Dielectrophoretic Characteristics of Microbeads Labeled with DNA of Various Lengths

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Abstract: Polymerase chain reaction (PCR) is one of the most sensitive and specific detection methods of bacterial and viral infections. The authors proposed a new electrical technique for rapid detection of DNA amplified by PCR using dielectrophoresis (DEP) of microbeads. The method is based on dramatic alteration of DEP characteristics of microbeads caused by DNA labelling. DNA labeled microbeads are trapped on a microelectrode under the action of positive DEP, whereas pristine microbeads are not. DEP-trapped microbeads can be measured impedimetrically to realize rapid and quantitative detection of the amplified DNA. In this study, it was aimed to reveal how DNA length affects DEP characteristic of DNA-labeled microbeads. Dielectrophoretic crossover from the negative to the positive was measured for microbeads labeled with DNA length in 204 bp, 391 bp and 796 bp. After theoretical fitting of DEP crossover data, it was revealed that the surface conductance increased when the length of labeled DNA increased.

1 INTRODUCTION

There are several methods to diagnose bacterial or viral infections in human and animals. A nucleic acid amplification test (NAT) is a highly sensitive and specific method for detecting DNA or RNA of a target pathogen. Polymerase chain reaction (PCR) is a type of NAT used to amplify specific regions of DNA or RNA via enzymatic reaction. PCR is a widespread application in several areas of genetic analysis (Storch, 2000; Malorny, 2003; Chung et al., 2006).

DNAs amplified by PCR, amplicons, are generally separated by size and detected by agarose gel electrophoresis. Although this method is well established and reliable, it requires rather complicated and time-consuming manual operations by experts. To overcome this drawback, real-time PCR has emerged as an improvement for rapid analysis. Real-time PCR optically detects amplicons during PCR using a fluorescent probes that bind to DNA. The fluorescence intensity increases with the number of amplicons during the amplification. However, the apparatus for real-time PCR is expensive, almost 10 times the cost of a general PCR equipment. Moreover, special knowledge and experience are required to design fluorescent DNA probes for optical detection (Mackay, 2002). Hence, rapid, simple and economical amplicons detection method was required.

The authors develop and demonstrate a novel electrical method for detection of amplicons by dielectrophoresis (DEP) of microbeads (Nakano et al., 2014). In the method (Figure 1), the amplicons are chemically immobilized on dielectric microbeads so that DNA immobilization alters the DEP characteristics of the microbeads. DNA-labeled microbeads are trapped on a microelectrode under the action of positive DEP, whereas pristine ones are not trapped. Combining this dramatic alteration in DEP phenomena with impedance measurement allows rapid and quantitative detection of the amplicons. An electrical detection technique called dielectrophoretic impedance measurement (DEPIM), which was originally developed by the authors' group for bacterial and viral inspection, can be used for the impedance measurement (Suehiro et al., 1999). It was demonstrated that DNA-labeled microbeads were trapped in the electrode gap, which caused a detectable change in the electrode impedance in a few seconds, whereas pristine microbeads would repelled from the electrode gap, which resulted in no impedance change (Nakano et

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al., 2014). Hence, this method would provide rapid detection of DNAs amplified by PCR, which may be applicable to rapid, quantitative, and automated diagnosis of bacterial and viral infections. However, this method still required separation and selective detection of PCR amplified DNA of different length.

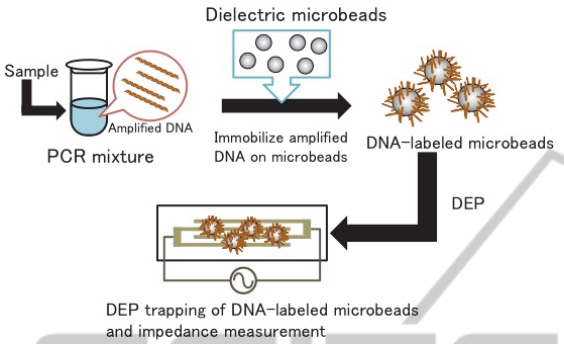


Figure 1: Schematic illustration of the microbeads-based detection of amplicons. After PCR amplification, amplicons (amplified DNA) are chemically immobilized on the microbeads. The DNA-labeled microbeads behaves positive DEP, whereas the pristine microbeads behaves negative DEP. The DEP-trapped microbeads can be detected by DEPIM

This study aim to reveal how the length of labelling DNA affects DEP characteristics of DNA labeled microbeads. Alteration of DNA-labeled microbeads DEP characteristics, which is affected by the length of labeled DNA, can lead to rapid separation and selective detection of PCR amplified DNA of different length by impedance measurement. Crossover frequency of the DEP for microbeads labeled with DNA of different length were measured at different suspending medium conductivities. The alteration of DEP charateristics was analysed using theoritical fitting of measured data.

2 THEORY

DEP is the electrokinetic motion of dielectrically polarized materials in non-uniform electric fields, and it is currently an active area of research for manipulation of biological particles and nanomaterials, including bacterial cells and DNA molecules (Pethig, 2010, Hughes, 2000, Ausbury et al., 2002,). The DEP force acting on a spherical dielectric particle of radius r suspended in a medium of absolute permittivity ε_m is given by as follows

$$F_{DEP} = 2\pi r^3 \varepsilon_m \mathrm{Re}[K(\omega)]\nabla E^2 \quad (1)$$

where E is the magnitude of the applied field. Re[$K(\omega)$] is the real component of the Clausius–Mossotti (CM) factor, given by

$$K(\omega) = \frac{\varepsilon_p^* - \varepsilon_m^*}{\varepsilon_p^* + 2\varepsilon_m^*} \quad (2)$$

where ε^*_p and ε^*_m are the complex permittivities of the particle and the surrounding medium, respectively. For a real dielectric, the complex permittivity is defined as $\varepsilon^* = \varepsilon\text{-}j(\sigma/\omega)$, where ε is the permittivity, σ is the conductivity of the dielectric, and ω is the angular frequency of the applied electric field. When Re[$K(\omega)$] has a positive value, the particle is propelled toward the high field region (positive DEP, p-DEP). With a negative value of Re[$K(\omega)$], the particle is repelled from the high field region (negative DEP, n-DEP). The crossover frequency f_x is defined as the value of the applied frequency which results in the cessation of particle motion. Therefore, measurement of the crossover frequency can used to characterize the dielectric properties of single particle.

The conductivity of a solid dielectric particle, σ_p, can be expressed by the following equation (Ermolina and Morgan, 2005).

$$\sigma_p = \sigma_b + \frac{2K_s}{r} \quad (3)$$

where σ_b and K_s are the bulk conductivity and the surface conductance of the particle. Equations 1–3 imply that the dielectric properties and the resultant DEP force acting on a smaller particle should be more dependent on the surface conductance K_s. Hughes *et al.* reported that antibody (protein) coating of submicrometer latex spheres altered the surface conductance and DEP spectrum of the particles, enabling the separation of unlabeled and protein-labeled particles (Hughes and Morgan, 1999). Zhou *et al*. found that the dielectric properties of microbeads were modified by coating with bacterial biofilms, resulting in an altered electrorotation spectrum (Zhou et al., 1995).

3 EXPERIMENTS

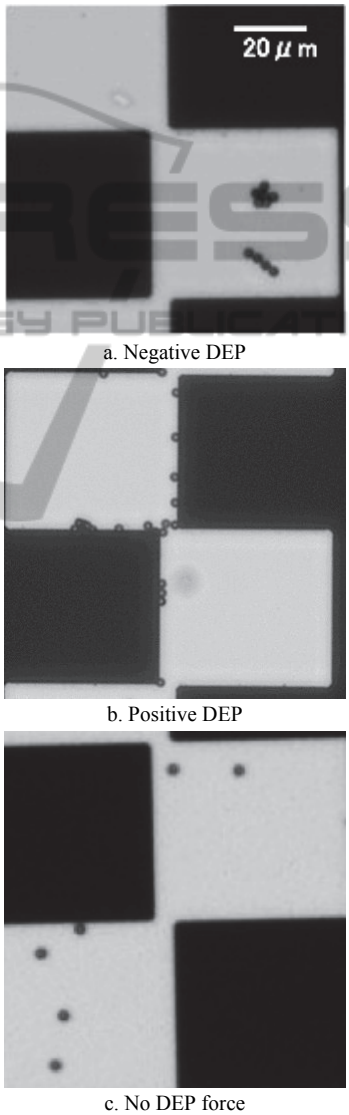
We used pUC 19 DNA as template for PCR. The 5' end of forward primers, which were designed for amplifications of 204 bp DNA, 391 bp DNA and 796 bp DNA from pUC 19, were tagged with biotin. As the results of PCR, 204 bp DNA, 391 bp DNA and 796 bp DNA were amplified and these amplicons were confirmed by standard agarose gel electrophoresis.

Magnetic microbeads (Dynabeads® M-280, Life Technologies 2.8 μm in diameter) were used in this experiment. The surface of the microbeads is coated with streptavidin, which binds specifically to biotin. Microbeads ($3x10^4$ beads/μl) were mixed with the reaction solution (5 mM Tris-HCl (pH 7.5), 0.5 mM EDTA, 1 M NaCl), including the amplicons of 204 bp, 391 bp and 796 bp separately. The amplicon concentrations in the solution were approximately $3.5x10^{10}$ DNA/μl. The mixtures of the amplicons and microbeads were incubated at room temperature for 15 minutes. Hence, the microbeads were functionalized with amplicons via biotin-streptavidin interaction. Then, the DNA labeled microbeads were suspended in deionized water (conductivity 2×10^{-4} S/m).

A castle-walled microelectrode having the narrowest gap of 5 μm, which is shown as black part in Figure 2, was used. DEP behaviors of microbeads labeled with DNA of different length were observed with an inverted microscope equipped with a CCD camera. The DNA-labeled microbeads were suspended in NaCl solution of concentration range from 5 μM to 1 mM. Then, 5μl of the solution containing the DNA-labeled microbeads was placed on the microelectrode and covered with a cover slip. An AC voltage of 20 $V_{peak\text{-}to\text{-}peak}$ was applied to the microelectrode to generate DEP force. The DEP crossover frequency was measured by observing DNA-labeled microbeads motion with varying applied voltage frequency. The DEP force changed from negative DEP, where microbeads were repelled from the electrode gap, which is the high electric field (Figure 2. a.) to positive DEP, where microbeads were trapped in the electrode gap (Figure 2. b.) along with the decreasing of applied voltage frequency. The DEP crossover frequency was determined as the frequency when the DEP force changed from n-DEP to zero (Figure 2. c.).

4 RESULTS AND DISCUSSION

The crossover data for microbeads labeld by DNA of 204 bp, 391 bp and 796 bp in length are shown in Figure 3. The data are plotted for DNA-labeled microbeads suspended in NaCl solution of different conductivities range from 10^{-4} to 10^{-1} S/m. At high suspending medium conductivities the DNA labeled microbeads experienced only negative DEP. At suspending medium conductivities below 10^{-3} S/m, the crossover frequency was clearly dependent on the length of labeled DNA. For example, Figure 4 shows that at suspending medium conductivity of $2x10^{-4}$ S/m, the crossover frequency became higher when the length of labeled DNA increased. This suggests that applying voltage of appropriate frequency can separate microbeads labeled by DNA of different length. For example, as shown in Figure 3, in suspending medium conductivity of $2x10^{-4}$ S/m, if the frequency between 3.1 x 10^6 Hz and 3.8 x 10^6 Hz is applied, 796 bp-DNA-labeled microbeads would experience positive DEP, while 391 bp-DNA-labeled microbeads and 204 bp-DNA-labeled microbeads would experience negative DEP.



a. Negative DEP

b. Positive DEP

c. No DEP force

Figure 2: Optical images showing DEP behaviors of DNA-labeled microbeads.

The solid lines in Figure 3 are the best fit to model described by Equations 1 - 3 and the fitting data are summarised in Table 1.

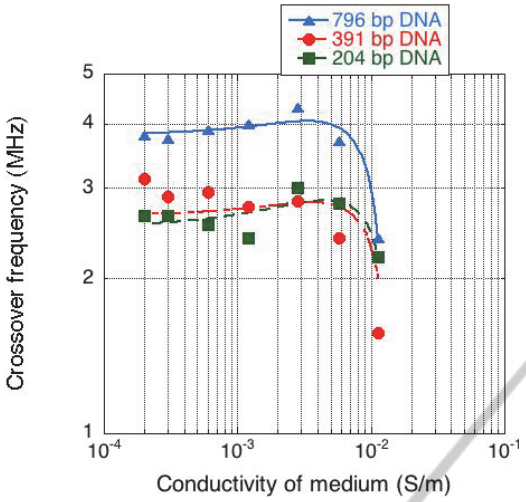


Figure 3: Crossover frequency for microbeads labeled by DNA length in 204 bp, 391 bp and 796 bp plotted as a function of suspending medium conductivity. Solid lines are the best fir to the model described by Equation 1- 3. The fitting data are summarized in Table 1.

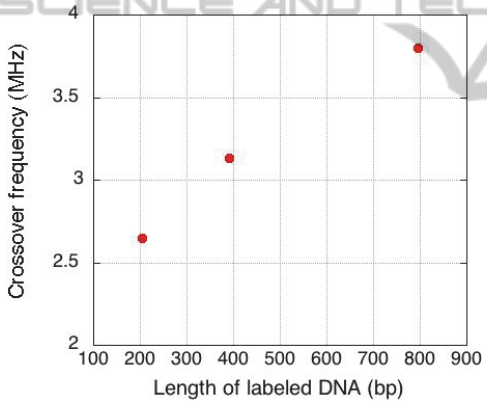


Figure 4: Crossover frequency plotted as a function of labeled DNA length.

Table 1: Fitting data of surface conductance of microbeads labeled by DNA length in 204 bp, 391 bp and 796 bp.

Labeled DNA length (bp)	204	391	796
Surface conductance (nS)	9.45	10.29	11.28

Table 1 shows that the surface conductance of DNA labeled microbeads increased with the DNA length. This was because of the negative electrical charges of DNA. Longer DNA has lager negative electrical charges, therefore, the surface conductance of DNA labeled microbeads will increase when the length of labeled DNA increases, which will cause the alteration of crossover frequency of DNA-labeled microbeads.

5 CONCLUSIONS

Dielectrophoretic crossover frequency of microbeads labeled by DNA of virous lengths were measured at different suspending medium conductivities. At suspending medium conductivities below 10^{-3} S/m, the crossover frequency was clearly dependent on the length of labeled DNA that the crossover frequency became higher when the length of labeled DNA increased. After theoretical fitting of DEP crossover data, it was revealed that the surface conductance increased when the length of labeled DNA increased. Hence, DEP characteristics of DNA-labeled microbeads altered with DNA length. Therefore, if voltage of appropriate frequency were applied, longer DNA would experience positive DEP while shorter DNA would experience negative DEP, which could lead to rapid separation and selective detection of PCR amplified DNA of different length by impedance measurement. Hence the proposed microbead-based assay may provide rapid detection of DNAs amplified from multiplex PCR, which may be applicable to rapid, quantitative, and automated diagnosis of bacterial and viral infections.

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