the practical POCT application in the field.

Recently, some research groups measured Polymerase Chain Reaction (PCR) products by deposit a positively-charged layers such as poly-L-lysine (PLL) on electrode to attract the negatively-charged DNA. But, this method also has several drawbacks such as difficulties of constant surface coating or regeneration, in particular non-specific binding of unwanted primers or PCR products which may reduce sensitivity and reproducibility of quantification [3], [9], [11].

Previously we developed an integrated BioFET device and detection protocol for DNA oligonucleotides and PCR product with non-surface-binding detection technique [13, 14]. It was fast and continuous monitoring method for DNA quantification. But in case of PCR product detection, laborious sample preparation process such as membrane purification or overnight desalting step was necessary. Moreover, it is impossible to identify PCR products, which is important to field application such as molecular diagnostics and laboratory uses.

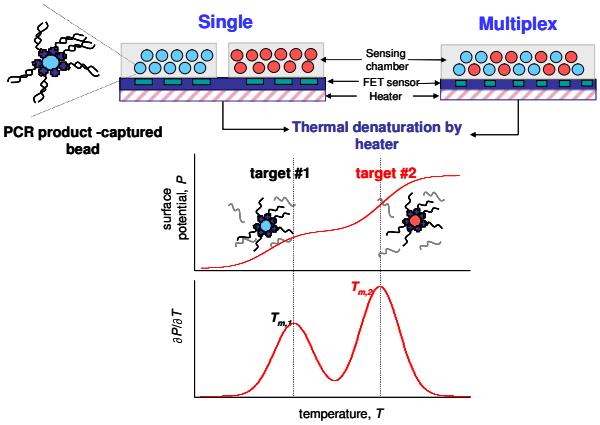

Fig 1. Schematic of PCR product detection using PCR product-captured bead

In this paper, we propose a new detection protocol using PCR product-captured microbeads with BioFET to solve

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the aforementioned problems (Fig 1). Streptavidin-coated microbeads are used for capturing biotinylated PCR products. The PCR product-captured beads are heated in the BioFET sensing chambers and the denatured DNAs result in signal change of BioFET. We could successfully discriminate three kinds of PCR products due to the difference in their melting temperatures (*Tm*). Purification process was easily finished just by several times washing the PCR product-captured beads.

2 EXPERIMENTAL

2.1 Device

Figure 2 (A) shows the proposed FET DNA sensor. The device is fabricated by using standard 1.0μm CMOS processes with an additional etching step in poly silicon gate region. The gate oxide (about 25nm) is exposed to sample solution as a sensing layer. Platinum layer is deposited and patterned to be used as a reference electrode, which applies bias voltage to the gate through electrolyte solution. Details about readout circuit design and pixel architecture were reported in previous research [13].

Figure 2 (B) shows a schematic of experimental apparatus. The sensor chip is located on the aluminum block of temperature control unit and covered by customized PMMA jig. A silicon pad which is pressed between jig and sensor chip forms four fluidic chambers, which make it possible to measure four different samples simultaneously. While the chip and the injected solution are heated by temperature control unit, holes are closed by PMMA cap and silicon pad for preventing evaporation of the solution.

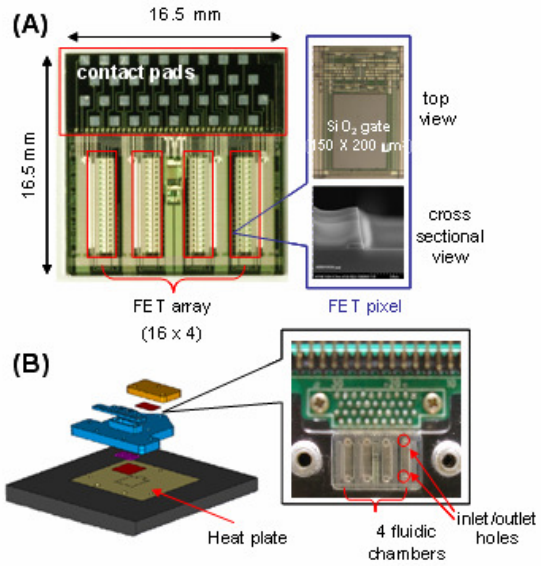

Fig 2 .Sensor chip design (A) and customized jig (B)

2.2 Preparation of PCR products and beads

We prepared three kinds of biotinylated PCR products which have different Tm (below table). PCR mixture includes 0.2 uM of forward and reverse primers, 200uM of dNTP in Tris-HCl (pH 9.0) with 1.5 mM of $(NH_4)_2SO_4$, 2.5 mM of $MgCl_2$ and 1 mg/ml BSA and 0.1 U Taq polymerase. Following PCR, the amplified DNA was quantified using an Agilent Bioanalyzer 2100 (Agilent Technologies, Palo Alto, CA). PCR product-captured microbeads are prepared as follows: 2.8 μm streptavidin-coated magnetic microbeads (Dynabeads M-270) purchased from Invitrogen (Invitrogen Dynal AS, Oslo, Norway) are washed several times with 100 mM KCl (pH 4.5) and 0.1mM KCl (pH 3.9) which is optimized buffer for BioFET detection. To immobilize the PCR products on the beads, pre-washed beads are added to 200 ㎕ of 5 ng/㎕ unpurified biotinylated PCR products and the mixed solution is incubated for 10 minutes at room temperature. Beads were washed with 0.01 mM KCl (pH 3.9). Finally, The PCR product-immobilized beads are re-suspended in 50 μl of 0.01 mM KCl (pH 3.9) for the BioFET test.

PCR product	Primer set (forward & reverse primer)	Template DNA
1 (198 bp)	5'-(biotin)-GTCCAAAGC AAAATGCAT GA-3' 5'-CCTTATATAAACCC CCTC AGACA-3'	Human cDNA related with fibroblast activation protein
2 (412bp)	5'-(biotin)-CACGAGTGA CGGAAACACCTC-3' 5'-TTAAAGAACTCTAA GCGGAGAC-3'	*Mycoplasma pnemoniae*
3 (129bp)	5'-(biotin)-GGATGGCAA GCA GACACTG -3' 5'-TGATGATGATGGCT CTGCT - 3'	Human cDNA related with cytochrome c-1

3 RESULT & DISCUSSION

Figure 3 (A) shows the potential change when the 412bp PCR product-captured bead was applied in sensing chamber. After raising temperature upto denaturation point with ramping rate 0.05℃/sec, potential of BioFET is changed by mobile DNA in solution from PCR product-captured beads (solid line). By differentiating potential change with respect to temperature change, melting temperature (*Tm*=46.7℃) can be determined (bold line). Figure 3 (B) shows the control experiment with streptavidin coated beads which are not applied 412bp PCR product. In this case, there are not significant peak around *Tm*.

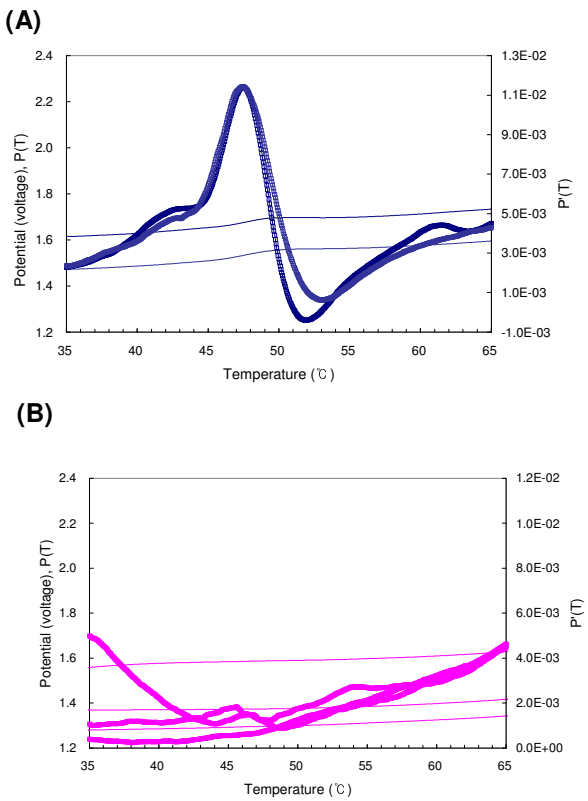

Fig 3. Identification of 412 bp PCR product (A) and control experiment (B)

In fact, we can estimate whether PCR is successfully performed or not from the magnitude of potential change. But in order to discriminate various kinds of PCR product, identification of PCR product is necessary. It is identical with the size separation process using the gel electrophoresis or melting curve analysis of real-time PCR with SYBR Green I dyes [15].

So we tried to discriminate three kinds of PCR product by melting temperature analysis like SYBR Green I assay. Prior to the discrimination test, melting temperatures of PCR products were measured by UV-spec (data are not shown). As shown in Fig 4, the result clearly shows that PCR products can be discriminated by melting temperature analysis with label-free bioFET [(129bp, *Tm*=53℃), (412bp, *Tm*=46.7℃) and (198bp, *Tm*=44℃)]. Identification method based on differentiation of potential change with respect to temperature change is similar to general analysis method of SYBR Green I assay, but our method is not optical detection and dose not need dye labeling [15].

Discrimination of three kinds of PCR product was reputably tested over 10 times and the results were statistically analyzed using MINITAB® statistical software (Minitab Inc, PA). The result of logistic regression shows that PCR products can be discriminated from each other with 99.9% discrimination power (Fig 5).

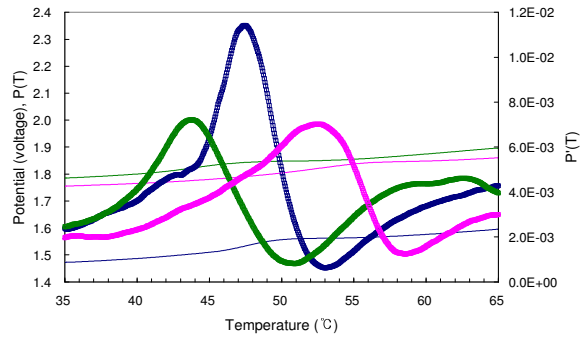

Fig 4. Discrimination of three kinds of PCR products by melting temperature analysis.

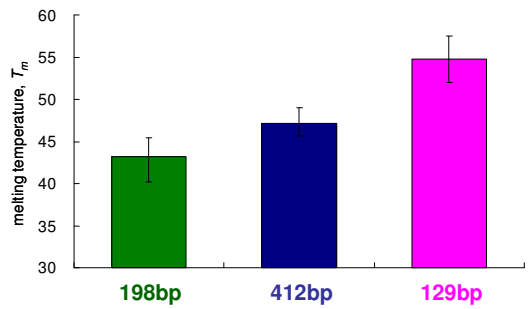

Fig 5. Melting temperature of three kinds PCR products (10 times repetitation test)

Limit of detection (LOD) can be lowered by concentrating PCR products using microbead. As mentioned in section 2.2, 200 ㎕ of 5 ng/㎕ biotinylated PCR products were mixed with streptavidin-coated microbeads. There are sufficient biotinylated site on microbead surface, the most of the PCR products are captured to the beads. When the PCR product-captured beads are resuspended in smaller volume than the original volume, concentration of the final sample could be much higher than that of the original PCR products. From this fact, we can deduce that the PCR products at low concentration can be detected even though the initial concentration is too low. Figure 6 shows the melting curve of 412bp PCR product which concentration is 0.5 ng/㎕. Peak of Tm is clearly founded. From the result, PCR product detection by our method can be possible to lower the LOD because this result is more sensitive than many other groups [3, 9]. Their methods focused on detection of the amount of PCR product, but they could not discriminate the kinds of PCR products and have limitation to improve sensitivity. We expect that our method can be applied to real field applications such as molecular diagnostics and

general laboratory uses which are needed higher sensitivity and specificity.

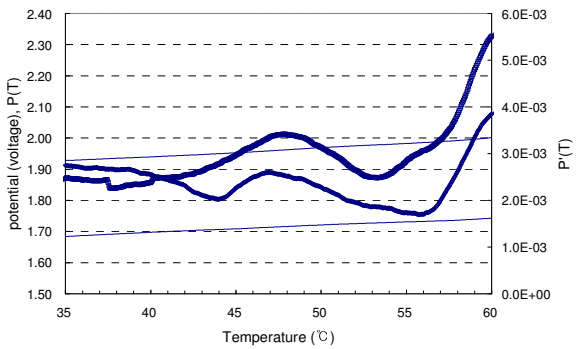

Fig 6. Detection of 412bp PCR product (Concentration: 0.5 ng/$\mu\ell$)

5 CONCLUSION

Using integrated BioFET, we could, for the first time to the best of our knowledge, demonstrate identification method of PCR product by electrical label-free detection. Moreover, easy PCR product purification and lowering limit-of-detection can be possible as a result of utilizing microbeads. Further study, we try to identify the multiplex PCR samples and expect to be used in a real time identification of multiplex PCR product. As such, our approach establishes a foundation for PCR based biotechnologies with microelectronics.

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