Capillary Electrophoresis with Nanoparticle Matrix for DNA Analysis

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Biopolymer such as DNA is important in living cells since its genetic information provides many building blocks for cell components. Also, DNA is employed for diagnosis of genetic disease,[1,2] DNA sequencing,[3] forensic investigation,[4] and post-human genome project.[5]

Conventional methods such as slab gel electrophoresis (SGE) and ion chromatography (IC)[6,7] for DNA analysis suffered long separation time and low resolution. However, recently developed capillary gel electrophoresis (CGE) showed high speed and high separation efficiency for especially DNA separation.[8]

Capillary electrophoresis (CE) without sieving medium does not have enough separation power for DNA sample with different lengths since each DNA has very similar charge-to-mass ratio, which deteriorates mobility differences for various sizes of DNA under electric field.[9] Therefore, development of the sieving medium is the key for efficient DNA analysis.

In early era for the capillary format, agarose or cross-linked polyacrylamide had been used at the expense of bubble formation at high electric field, gel instability, and no room for gel replacement.[10,11] Later, water-soluble polymers such as cellulose derivatives including methyl cellulose (MC),[12] hydroxyethyl cellulose (HEC),[13] and hydroxypropylmethyl cellulose (HPMC)[14] had been employed for relatively short DNA fragment analysis. These polymers exhibited strong stability under high electric field and ease of replacement. Liner type polymers such as linear polyacrylamide (LPA),[15] polyethylene oxide (PEO),[16] polyvinyl pyrrolidone (PVP),[17] polyvinyl alcohol (PVA)[18] also provided similar effects on DNA separation. However, either cellulose or linear type polymers had relatively high solution viscosity for high resolution of DNA samples, which made it difficult for automation and fast analysis.[19]

In high throughput analysis for DNA sequencing, single nucleotide polymorphism (SNP), and polymerase chain reaction (PCR)[20] products, effective but low viscosity sieving medium is essential for high sieving ability and easy replacement. Recently, mannitol added poly(N-isopropylacrylamide) (PNIPAM)[21] or gold nanoparticle (GNP)[22,23] as the sieving medium showed the potential for this purpose. In GNP case, the production of GNP took long time and the cost was high for preparation.

In this paper, we have employed relatively cheap silica or α-alumina nanoparticle as the sieving medium. These nanoparticles were successfully applied for DNA analysis with the size range of 500 bp to 5000 bp.

Figure 1 shows the electropherograms of 500 bp step ladder by capillary electrophoresis with different sieving matrices. Since the direction of the electosmtic flow (EOF) caused by surface silanol groups on fused silica capillary is toward negative electrode (opposite compared to migration of DNA) and the mangnitude of EOF is usually larger than that of electrophoretic mobility of DNA, the capillary surface needs to be covered in order to reduce EOF. The capillary surface in this experiment was coated either permanently (Figure 1(a) and (b)) or dynamically (Figure 1(c) to (e)). Figure 1(a) exhibited the significance of sieving

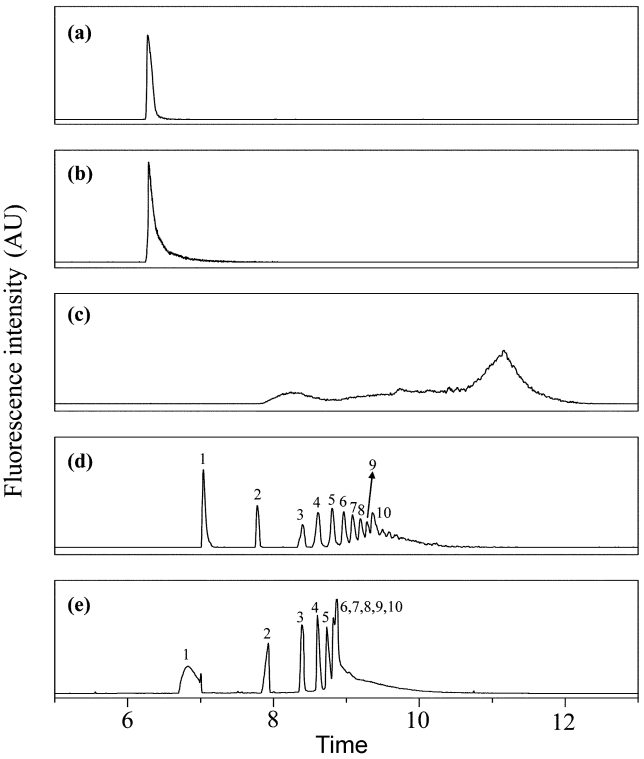

Figure 1. Separations of 500 bp DNA step ladder with (a) no sieving medium, capillary wall-coated by Hjerten's method, (b) 0.01% silica nanoparticle only, capillary as in (a), (c) 0.05% PEO only, capillary wall-dynamically coated, (d) 0.01% silica nanoparticle and 0.05% PEO, capillary as in (c), and (e) 0.01% silica nanoparticle and 0.10% PEO, capillary as in (c). Conditions: DNA sample concentration, 200 ng/μL; 1XTBE (pH 8.4) containing 3.0 ng/μL ethidium bromide, electrokinetic injection at 4 kV for 4s, separation at 5.4 kV in a 30 cm long (22 cm to the detector) fused-silica capillary with 375 μm OD and 75 μm ID. Peak assignment, 1 = 500 bp. 2 = 1000 bp. 3 = 1500 bp. 4 = 2000 bp. 5 = 2500 bp. 6 = 3000 bp. 7 = 3500 bp. 8 = 4000 bp. 9 = 4500 bp. 10 = 5000 bp.

matrix, showing that without any sieving matrix, 500 bp step ladder containing 10 different fragment lengths was not separated at all. When only silica nanoparticle was used as the sieving matrix, any partial separation was not obtained (Figure 1(b)), meaning that pores for DNA separation was not adequately formed. In Figure 1(c), only partial separation of 500 bp step ladder was observed with elongated migration time by 0.05% PEO matrix. In fact, the major function of PEO in tour experiment was to dynamically coat the capillary surface through hydrogen bonding. It was clear that PEO, at this very low concentration (0.05%), did not provide enough sieving power to separate the mixture of DNA fragments (partial separation), but enough to coat the capillary surface (presence of DNA peaks). With dynamic coating by PEO and silica nanoparticles (average particle size, 7 nm) as the sieving matrix, much improved separation efficiency was obtained as shown in Figure 1(d). An interesting fact was that 10 different fragment sizes ranging from 500 bp to 5,000 bp were successfully separated with the resolution at least more than 0.5. We like to note that the viscosity of the silica nanoparticle solution was quite low, therefore the pumping time for this matrix into the capillary was much reduced down to 2 min, compared to more than 20 min of pumping by using conventional PEO or cellulose sieving matrices.

In Figure 1(d), although PEO was mainly used as the dynamic coating reagent, we believe that PEO also acted as the agent for the formation of the silica nanoparticle composite. According to Eisenburg's model,[24] nanoparticle and polymer has certain degree of interaction, causing the limited movement of both nanoparticle and polymer. Therefore, it leads to the formation of immobilized and restricted mobility regions around the nanoparticle, resulting in the formation of the effective pores for DNA fragment separation. However, it turned out that pronounced immobilization caused by increased concentration of PEO did not help the matrix system perform the proper separation of DNA fragment mixture as shown in Figure 1(e).

Figure 2 represents the scanning electron microscopic images of silica (Figure 2(a)) and α-alumina (Figure 2(b)) nanoparticles. Unlike GNPs, the shape of nanoparticle is not spherical, rather irregular and coarse. However, as shown in Figure 1(c), the separation of DNA 500 bp step ladder was successful, meaning that the shape of the particle may not the issue for this DNA size range.

The contribution to DNA fragment separation by average nanoparticle sizes was tested as shown in Figure 3. It was observed that for the same 0.005% concentration of nanoparticles, smaller size particle (silica, 7 nm) provided better separation efficiency compared to larger one (α-alumina, 250 nm) as shown in Figure 3(a) and (d). This effect was more pronounced at 0.01% nanoparticle concentration as shown in Figure 3(b) and (e). Although it is not clear how exactly nanoparticle and PEO interact in the buffer and form nanocomposites, it is obvious that effective pores were more efficiently constructed by smaller size nanoparticles probably due to the improved interaction of silica nanoparticle with the unit PEO chain length. However, the concentration of PEO should be carefully controlled as shown in Figure 3(c). In this case, it seemed that the effective pores were too small to give enough sieving power for DNA fragment mixtures.

Compared to GNPs, silica nanoparticle is cheaper and easier to prepare in the buffer. Since the viscosity of the sieving buffer is much smaller than that with other sieving polymer, the potential for automation and multiplexing for SNP or PCR product analysis is great. Application of this sieving medium to genetic disease diagnosis with PCR products and lab-on-a-chip is under progress in our laboratory.

Experimental Section

Chemicals. Bare fused silica capillaries with 75 μm i.d. and 375 μm o.d. were purchased from Polymicro Technologies (Phoenix, AZ, USA). A detection window was prepared by burning the polyimide coating with a hot sulfuric acid. Total lengths of the capillary were either 30 cm with the effective lengths of 22 cm. The platinum electrode (0.5 mm Φ) was obtained from Aldrich Co. (MO, USA).

Tris base, boric acid, and ethylenediaminetetraacetic acid

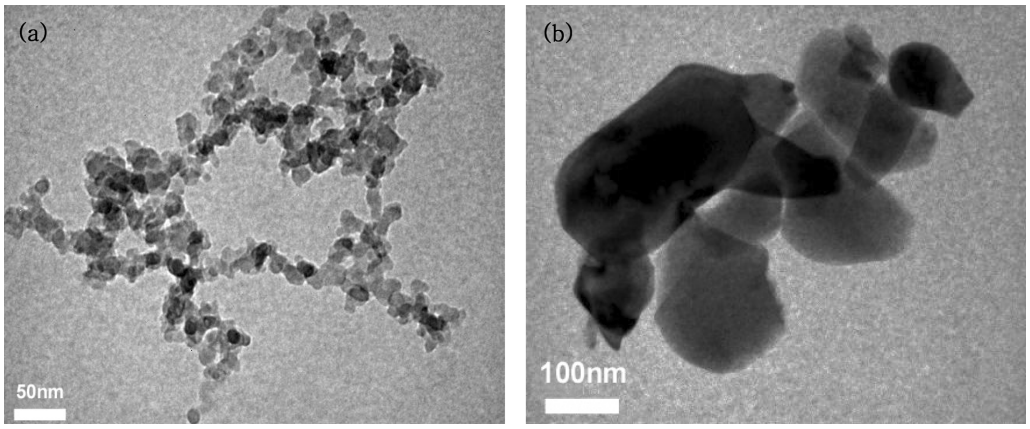

Figure 2. Transmission Electron Microscopy (TEM) image of nanoparticles. (a) silica nanoparticle (average particle size, 7 nm), (b) α-alumina nanoparticle (average particle size, 250 nm). TEM acceleration voltage; 200 kV.

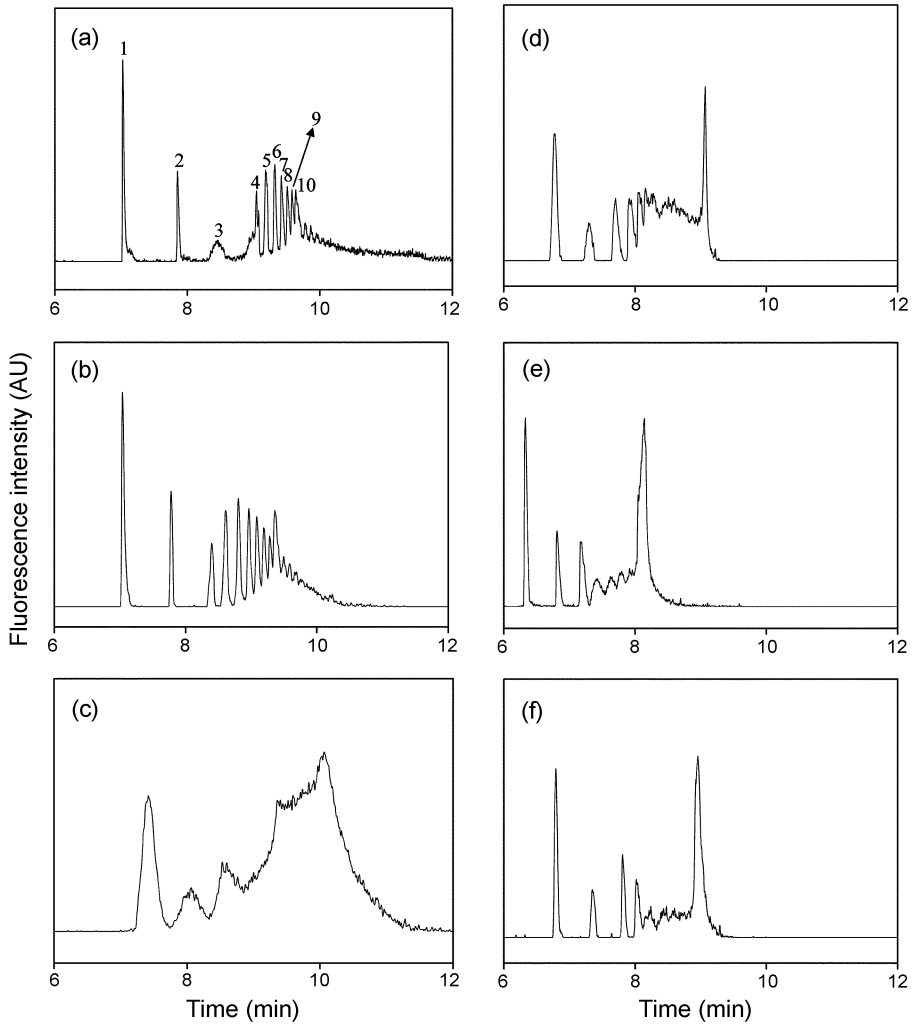

Figure 3. Separation of 500 bp DNA step ladder using (a) 0.005% silica nanoparticle, (b) 0.01% silica nanoparticle, (c) 0.03% silica nanoparticle, (d) 0.005% alumina nanoparticle, (e) 0.01% alumina nanoparticle and (f) 0.03% alumina nanoparticle. Condition: DNA sample concentration, 200 ng/μL; PEO was in 1XTBE (pH 8.4) at 0.05%, 3.0 ng/μL ethidium bromide, electrokinetic injection at 4 kV for 4 s, separation at 5.4 kV in a 30 cm long (22 cm to the detector) fused-silica capillary with 375 μm OD and 75 μm ID. Peak assignment; the same as in Figure 1.

(EDTA) (all from Aldrich Co.) were used for TBE buffer preparation. A monomeric dsDNA intercalating dye, ethidium bromide (EB) was purchased from Aldrich. Since EB is mutagenic and carcinogenic, a pair of lab-glove should be worn during treatment. Polyethyene oxide (PEO, M_r = 8,000,000) were obtained from Aldrich.

Fumed silica nanoparticle (average particle size, 7 nm, AEROSIL R 812) was from Degussa AG, Düsseldorf, Germany, and α-alumina nanoparticle (average particle size, 250 nm) was kindly donated from the ceramic nano powder group by Prof. Jong Bong Kang at Kyungnam University.

Standard dsDNA fragments, 500bp ladder (Takara Bio Inc., Japan) with the concentration of 50-125 ng/mL were employed for the experiment. Deionzed water (Mili-Q reagent water system, MA, USA) was used throughout the experiment.

Capillary electrophoresis with nanoparticle. A high-voltage power supply (−30 kV, Spellman, NY, USA) was used for electrophoresis with the electric field strength of 100 V/cm−300 V/cm. A 1.5 mW He-Ne laser (Edmund Scientific Co., NJ, USA) with 543.6 nm output was used for the excitation of DNA labeling dye, EB. Two RG610 optical filters were used to block scattered laser light. The fluorescence signal was collected with a 10X microscope objective (Nikon, Japan) into the photomultiplier module (H5784-02, Hamamatsu, Shinzuka, Japan) and transferred directly through a low-pass filter to an A/D interface board (National Instrument Co., TX, USA). The control of the high-voltage power supply and data collection at 7 Hz was performed by in-house LabView program with an IBM compatible computer.

For the permanent coating of the capillary wall, Hjerten's method was employed.[25] Briefly, 0.004% of γ-methacryloxypropyltrimethoxysilane (MAPS, Aldrich Co., Mo, USA) was introduced into the capillary for 1.5-2 h for the activation of surface silanol group. Then, acrylamide (3.5%) solution containing 1.0 mg/mL $K_2S_2O_8$ and *N,N,N',N'*-tetramethylene theylenediamine (TEMED) was pushed into

the capillary for 3 h. For dynamic coating of the capillary wall, the capillary was firstly flushed with 10 mM HCl for 20 min. Then, TBE containing PEO (0.05%) was introduced with the positive pressure on one side with the syringe and the negative pressure on the other side with the vacuum pump for 5-10 min. The capillary coated with this method could survive more than 4 weeks if stored in neutral water when not in use. It was found that migration time for each DNA at the given condition after coating showed error less than 2%.

A 1X TBE buffer (90 mM Tris, 90 mM borate, and 2 mM EDTA, pH 8.4) was filtered once with 0.25-μm membrane filter paper (Milipore Co., MA, USA). A stock solution of nanoparticle (0.1%, w/v) in 1X TBE prepared after ultrasonication for 30 min. Then, it was diluted for the concentration of 0.005%-0.01% by 1X TBE containing PEO (0.05%, w/v, M_r 8,000,000). This solution was homogeneously mixed by vigorous stirring for 4 h. Then, it was degassed by vacuum. The TBE buffer containing nanoparticle was pushed into the capillary with the positive pressure for 1-2 min. Two glass vials for TBE buffer containing nanoparticle were placed on both ends of the capillary. The capillary was electrophoretically equilibrated for 10 min before sample injection. The injection for DNA sample was performed at 4 kV for 4 s. After each run, the capillary was flushed with water for 15-30 min, and then a new nanoparticle buffer was introduced.

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