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Yang-Wei Lin Ming-Feng Huang Huan-Tsung Chang

Department of Chemistry, National Taiwan University, Taipei, Taiwan, R.O.C.

Nanomaterials and chip-based nanostructures for capillary electrophoretic separations of DNA

Capillary electrophoresis (CE) and microchip capillary electrophoresis (MCE) using polymer solutions are two of the most powerful techniques for the analysis of DNA. Problems, such as the difficulty of filling polymer solution to small separation channels, recovering DNA, and narrow separation size ranges, have put a pressure on developing new techniques for DNA analysis. In this review, we deal with DNA separation using chip-based nanostructures and nanomaterials in CE and MCE. On the basis of the dependence of the mobility of DNA molecules on the size and shape of nanostructures, several unique chip-based devices have been developed for the separation of DNA, particularly for long DNA molecules. Unlike conventional CE and MCE methods, sieving matrices are not required when using nanostructures. Filling extremely low-viscosity nanomaterials in the presence and absence of polymer solutions to small separation channels is an alternative for the separations of DNA from several base pairs (bp) to tens kbp. The advantages and shortages of the use of nanostructured devices and nanomaterials for DNA separation are carefully addressed with respect to speed, resolution, reproducibility, costs, and operation.

 Keywords:
 DNA / Microchip capillary electrophoresis / Nanomaterials / Nanostructures /

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Correspondence: Dr. Huan-Tsung Chang, Department of Chemistry, National Taiwan University, Taipei, Taiwan, R. O. C. E-mail: changht@ntu.edu.tw Fax: +11-886-2-23621963

Abbreviations: GNP, gold nanoparticles; **MCE**, microchip capillary electrophoresis; **PDMA**, poly(*N*,*N*-dimethylacrylamide); **PEO**, poly(ethylene oxide)

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1 Introduction

Rapid, efficient, and sensitive separation techniques are important for DNA analysis. Numerous capillary electrophoresis (CE)- and microchip capillary electrophoresis (MCE)-based approaches have been tested and validated for DNA sequencing [1, 2], genotyping [3, 4], mutation analysis [5, 6], characterization of single nucleotide polymorphisms (SNPs) [7, 8], forensic human identification [9, 10], diagnosis of diseases [11, 12], and other applications [13, 14]. When compared to slab-gel electrophoresis, these techniques provide the advantages of comparable resolving power, rapidity, high throughout, minute sample requirement, and ease of integration [10, 15, 16]. Using these techniques, small separation channels/capillaries are often filled with polymer solutions that are advantageous over cross-linked gels, including relatively low viscosity, ease of preparation, and flexibility. Polymer solutions are commonly prepared from linear polymers, including cellulose and its derivatives [17], linear poly-(acrylamide) (LPA) [18], poly(ethylene oxide) (PEO) [19], and poly(vinylpyrrolidone) (PVP) [20]. In order to provide great resolving power for small DNA fragments, the concentration of a polymer solution is usually higher than its

entanglement (overlap) threshold (c^*). It is noted that at the concentration above c^* the interaction of the polymers begins to affect bulk solution properties such as viscosity. Although these techniques are powerful for DNA analysis, two main problems still remain: (i) the separation sizes are usually limited to less than several thousands DNA base pairs (bp), and (ii) filling a small separation channel with a high-viscosity polymer solution is difficult.

The analysis and fractionation of long DNA molecules plays a key role in many genome projects, such as investigation of tumorigenesis by monitoring the variations of telomeric length (7-10 kbp tandem repeats of 5'-TTAGGG-3' in human) [21]. Although pulsed-field gel electrophoresis (PFGE) has been widely accepted for the analysis of large DNA molecules, it is very slow (10-200 h) and difficult for automation (detection and sample recovery) [22, 23]. It also needs a large sample volume, usually several µL. To overcome some of the problems, pulsed-field capillary gel electrophoresis (PFCGE) was developed [24-26]. Despite its advantages of speed, detection sensitivity, and ease of automation, PFCGE has not been in the position to replace PFGE as a powerful separation tool for the analysis of large DNA, mainly because of its poor reproducibility and small sample handling capability. In addition, in order to achieve high resolution for long DNA molecules, PFCGE has to be conducted at low electric field, leading to long separation times (tens minutes to hours). PFGE and PFCGE also share one same problem that the separation resolution depends on gel matrices; resolution decreases with increasing DNA size and eventually losses when the DNA fails to enter the gel or is trapped by the sieving matrix [27].

Using ultradilute hydroxyethylcellulose (HEC) (0.002%; this concentration is far below its c^* of 0.40%), the separation of dsDNAs (2-23.1 kbp) has been achieved in CE under a high electric field (270 V/cm) [28]. The separation is according to the transient entanglement mechanism, which is suggested by Barron et al. [28] and modeled by Hubert et al. [29]. As a result of the drag of DNA molecules by the polymer molecules when they are encountered during migration, their mobility decreases, showing size dependence of mobility in certain ranges. The interactions between DNA and polymer molecules have been confirmed by another study that shows dynamic formation and deformation of U-shape in DNA conformation [30]. Although ultradilute polymer solutions have been used for the separation of long DNA under a constant electric field [31-36], they do not provide highresolving power for small DNA fragments.

Alternatively, the analysis of large DNA fragments ranging in size from 5 to 40 kbp was completed in 6 min by CE in the presence of electroosmotic flow (EOF) using HEC solution [37]. Unlike the transient mechanism, HEC solution at a

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concentration (1.5%) higher than its entanglement threshold was used for separating long DNA molecules. In addition to sieving, the variation in DNA conformation due to pH changes and migration of DNA against EOF account for better resolution of long DNA molecules. A stepwise technique in CE has been developed for separating DNA with a wide size range (8-23.1 kbp) via changing PEO and ethidium bromide (EtBr) concentrations in the course of separation [38]. Different concentrations (0.5-1.5%) of PEO solutions are injected to the polyethylene tubes by pressure means, where they enter the capillary by EOF. Because the large DNA fragments migrate faster (slower to against EOF) toward the cathode end under counterflow conditions, 0.5% PEO solution containing less amounts of EtBr is injected first after sample injection. It is noted that filling the capillaries with high-viscosity HEC or PEO solution is not a problem since they migrate to the capillaries by EOF. Long equilibrium times for obtaining high and reproducible EOF and instabilities of HEC and PEO solutions under highly alkaline conditions (pH > 10.0) as well as loss of resolution for long DNA molecules are problematic.

Recently, several new separation approaches for DNA analysis using nanostructures have been developed, which provide different idea for DNA separation from the conventional sieving mechanism and the transient mechanism [39–47]. Some representative chip-based nanostructures developed for DNA separation are summarized in Fig. 1. By taking the advanced lithographic and wet chemical etching techniques, one can easily control the dimension of obstacles or retarding matrix for varying separation resolution and speed. The sample volume used in the nanofluidic channel systems is extremely low (fL to pL), which is especially useful for DNA analysis when the sample is limited and expensive. In addition, recovery of DNA molecules after separation is straightforward because polymer solution is not required.

Nanoseparation techniques are still in their early developing stages and their practical use in the life science is still not widely demonstrated. The purpose of this review is to deal with DNA separation using nanostructures and nanomaterials. The basic theory, designs, advantages, and shortages of these recently developed techniques are discussed. Developing novel nanoseparation techniques based on the advanced nanotechnologies is focused. We also emphasize the importance of the nanoseparation techniques for DNA analysis by providing some interesting examples and their potential for separating other biomolecules of interest, such as proteins and peptides. It is not our intent to cover all CE and MCE separation techniques for DNA analysis in this review. Thus, for those who are interested in other CE and MCE techniques for DNA analysis should refer to other review papers [47-49].



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(D)



Detection Point (Nanopillar-free region) E₁ 25 μm E₄ E₄ Construction Point (Nanopillar-free region) 180 μm 35 μm 500 μm 500 μm 500 μm 500 μm 500 nm 2700 nm



Figure 1. Schematic diagrams for different chip-based devices. (A) Scanning electron microscopy (SEM) surface image of Ni nanopattern on Si wafer; (a) etching process; (b) topography of Ni nanopattern; (c) elemental dispersion analysis. (B) Cross-sectional diagram of an entropic trap chip. (C) Fluidic device contains nanopillars (60 nm in height). (D) Microchannel equipped with nanopillars. (E) SEM image of obstacle course. (F) DNA prism. (G) Columnar structure formed by a suspension of superparamagnetic particles. (A)–(G) are reprinted with permission from [39]–[44], and [69], respectively.

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2 Chip-based nanostructures for DNA separation

2.1 Surface electrophoresis

By controlling different degrees of adsorption of various lengths of molecules on an attractive surface, Pernodet and co-workers [50-52] demonstrated the separation of DNA molecules on a flat surface without any restrictions or any sieving matrixes. For example, the separation of 1 kbp DNA ladders (range from 2 to 10 kbp) was accomplished within 80 min [50]. The variety of the electrophoretic mobility of DNA is dependent on the changes in its conformation as a result of the interaction with the flat surface. However, it is difficult to control the strength of the interaction between DNA molecules and the surface. If the surface has a strong attraction to DNA, the large DNA molecules are fully adsorbed and there is no difference between their conformations (no separation). On the other hand, if the interaction is too weak, small DNA molecules are desorbed from the surface and can not be separated.

To control the interaction between the surface and DNA, Seo et al. [39] have designed a nanopattern of Ni patches superimposed upon a Si matrix (Fig. 1A). The function of the nanopattern is to increase the mobility sensitivity to changes in DNA conformation, thereby allowing the separation of a broad range of DNA molecules such as λ -*Hind*III digests (0.12–23.1 kbp), λ -phage DNA (48.5 kbp), T2-DNA (164 kbp), and S. Pombe DNA (3.5, 4.7, and 5.7 Mbp). Although the separation speed and resolving power are not quite impressive, this technique requires no sieving matrix, very low sample loading amounts (approximately 1 ng to 0.04 µg), and low operating voltages, making it amenable for incorporation into chip-based portable devices. Theoretically, the separation efficiency and the DNA separation length can be further improved by functionalizing the Ni and Si surfaces and by changing the shape of the nanopatterns. In addition, since this method discerns the conformations of the adsorbed chains, it can in principle be used to separate macromolecules of identical molecular weight but different structure, such as circular or supercoiled DNA molecules.

2.2 Entropy-based separation systems

The separation based on entropic effect is rooted in the process of entropic trapping (ET) that was first introduced as a generic concept for diffusion of polymer chains in random media, such as gel and polymer solutions, over two decades ago by Baumgärtner and Muthukumar [53–55]. The entropy effect is related to the separation environments (e.g., electric field and sieving matrix) and the

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internal conformational entropy, one of the dominant properties of flexible macromolecules, such as DNA, which is directly proportional to the molecular contour length. Thus, the use of nanofabricated devices to control the entropic effects to achieve size separation of DNA fragments is highly encouraged. Craighead's group [40, 56–58] demonstrated ET of long DNA in a microfabricated device and established a model to describe the observed escape of DNA molecules from an entropic trap.

If a stretched DNA molecule meets an open space larger than or comparable to its relaxed volume, it will relax to form a spherical shape. When it undergoes a constriction much less than the radius of gyration of DNA (R_0), it has to be deformed from its equilibrium shape to fit into the constriction. To provide selectivity (resolution), the separation channel consists of alternating thick and thin regions, where the thickness of the thin region was as small as 90 nm (Fig. 1B). Since this deformation is entropically unfavorable, a certain driving force such as electric force is required to force a DNA molecule to enter the constriction. Without filling a sieving matrix into the separation channel, the separation of long DNA molecules (range from 5 to 164 kbp) under a direct current (dc) field (24.5 V/cm) within 30 min has been demonstrated [57]. To observe the ET effect, the electrophoretic mobility of long DNA molecules in the channel has to be measured as a function of the applied electric field. Because the R_0 values of DNA molecules are much larger than the thin gap (90 nm), DNA molecules are trapped when they move from the thick to the thin region. The trapping efficiency determines the mobility of DNA in this separation mode. We note that longer DNA molecules (T2: 164 kbp) move faster than shorter DNA molecules (T7: 37.9 kbp) in the channel (Fig. 2). This is likely due to the fact that a larger DNA molecule is easier to escape from the entropic traps as a result of greater contact area in the thin slit.



Figure 2. Separation of long DNA molecules using an entropic trap array. T2-T7 DNA mixture is separated through a channel with 90 nm thin regions, 650 nm thick regions, and 4 μ m channel period. Reprinted from [57], with permission.

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The separation of DNA based on ET has been further demonstrated by using a chip-based device with densely spaced nanopillars between the floor and ceiling as shown in Fig. 1C [41, 59]. The dense pillars act as an artificial gel or mesoporous solid, and thus filling the separation channel with polymer solution is not required. Figure 3 shows the motion of DNA in the presence and absence of electric field in pillared and pillar-free regions. When molecules of dissimilar length are present, shorter strands will clear the interface in a shorter time than longer strands. When the field is removed, those molecules with some portion still in the pillar-free region will recoil, affecting a spatial separation from the shorter molecules that clear the interface. The separation result of T2 and T7 clearly supports that the entropic recoil separation is according to length and the technique holds good potential for separating long DNA molecules.

Due to steep entropic barrier, it is difficult to introduce large DNA molecules into a nanometer-scale channel directly form the macroscale world. Cao *et al.* [60] presented a simple technique using optical lithography (diffraction gradient lithography) to fabricate continuous spatial gradient nanoscale fluidic structures. DNA molecules are partially uncoiled when they enter the gradient area, and slow down at the edge of the nanochannels due to "uphill" entropy, however, larger DNA molecules move into the nanochannels continuously and remain stretched, with significantly improved efficiency. Based on the fact that an electrically polarizable object, such as DNA, will be trapped in a region of a focused electric field, provided there is sufficient dielectric response to overcome thermal energy and the electrophoretic force, Chou et al. [61] demonstrated electrodeless dielectrophoretic (EDEP) trapping using insulating constrictions in the audio frequency range. The devices were fabricated on quartz wafers using UV lithography and reactive ion etching techniques, and sealed with a glass coverslip coated with an elastomer thin film to act as a sealing gasket. At a given trapping voltage applied, the dielectrophoretic force dramatically increases with the increase of the length of the DNA molecule. Thus, by appropriate choice of parameters, one can envision selectively trapping one range of DNA molecules while removing others. This allows concentration of DNA molecules to very tight bands before launch into a fractionating media, PCR cleanup, concentration of DNA in gene array chips to enhance sensitivity, and acceleration of gene hybridization rates by concentration of single-stranded DNA. Because EDEP trapping occurs in high field gradient regions, EDEP allows easy patterning of DNA by an appropriate geometrical obstacle design.



Figure 3. Entropic recoil of long DNA molecules. (A) the electric field pulls DNA fragments in the lowentropy pillared region into the high-entropy pillared region. (B) The field is turned off, and molecules that straddle the interface rapidly recoil to return in the open region. Reprinted from [41], with permission.

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By taking the advanced nanofabrication technique (electron beam lithography technique), nanopillars (2700 nm in height, 500 nm in width, and 500 nm in spacing) with a high aspect ratio (height/width 5.4) inside a microchannel on a quartz chip (Fig. 1D) have been fabricated and used for DNA separation [42]. When DNA molecules intrude into the nanopillar channels, they migrate at different velocities according to their molecular weights, indicating that the nanopillars produce a molecular sieving effect and work as a DNA sieving matrix. DNA fragment (range from 1 to 38 kbp) separation as clear bands at the detection window of 1450 μm from the entrance of the nanopillar channel (25 μ m in width and 2.7 μ m in height) has been achieved in 170 s. It is interesting to note that the technique has been applied to the separation of long DNA molecules (λ -phage DNA, T4-DNA: 165.6 kbp) within only 10 s under a dc electric field. Theoretical plate numbers of the channels (380-1450 µm long) are 1000-3000 (0.7- 2.1×10^6 plates/m). Since there is no need to fill microchannels with sieving matrixes, nanopillar chips also hold the potential for integrated bioanalysis, which implicate whole process of DNA extraction from cells, purification, DNA amplification, separation, detection, and collection on one chip.

2.3 Obstacle array

DNA molecules are sorted by diffusion as they flow through a microfabricated array of asymmetrically arranged obstacles (Fig. 1E) [43, 62]. The basic concept is to use a regular lattice of asymmetric obstacles to rectify the lateral Brownian motion of the molecules so that species of different sizes follow different trajectories through the device [63]. Molecules that diffuse very slowly are likely to travel straight through the sieve, without being deflected by the obstacles. A nominal 6% resolution by length of DNA molecules in the size range of 15-30 kbp was achieved in a 4-inch silicon wafer. A major advantage of the Brownian ratchet array over the entropic trap array is that the ratchet array does not require stretching of the molecules. However, the separation of large molecules in a microfabricated Brownian ratchet array is slow, because it relies on diffusion, an intrinsically slow process. By tilting the electrophoretic flow relative to the vertical axis of the array, the separation of large molecules can be improved dramatically [64]. This is because a higher fraction of the diffusing molecules is ratcheted at each step in the array. Using a 12 mm long array, DNA molecules of 48.5 and 164 kbp length were separated in 70 min with a resolution of 3.8. Based on a Brownian ratchet mechanism, DNA molecules were also transported and separated by an interdigitated electrode array device [65, 66]. The migration is produced by periodic formation of an asym-

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metric sawtooth electric field in the device. Since the transport rate depends primarily on the diffusion constant of the molecules in free solution, the device could be used for size-dependent DNA separations, such as DNA genotyping in aqueous buffer [67]. As compared with traditional methods, such as gel electrophoresis, these approaches provide the advantage of continuous sorting and integration that allows sample preparation and subsequent analytical steps on a single chip. Moreover, by obviating the need for a viscous sieving medium, the methods facilitate automation and offers new levels of convenience. From the theoretical point of view, the separations of proteins, colloidal particles, and cells are also possible by controlling obstacle dimensions, flow velocity, and supporting solvents.

A thumbnail-sized device (DNA prism) has been fabricated and used for the separation of long DNA molecules prepared from bacterial artificial chromosomes [44]. The DNA prism consisted of hexagonally packed arrays of µm-scale posts array as the sieving matrix, sample injection, and extraction channels, and structures for shaping uniform electric field (Fig. 1F). Asymmetric pulsed fields are applied for continuous-flow operation, which sorts DNA molecules in different directions according to their molecular masses, such as a prism deflects light of different wavelengths at different angles (Fig. 4). This technique allows to sort large DNA fragments (range from 61 to 209 kbp) in 15 s, which is 1000 times faster than conventional PFGE (10-240 h). Furthermore, the prism device has better resolution (~13%) than asymmetric obstacle arrays [43] and entropic trap arrays [56].

In order to create equivalent migration paths for each molecule in a mixture, thereby eliminating multipath zonebroadening, a device was microfabricated in silicon consisting of a matrix of obstacles [68]. The horizontal obstacle spacing λ is 8 μ m, the row-to-row spacing is 8 μ m, and the gap width *d* is 1.6 μ m. Each row is shifted laterally by 0.1 λ , providing ten lanes. Particles, such as beads and DNA, are injected from a 10 µm wide channel at the top of the matrix and are carried across the matrix by fluid flow. Fluid emerging from a gap between two obstacles will encounter an obstacle in the next row and will bifurcate as it moves around the obstacle. When using such a device, a particle chooses its path deterministically on the basis of its size. Fluorescence beads and bacterial artificial chromosomes from Escherichia coli were used to test the hypothesis in the presence of hydrodynamic flow (by a driving pressure of 3 kPa) and electric field (12 V/cm), respectively, because the bacterial artificial chromosomes tend to coil up and can thus be approximated as soft spheres. When electric fields are used to drive the molecules through the matrix, they cre-



Figure 4. Separation of DNA molecules in a DNA prism. (A) Schematic showing the behavior of small and large DNA molecules in microfabricated arrays through a full cycle of asymmetric electric fields of alternating angles. (B)–(D) Fluorescence micrographs of continuous DNA separation under different field strengths. Reprinted from [44], with permission.

ate electric currents, which are bifurcated in the matrix in the same manner as fluid flow. Bacterial artificial chromosomes of 61 and 158 kb were separated in 10 min. When compared to the separation of the fluorescence beads, the separation efficiency is relatively low as a result of random deformation and stretching of DNA in higher fields and diffusion (a long separation time at a low flow rate).

2.4 Magnetic self-assembling sieves

Fabrication processes of the above-mentioned nanodevices are sophisticated and the nanotechniques used are not accessible to most labs. A promising new alternative is the use of quasi-regular arrays of columns as depicted in Fig. 1G [69]. The quasi-regular arrays of columns are formed by confining a suspension of superparamagnetic particles (with a diameter on the order of a few micrometers) between two parallel flat plates under the application of a homogeneous magnetic field (> 10 mT). In the system, the polymer forms a pulley-like structure, where the two downfield arms of the polymer compete to release the molecule as a result of polymer post entrapment [70]. The time for disentangling from the post is a function of the molecular contour length and hence separation is possible [71, 72]. Doyle et al. [69] employed self-assembled posts of Fe₂O₃ ferrofluids with inter-post spacing of 5 μm to effectively separate $\lambda\text{-phage DNA}$ and associated fragments of 15 and 33.5 kbp in 10 to 15 min. The λ -phage DNA electropherograms are reproducible within approximately 6% with each subsequent replacement of the sieving medium.

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3 Nanomaterials for DNA separation

3.1 Polymer solutions containing nanoparticles

In order to solve the problem of filling capillaries with highviscosity polymer solution for separating small DNA fragments, poly(N,N-dimethylacrylamide) (PDMA, M_w 100 000) containing montmorillonite clay was tested [73]. The addition of 5.0×10^{-5} g/mL clay into the 5% w/v PDMA greatly enhanced both the efficiency and resolution of DNA separation without losing the speed. Montmorillonite clay functions like a dynamic cross-linking plate for the PDMA chains and effectively increases its apparent molecular mass. However, using clay to enhance the DNA separation has some limitations: it is hydrophobic and only interacts with some polymers. Alternatively, PEO solution containing gold nanoparticles (GNPs) was used for the analysis of dsDNA fragments by CE [74, 75]. The separation of DNA ranging in size from 8 to 2176 bp was accomplished in 5 min using 0.2% PEO $(M_w 8000000)$ containing 56 nm GNPs (Fig. 5). Using 0.05% PEO (M_w 2000000) containing 13 nm GNPs or 0.05% PEO (M_w 4 000 000) containing 32 nm GNPs, DNA fragments ranging from 5 to 40 kbp were completely resolved [75]. The advantages of using PEO containing GNPs include ease of filling the capillary with such lowviscosity solutions (< 15 cP), rapidity, high resolving power (single-base resolution), and excellent reproducibility. When compared to montmorillonite clay, GNPs are hydrophilic after being capped with capping agents such as citrate and have narrower size distributions. In addition, their chemical and physical properties are well-



Figure 5. Separations of 10 μ g/mL DNA markers V and VI using (A) 0.2% PEO (M_w 8 000 000) and (B) 0.2% PEO (M_w 8 000 000) containing 0.3 × 56 nm GNPs. Reprinted from [74], with permission.

known, allowing ease of modification. There are also many reliable methods available for synthesizing highquality GNPs with different sizes and shapes that may be added to PEO solutions for further optimizing resolution and speed for DNA. However, a great effort must be paid to decrease the size distribution of GNPs and to increase their stability.

0.2% PEO (M_w 8 000 000) containing 0.3 × 32 nm GNPs was used to separate DNA fragments ranging in size from 8 to 23 130 bp, with high resolving power and speed (7 min). Changes in the PEO morphologies in the presence of GNPs likely account for improved resolution. The reproducibility of the method is excellent (relative standard deviations for the migration times less than 0.5%) when using a capillary dynamically coated with 5.0% PVP. It is noted that reproducibility and high efficiency are also due to minimized DNA adsorption on the capillary wall in the presence of GNPs that are strongly adsorbed on the wall. When compared to the separation of the same DNA sample by using a stepwise technique (changes in PEO concentration), this technique is faster and provides better resolution for long DNA fragments [38]. This approach

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is advantageous over the use of ultradilute polymer solution [28], including reproducibility, resolving power, and speed.

3.2 Polymers adsorbed on gold nanoparticles

Very recently, Chang's group has presented the first example of the analysis of long double-stranded (ds) DNA molecules by nanoparticle-filled capillary electrophoresis (NFCE) [76]. The GNPs were modified with PEO molecules via noncovalent bonding to avoid aggregation of GNPs that allow strong interactions with the DNA molecules. As in the transient entanglement mechanism, a DNA molecule temporarily intertwines with the polymers adsorbed on a GNP with which it collides during electrophoretic separation. The nearly neutral GNPPs (PEOmodified GNPs) (Fig. 6A) resist the flow and slow down the migration of the DNA. It is noted that the GNPP is heavier (> 2.0×10^8 Da per particle) than PEO $(M_w 8\,000\,000)$. When compared with the free linear polymers, the polymers adsorbed on the GNPs are stiffer and less extended, depending on the size of the GNPs and the



Figure 6. Cartoons for (A) GNPPs and (B) core-shell type nanosphere structures. (B) Reprinted from [77], with permission.

length of the polymer chain, and, thus, become slightly deformed under the flow. The separation of λ -*Hin*dIII DNA fragments by NFCE was successful at pH 7.0 using 10 × (10 times the concentration of the original GNPs) GNPPs containing EtBr (2.0 µg/mL). By using NFCE, the DNA fragments with sizes ranging from 8.2 to 48.5 kbp were well resolved within 7 min except for the 8.2 and 8.6 kbp fragments, with theoretical plate numbers of 1.8×10^6 , 2.2×10^6 , and 2.1×10^6 for the 10.1, 24.8, and 48.5 kbp fragments, respectively. This approach offers the advantages of speed for separating long DNA molecules when compared to the use of PEO-containing GNPs that must be conducted at low electric fields. However, preparation of GNPPs is not easy and NFCE is only useful for separating long DNA molecules.

3.3 Nanopacking medium

Baba's group [77] utilized a nanoparticle medium, coreshell type nanospheres, in conjunction with a double pressurization technique for the separation of DNA by MCE. After filling the separation channel with a 1% nanosphere solution (Fig. 6B), the sample was injected from the vertical direction by initial pressure (P1st) application. Just before electrophoretic separation, a secondary pressure (P_{2nd}) was applied for a few seconds to the separation channel, which causes the sample at the cross section to advance further as a dispersed broad. Subsequently, electrophoresis was conducted at 220 V/cm without pressure. In the presence of the nanospheres, the sample zones were stacked, resulting sharp peak profiles and high efficiency. The application of the 2nd pressure also caused a faster separation speed; the separation of 100 to 1000 bp DNA fragments was completed in 60 s, while that for 1 to 15 kbp DNA ladder was completed in

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100 s using the nanosphere medium. As the use of PEOcontaining GNPs, the core-shell type nanospheres are useful for separating small and long DNA molecules. Advantages of using the core-shell type nanospheres over PEO-containing GNPs in MCE are ease of filling (low viscosity) and speed.

4 Conclusions

The analysis of DNA will remain one of the most important issues in many fields, such as life science and analytical chemistry after the post human genome era. Although numerous DNA analysis techniques, such as DNA microarray, gel electrophoresis, and denatured high-performance liquid chromatography, are powerful, CE- and MCE-based separation techniques will still play a strong role in the DNA analysis. The use of conventional polymer solutions in CE and MCE is, no doubt, the main technique for DNA analysis. However, their shortages for separating long DNA fragments and narrow separation ranges have put a great demand for new materials and devices. In this review, we have addressed the basic principle, examples, and features of some important techniques, such as entropic trap, nanopillars, and obstacle entrapment for the separation of long DNA fragments. These techniques offer common advantages: no need of using sieving matrixes, and high speed. However, they also share some common disadvantages: poor resolution for small DNA molecules, and requirements of complicated fabrication processes and a sensitive detection system. We also reviewed several successful DNA separations using nanomaterials. Unlike the use of nanostructured devices, CE and MCE with nanomaterials offer great advantages of simplicity, a broad size separation range, and capability of separating small DNA fragments. However, preparation of nanomaterials that provide high separation efficiency for DNA separation is a complex and time-consuming process. When compared to conventional polymer matrices, the costs and accessibility of the nanoparticles are problematic.

The works of nanomaterials and nanostructured devices reviewed in this article show new trends in the analysis of DNA. The combination of nanomaterials and nanostructured devices should be desired for separating DNA molecules with a broad size range (e.g., several bp to Mbp). In order to prevent the difficulty of filling sieving matrixes to the nanostructured channels, the use of extremely low-viscosity nanomaterials is required. By taking the advanced synthetic techniques, nanomaterials, such as organic polymers, metallic nanoparticles, and functionalized nanoparticles, can be prepared and tested for DNA separation. It is also possible to fabricate biofunctional nanostructured devices for DNA analysis. Although only DNA separations using nanomaterials and nanodevices are reviewed in this article, the possibility of using these techniques for separating proteins and small analytes should not be ruled out. Using GNP-coated capillaries and microchannels, the separations of isomeric compounds with high efficiency have been demonstrated [78, 79]. The separation of amines and their metabolites (acids) using silica nanoparticles has also been recently demonstrated by Chang's group (unpublished results).

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5 References

- [1] Voss, K. O., Roos, H. P., Dovichi, N. J., Anal. Chem. 2001, 73, 1345–1349.
- [2] Liu, S., Electrophoresis 2003, 24, 3755–3761.
- [3] Vreeland, W. N., Meagher, R. J., Barron, A. E., Anal. Chem. 2002, 74, 4328–4333.
- [4] Emrich, C. A., Tian, H., Medintz, I. L., Mathies, R. A., Anal. Chem.2002, 74, 5076–5083.
- [5] Lin, Y.-W., Huang, M.-J., Chang, H.-T., J. Chromatogr. A 2003, 1014, 47–55.
- [6] Buch, J. S., Kimball, C., Rosenberger, F., Highsmith, W. E. Jr., DeVoe, D. L., Lee, C. S., Anal. Chem. 2004, 76, 874–881.
- [7] Russom, A., Ahmadian, A., Andersson, H., Nilsson, P., Stemme, G., *Electrophoresis* 2003, 24, 158–161.
- [8] Doi, K., Doi, H., Noiri, E., Nakao, A., Fujita, T., Tokunaga, K., *Electrophoresis* 2004, 25, 833–838.
- [9] Hu, S., Dovichi, N. J., Anal. Chem. 2002, 74, 2833-2850.
- [10] Goedecke, N., McKenna, B., El-Difrawy, S., Carey, L., Matsudaira, P., Ehrlich, D., *Electrophoresis* 2004, 25, 1678– 1686.
- © 2005 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim

- [11] Shortreed, M. R., Li, H., Huang, W.-H., Yeung, E. S., Anal. Chem. 2000, 72, 2879–2885.
- [12] Chang, P.-L., Kuo, I-T., Chiu, T.-C., Chang, H.-T., Anal. Bioanal. Chem. 2004, 379, 404–410.
- [13] Breadmore, M. C., Wolfe, K. A., Arcibal, I. G., Leung, W. K., Dickson, D., Giordano B. C., Power, M. E., Ferrance, J. P., Feldman, S. H., Norris, P. M., Landers, J. P., *Anal. Chem.* 2003, 75, 1880–1886.
- [14] Tseng, W.-L., Lin, Y.-W., Chen, K.-C., Chang, H.-T., *Electro-phoresis* 2002, 23, 2477–2484.
- [15] Hataoka, Y., Zhang, L., Mori, Y., Tomita, N., Notomi, T., Baba, Y., Anal. Chem. 2004, 76, 3689–3693.
- [16] Hisamoto, H., Nakashima, Y., Kitamura, C., Funano, S., Yasuoka, M., Morishima, K., Kikutani, Y., Kitamori, T., Terabe, S., Anal. Chem. 2004, 76, 3222–3228.
- [17] Gelfi, C., Vigano, A., Palma, S. D., Righetti, P. G., Righetti, S. C., Corna, E., Zunino, F., *Electrophoresis* 2002, *23*, 1517–1523.
- [18] Zhou, H., Miller, A. W., Sosic, Z., Buchholz, B., Barron, A. E., Kotler, L., Karger, B. L., *Anal. Chem.* 2000, 72, 1045–1052.
- [19] Chang, H.-T., Yeung, E. S., J. Chromatogr. B 1995, 669, 113– 123.
- [20] Gao, Q., Yeung, E. S., Anal. Chem. 2000, 72, 2499-2506.
- [21] Moyzis, R. K., Buckingham, J. M., Cram, L. S., Dani, M., Deaven, L. L., Jones, M. D., Meyne, J., Ratliff, R. L., Wu, J.-R., *Proc. Natl. Acad. Sci. USA* 1988, 85, 6622–6626.
- [22] Lalande, M., Noolandi, J., Turmel, C., Rousseau., J., Slater, G. W., Proc. Natl. Acad. Sci. USA1987, 84, 8011–8015.
- [23] Slater, G. W., Desruisseaux, C., Hubert, S. J., Mercier, H.-F., Labrie, J., Boileau, J., Tessier, F., Pépin, M. P., *Electrophoresis* 2000, *21*, 3873–3887.
- [24] Sudor, J., Novotny, M. V., Anal. Chem. 1994, 66, 2446–2450.
- [25] Kim, Y., Morris, M. D., Anal. Chem. 1994, 66, 3081–3085.
- [26] Kim, Y., Morris, M. D., Anal. Chem. 1995, 67, 784–786.
- [27] Mitnik, L., Salomé, L., Viovy, J.-L., Heller, C., J. Chromatogr. A 1995, 710, 309–321.
- [28] Barron, A. E, Blanch, H. W., Soane, D. S., *Electrophoresis* 1994, 15, 597–615.
- [29] Hubert, S. J., Slater, G. W., Viovy, J.-L., *Macromolecules* 1996, 29, 1006–1009.
- [30] Shi, X., Hammond, R. W., Morris, M. D., Anal. Chem. 1995, 67, 1132–1138.
- [31] Sunada, W. M., Blanch, H. W., *Electrophoresis* 1998, 19, 3128–3136.
- [32] Schwinefus, J. J., Hammond, R. W., Oana, H., Wang, S.-C., Carmejane, O. D., Bonadio, J., Morris, M. D., *Macro-molecules* 1999, *32*, 4625–4630.
- [33] Desruisseaux, C., Drouin, G., Slater, G. W., *Macromolecules* 2001, 34, 5280–5286.
- [34] Todorov, T. I., Morris, M. D., *Electrophoresis* 2002, 23, 1033– 1044.
- [35] Cretich, M., Chiari, M., Rech, I., Cova, S., *Electrophoresis* 2003, 24, 3793–3799.
- [36] Yamaguchi, Y., Todorov, T. I., Morris, M. D., Larson, R. G., *Electrophoresis* 2004, 25, 999–1006.
- [37] Chiu, T.-C., Chang, H.-T., J. Chromatogr. A 2002, 979, 299– 306.
- [38] Kuo, I.-T., Chiu, T.-C., Chang, H.-T., *Electrophoresis* 2003, 24, 3339–3347.
- [39] Seo, Y.-S., Luo, H., Samuilov, V. A., Rafailovich, M. H., Sokolov, J., Gersappe, D., Chu, B., *Nano Lett.* 2004, *4*, 659– 664.
- [40] Han, J., Craighead, H. G., J. Vac. Sci. Technol. A 1999, 17, 2142–5147.

- 330 Y.-W. Lin et al.
- [41] Turner, S. W. P., Cabodi, M., Craighead, H. G., Phys. Rev. Lett. 2002, 88,128103-1–128103-4.
- [42] Kaji, N., Tezuka, Y., Takamura, Y., Ueda, M., Nishimoto, T., Nakanishi, H., Horiike, Y., Baba, Y., *Anal. Chem.* 2004, 76, 15–22.
- [43] Chou, C.-F., Bakajin, O., Turner, S. W. P., Duke, T. A. J., Chan, S. S., Cox, E. C., Craighead, H. G., Austin, R. H., Proc. Natl. Acad. Sci. USA1999, 96, 13762–13765.
- [44] Huang, L. R., Tegenfeldt, J. O., Kraeft, J. J., Sturm, J. C., Austin, R. H., Cox, E. C., *Nature Biotech.* 2002, 20, 1048– 1051.
- [45] Bakajin, O., Duke, T. A. J., Tegenfeldt, J., Chou, C.-F., Chan, S. S., Austin, R. H., Cox, E. C., *Anal. Chem.* 2001, 73, 6053– 6056.
- [46] Chou, C.-F., Austin, R. H, Bakajin, O., Tegenfeldt, J. O., Castelino, J. A., Chan, S. S., Cox, E. C., Craighead, H., Darnton, N., Duke, T., Han, J., Turner, S., *Electrophoresis* 2000, *21*, 81–90.
- [47] Austin, R. H., Tegenfeldt, J. O., Cao, H., Chou, S. Y., Cox, E. C., *IEEE Trans. Nanotechnol.* 2002, 1, 12–18.
- [48] Mitnik, L., Novotny, M., Felten, C., Buonocore, S., Koutny, L., Schmalzing, D., *Electrophoresis* 2001, 22, 4104–4117.
- [49] Sartori, A., Barbier, V., Viovy, J.-L., *Electrophoresis* 2003, *24*, 421–440.
- [50] Pernodet, N., Samuilov, V., Shin, K., Sokolov, J., Rafailovich, M. H., Gersappe, D., Chu, B., *Phys. Rev. Lett.* 2000, *85*, 5651–5654.
- [51] Seo, Y.-S., Samuilov, V., Sokolov, J., Rafailovich, M. H., Tinland, B., Kim, J., Chu, B., *Electrophoresis* 2002, *23*, 2618– 2625.
- [52] Luo, H., Gersappe, D., *Electrophoresis* 2002, 23, 2690– 2696.
- [53] Baumgärtner, A., Muthukumar, M., J. Chem. Phys. 1987, 87, 3082–3088.
- [54] Baumgärtner, A., Muthukumar, M., *Macromolecules* 1989, 22, 1937–1941.
- [55] Baumgärtner, A., Muthukumar, M., *Macromolecules* 1989, 22, 1941–1946.
- [56] Han, J., Turner, S. W. P., Craighead, H. G., Phys. Rev. Lett. 1999, 83, 1688–1691.
- [57] Han, J., Craighead, H. G., Science 2000, 288, 1026–1029.
- [58] Han, J., Craighead, H. G., Anal. Chem. 2002, 74, 394–401.
- [59] Cabodi, M., Turner, S. W. P., Craighead, H. G., Anal. Chem. 2002, 74, 5169–5174.
- [60] Cao, H., Tegenfeldt, J. O., Austin, R. H., Chou, S. Y., Appl. Phys. Lett. 2002, 81, 3058–3060.

- [61] Chou, C.-F., Tegenfeldt, J. O., Bakajin, O., Chan, S. S., Cox, E. C., Darnton, N., Duke, T., Austin, R. H., *Biophys. J.* 2002, 83, 2170–2179.
- [62] Cabodi, M., Chen, Y.-F., Turner, S. W. P., Craighead, H. G., Austin, R. H., *Electrophoresis* 2002, 23, 3469–3503.
- [63] Huang, L. R., Silberzan, P., Tegenfeldt, J. O., Cox, E. C., Sturm, J. C., Austin, R. H., Craighead, H., *Phys. Rev. Lett.* 2002, 89, 178301-1–178301-4.
- [64] Huang, L. R., Cox, E. C., Austin, R. H., Sturm, J. C., Anal. Chem. 2003, 75, 6963–6967.
- [65] Bader, J. S., Hammond, R. W., Henck, S. A., Deem, M. W., McDermott, G. A., Bustillo, J. M., Simpson, J. W., Mulhern, G. T., Rothberg, J. M., *Proc. Natl. Acad. Sci. USA*1999, *96*, 13165–13169.
- [66] Hammond, R. W, Bader, J. S., Henck, S. A., Deem, M. W., McDermott, G. A., Bustillo, J. M., Rothberg, J. M., *Electro-phoresis* 2000, *21*, 74–80.
- [67] Bader, J. S., Deem, M. W., Hammond, R. W., Henck, S. A., Simpson, J. W., Rothberg, J. M., *Appl. Phys. A* 2002, 75, 275–278.
- [68] Huang, L. R., Cox, E. C., Austin, R. H., Sturm, J. C., Science 2004, 304, 987–990.
- [69] Doyle, P. S., Bibette, J., Bancaud, A., Viovy, J.-L., Science 2002, 295, 2237–2237.
- [70] André, P., Long, D., Ajdari, A., Eur. Phys. J. B 1998, 4, 307– 312.
- [71] Mayer, P., Bibette, J., Viovy, J.-L., Mater. Res. Soc. Symp. Proc. 1997, 463, 57–66.
- [72] Sevick, E. M., Williams, D. R. M., Eur. Phys. Lett. 2001, 56, 529–535.
- [73] Liang, D., Song, L., Chen, Z., Chu, B., *Electrophoresis* 2001, 22, 1997–2003.
- [74] Huang, M.-F., Huang, C.-C., Chang, H.-T., *Electrophoresis* 2003, 24, 2896–2902.
- [75] Chiou, S.-H., Huang, M.-F., Chang, H.-T., *Electrophoresis* 2004, 25, 2186–2192.
- [76] Huang, M.-F., Kuo, Y.-C., Huang, C.-C., Chang, H.-T., Anal. Chem. 2004, 76, 192–196.
- [77] Tabuchi, M., Ueda, M., Kaji, N., Yamasaki, Y., Nagasaki, Y., Yoshikawa, K., Kataoka, K., Baba, Y., *Nature Biotech.* 2004, 22, 337–340.
- [78] Neiman, B., Grushka, E., Lev, O., Anal. Chem. 2001, 73, 5220–5227.
- [79] Pumera, M., Wang, J., Grushka, E., Polsky, R., Anal. Chem. 2001, 73, 5625–5628.