ORDER AND DISORDER IN NANOPOROUS MEDIA CONTROLS DNA SEPARATION EFFICIENCY

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ABSTRACT
The effect of order in separation media on DNA separation resolution is studied experimentally. A microfluidic technique based on colloidal self-assembly is employed to generate structures with different degrees of order. SEM images of the structures are employed to characterize the scale of order. The DNA separation resolution is quantified in each structure through calculation of peak separation distance and band broadening. DNA separation resolution under pulsed electrophoresis is found to change non-monotonically with the degree of order. Resolution is lowest in structures with the highest degree of disorder, reaching a maximum when there is a short range order.

KEYWORDS: DNA Separation, Colloidal Crystals, Pulsed Field Electrophoresis, Ordered Structures

INTRODUCTION
We have developed colloidal self-assembly of crystalline arrays of nanoparticles within microfluidic channels as a powerful tool for fabricating highly ordered, nanoporous media [1]. Asymmetric pulsed field electrophoresis (APFE) within arrays generates two-dimensional separation of DNA molecules [2]. Separation media with different degrees of disorder are easily fabricated by doping one particle size with another using colloidal self-assembly. This approach allows study of the effect of order and disorder in nanoporous media on DNA separation efficiency. We present the first experimental study on the effect of order on DNA APFE.

Three groups [3-5] have studied the effects of separation media order on DNA dynamics theoretically. Slater and co-workers [3] calculated the diffusion coefficient of DNA as a function of the degree of disorder in the medium, showing a non-monotonic change on going from disordered to full long range order. Shaqfeh and Patel [4] calculated that order produced poor separations compared to disorder, for sparse structured post arrays. Mohan and Doyle [5], using numerical techniques, concluded that local order in sparse arrays of obstacles should give better separation than fully ordered or disordered arrays. However, there is a lack of experimental data to test these models.

EXPERIMENTAL
DNA separation was conducted using a microfluidic chip filled with an array of nanoparticles as a sieving matrix. A schematic of the PDMS microchip is shown in Figure 1a.

![Figure 1: a) Schematic of DNA separation microchip. Separation chamber is filled with self-assembled nanoparticle arrays. b) Junction of separation chamber with injection channel. Three different sizes of DNA are mixed then continuously injected to the separation chamber. The white arrows represent the electric field directions. c) Two-dimensional separation of DNA molecules is achieved once the pulsed electric field is applied.](image-url)

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PDMS microchips were fabricated using a standard soft lithography technique, then sealed to clean glass slides prior to packing, as described in detail elsewhere [1]. Aqueous suspensions of silica colloids (Bangs Laboratories, Fishers, IN) of 330 nm, and 700 nm diameter were used to form the self-assembled nanoparticle array inside the microchips. DNA fragments (6, 10, 20 kbp, Fermentas Life Sciences) were stained by YOYO-1 (Molecular Probes) with dye-to-base ratio of 1:10. DNA samples were excited by a 488-nm argon ion laser beam, and the fluorescent emission was collected. Separation of DNA molecules was conducted by injecting DNA samples into the separation chamber inside the microchip. The fluorescence image shown in Figure 1b represents the junction of the injection channel and separation chamber. The separation chamber was connected to reservoirs where pulsed electric potentials were applied using platinum electrodes. The applied pulsed electric potentials generate asymmetric obtuse-angle pulsed fields, $E_1$ and $E_2$ across the separation chamber, where the angle between the pulsed fields is $\sim 135^\circ$ and $E_1 = 1.4E_2$ (as shown in Fig. 1b) in all our experiments. Once a DNA sample reaches the separation chamber, different sizes of DNA molecules separate from each other and form individual streams, as shown in Figure 1c. The separation mechanism of DNA molecules under obtuse-angle pulsed fields is as follows [6]: pulsed electric field causes DNA molecules to stretch and reorient periodically, with their head/tail repeatedly switched. Due to this periodic head/tail switching of the molecule, the net migration of DNA molecules is biased in different directions by the asymmetric fields; larger molecules are deflected farther from the injection angle compared to smaller ones. Monodispersed suspensions of silica particles of 320 nm and 700 nm were used to fabricate ordered packed structures inside the separation chamber in the microfluidic device. SEM images of these structures revealed homogenous, ordered structures where the pore size is around 15% of the particle size.

RESULTS AND DISCUSSION

In order to introduce defects and disrupt the regular crystalline geometry of homogenous packed structures, bidisperse suspensions of 320 and 700 nm silica beads with different volume fractions of 700 nm particles ($\chi_{700}$) were used to fabricate packed structures with different degrees of defects. This means that $\chi_{700} = 0$ and $\chi_{700} = 1$ represent ordered structures, whereas $0 < \chi_{700} < 1$ represent disordered structures. The disorder can be systematically controlled by changing the volume fraction ($\chi$) of each particle. The degree of disorder associated with each structure is quantified by both an orientational order parameter ($\psi$) and the radial distribution function ($g(r)$) calculated from SEM images, as shown in Figure 2 for different volume fractions of the larger particle in the structure ($\chi_{700}$). Equal volume fractions of 320 and 700 nm particles ($\chi_{700} = 0.5$) produced the highest degree of disorder (lowest value of $\psi$), while the best order obtained yielded $\psi = 0.93$.

![Figure 2](image_url)

_Figure 2: a) SEM images of the structures with different volume fraction of 700 nm particles, from the top $\chi_{700} = 0$, 0.09, 0.16, 0.5, respectively. b) Radial distribution function corresponding to each structure in (a). By increasing $\chi_{700}$, the number of peaks and peak values decreases which means the degree of disorder increases from top to bottom. c) Orientational order parameter ($\psi$) calculated for each structure in (a). By increasing $\chi_{700}$, $\psi$ decreases which means the degree of disorder increases from top to bottom._
DNA separations in each structure gave peak separation distances and band broadening between any two consecutive DNA bands that were a function of order ($\psi$). Resolution between any two DNA bands is defined as the ratio of the peak distance to the band broadening. Resolution changed in a non-monotonic fashion with the degree of order (Figure 3). Resolution is lowest in structures with the highest degree of disorder, reaching a maximum when there is short range order at $\psi = 0.5$ ($\chi_{700} = 0.09$). Band broadening follows a similar trend, being maximized at $\chi_{700}$ and $\chi_{320}$ close to 1 (dropping slightly at 1), and minimized at $\chi_{700} = 0.5$.

![Figure 3. Variation of DNA separation resolution in pulsed field electrophoresis as a function of degree of disorder ($\psi$) in separation media. $\psi = 1$ represents an ordered structure whereas $\psi < 1$ represent structures with different degrees of disorder.](image)

**CONCLUSION**

Our results demonstrate that coherent, ordered structures give more efficient DNA separation than random porous media, contrasting with reference 4. The non-monotonic behavior observed supports the theoretical analysis [5] that suggests short range order is preferable to 100% order, though the separation method is hooking-related, not ratchet-based in that theoretical study. Colloidal arrays provide a powerful means to explore a promising opportunity in separation science, the use of ordered materials.

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