DETECTION OF SINGLE-BASE MUTATION BY AFFINITY CAPILLARY ELECTROPHORESIS IN A PDMS-GLASS HYBRID MICRODEVICE

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Abstract
An extremely rapid on-chip separation of DNA from its single-base substituted mutant is described. By using affinity capillary electrophoresis (ACE) in a poly(dimethylsiloxane) (PDMS)-glass hybrid microdevice, we achieved the separation within 5 s.

Keywords: affinity electrophoresis, DNA, PDMS, single-base mutation

1. Introduction
Sequence-specific separation of DNA using electrophoresis is a promising candidate of rapid and reliable method for detection of gene point mutation and typing of single nucleotide polymorphisms (SNPs) [1]. Especially, ACE has unique advantages such as general applicability and high selectivity [2-6]. In this method, an affinity ligand DNA, complementary to a sequence around suspected mutation point of the normal type DNA, is immobilized onto the inner surface of a capillary [3], or is bound with polymer matrix such as polyacrylamide (Fig. 1) [5,6]. During electrophoretic migration, the normal type DNA and its single-base substituted mutant are separated by difference in affinity with the ligand DNA. Recently, our group demonstrated a gene mutation assay by ACE using a commercial apparatus of microchip electrophoresis [6]. For further optimization of on-chip ACE, we designed and fabricated a PDMS-glass hybrid microdevice, and achieved over ten times faster separation than the previous paper.

Figure 1. Schematic representation of the principle of affinity capillary electrophoresis.
2. Experimental

Design of the PDMS-glass hybrid microdevice is shown in Fig. 2. The microdevice contains a cross-shaped microchannel and four reservoirs for sample loading and electrodes. The PDMS part with surface relief was prepared by the replica-molding technique [7]. Four reservoirs were punched through the bulk PDMS, and it was reversibly bonded to a flat glass plate.

Two types of fluorescein isothiocyanate (FITC)-labeled oligonucleotides were mixed to make sample solution: the normal type has the same sequence as codon 10-13 of c-K-ras gene, while the mutant type has a single-base substitution (Fig. 3a). For the polymer matrix supporting the ligand oligonucleotide, we adopted poly(N,N-dimethylacrylamide) (PDMA) instead of polyacrylamide for suppression of electroosmotic flow [8]. As shown in Fig. 3b, the PDMA-oligonucleotide conjugate was synthesized through a similar process to the previous paper [5]. The ligand oligonucleotide has a complementary sequence to the middle part of the normal type oligonucleotide (Fig. 3b). Copolymerization was carried out between N,N-dimethylacrylamide and methacryloyl-modified oligonucleotide using ammonium persulfate (APS) and N,N,N',N'-tetramethylenediamine (TEMED) as a redox initiator couple.

After the polymerization mixture was diluted twice with 50 mM Tris-borate buffer (pH 7.4), the PDMA-oligonucleotide conjugate was introduced into the microdevice without any purification. The sample solution was pipetted into the sample reservoir, and platinum electrodes were dipped into all reservoirs. Two-step voltage control for sample injection and separation was carried out. Fluorescence of the sample was detected using a fluorescence microscope and a cooled CCD camera. The electrophoresis image was recorded by a digital video recorder.

![Figure 2](image-url)  

**Figure 2.** Schematic representation of the PDMS microdevice used in this work. The channel cross section is 100 μm (width) × 130 μm (height). (a) Cross sectional view of the PDMS microdevice attached on the glass plate. Solid lines indicate platinum electrodes (d = 0.5 mm) inserted into the reservoirs. (b) Top view. The circles indicate reservoirs: (1) sample, (2) injection waste, (3) buffer, and (4) waste.
Figure 3. (a) Sequences of the sample 12-mer oligonucleotides. (b) Synthesis of PDMA-oligonucleotide conjugates (APS, ammonium persulfate; TEMED, N,N',N'-'tetramethylethylenediamine; IPA, 2-propanol).

3. Results and discussion

Figure 4 shows video-clipped images of the ACE separation of the normal and the mutant types. They were clearly separated within 5 s. Because the normal type has stronger affinity with the ligand oligonucleotide than the mutant type does, the former migrated slower than the latter. In addition, no separation was observed when the ligand oligonucleotide had a non-complementary sequence for both normal and mutant types. Strict sequence-recognition of oligonucleotide was achieved. These results indicate that on-chip ACE is an extremely efficient and accurate technique for the gene mutation assay.

Figure 4. Separation of an oligonucleotide (12-mer) from its single-base substituted mutant on a PDMS-glass hybrid microdevice filled with PDMA-oligonucleotide conjugate. Conditions: electric field, 250 V/cm; buffer, 50 mM Tris-borate (pH 7.4) with 10 mM MgCl₂; sample concentration, 0.5 µM for each sample.
4. Conclusions

We demonstrated the separation of 12-mer oligonucleotide which has c-K-ras codon 10-13 and its single-base substituted mutant in a PDMS-glass hybrid microdevice filled with a PDMA-oligonucleotide conjugate within 5 s. The application of this detection system is very promising for the extremely rapid gene point mutation assay and typing of SNPs.

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References