CHEMISTRY

Self-Assembled Magnetic Matrices for DNA Separation Chips

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When a constant, homogenous magnetic field is applied to a suspension of superparamagnetic particles confined in a thin gap perpendicular to the field, the particles self-organize into a fixed, quasi-regular array of columns (Fig. 1A) (*1*). The array returns to a liquid suspension immediately upon field switch-off. The column spacing can be tuned from submicrometer to about 100 μm, by varying cell size and particle concentration. The pore size is reproducible within better than 5% and independent of the strength of the magnetic field, so long as it can hold the array in place during separation (typically >10 mT, which is achievable with ordinary permanent magnets).

We used here a self-assembled array with an average interparticle distance of 5.7 μm to separate large duplex DNA in a microchannel device prepared by soft lithography [preliminary demonstrations in a handmade cell were reported in (*2*)]. Such separations are currently performed in 12 to 24 hours with pulsed-field agarose gel electrophoresis (PFGE). Analysis of short DNA by capillary electrophoresis with polymer solutions was very successful, but DNA larger than a few kbp led to electrohydrodynamic instabilities (*3*). Microfabricated arrays of obstacles (*4*), microfluidic single-molecule counting devices, or entropic traps (*5*) provide an appealing alternative to gels but require high-resolution microlithography and very narrow channels (in the micrometer or submicrometer range), raising challenges in terms of cost and robustness to "real-life" samples.

Figure 1C shows electropherograms for intact λ DNA [48.5 kilobase pairs (kbp)] and fragments (15 and 33.5 kbp) created by Xho I digestion, at fields of 4.8, 7.0, and 10.0 V/cm. Three peaks associated with a single size of DNA, by peak intensity and spiking with monodisperse samples are clearly identified in only 10 to 15 min. The run-to-run reproducibility, with replacement of the matrix between each run, was better than 6%. λ-DNA concatemers (containing molecules of 48.5, 97, and 145.5 kbp and small amounts of larger DNA) were separated in 30 min, at a field strength of 3.2 V/cm (Fig. 1D). Such a separation is impossible in conventional DC field electrophoresis.

The theoretical plate number N was in the range of 1000 to 7000. Optimal resolution of the molecules in the size range of 15 to 33.5 kbp was obtained at 10 V/cm with a resolution length (RSL; measure of the smallest difference in DNA base pairs that can be resolved) of 5.5 kbp (RSL = 11.6 kbp for 33.5 to 48.5 kbp). For molecules of 33.5 to 48.5 kbp, optimal conditions were 4.8 V/cm with RSL = 7.2 kbp (RLS = 6.8 kbp for 15 to 33.5 kbp). For the larger concatemers, the RSL was 13 kbp in the range of 48.5 to 97 kbp and 22.2 kbp in the range 97 to 145.5 kb. These RSL and N are comparable to or better than that reported in (*5*) or found in PFGE (typically 10 kbp).

Mobility was studied as a function of field strength. At intermediate electric fields, mobility decreases with field strength increase, in contrast with the increase occurring in gel electrophoresis. The mobility of the 33.5-bp fragment drops from 2.55 to 2.13 × 10^{-8} m^2 V^{-1} s^{-1} between 2.7 and 10 V/cm, whereas the mobility of the 15-kbp fragment only drops from 2.55 to 2.39 × 10^{-8} m^{-2} V^{-1} s^{-1}. Using single molecule videomicroscopy, we observed the collision of DNA molecules with the columns of particles. At 1 V/cm, molecules migrate as random coils and bump into columns from time to time. Resolution is poor. At large electric fields, molecules get hooked and stretched and resume their motion by a "rope over a pulley" mechanism [see, e.g. (*6*)], which depends on chain size and yield size fractionation. At intermediate fields, hooking also occurs, but chain extension is incomplete, the larger chains being more extended because they are subject to a larger total force. This effect retards long chains more than short ones. It improves size fractionation, and it is also responsible for the decrease of mobility with field strength.

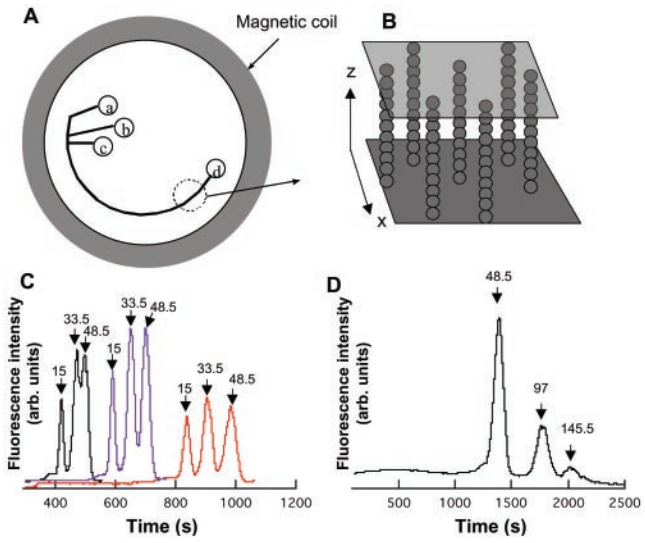

Fig. 1. (**A**) Schematic of the microchannel and magnetic coil. The poly(dimethylsiloxane) microchannel (height, 11 μm) with a "pinched injection" design was placed in the center of a magnetic coil mounted on an inverted microscope and filled before each run with a 2% volume fraction of 1-μm superparamagnetic particles (*7*). (**B**) Columnar structure formed by a suspension of superparamagnetic particles. (**C**) Fluorescence intensity at 10 mm from the injection zone versus time. Separation of a mixture of λ-phage DNA and λ-DNA digested with Xho I (Pharmacia) (the numbers refer to the size of the DNA fragments in kbp), at fields strengths of 4.8 (right), 7 (middle), and 10 left V/cm. (**D**) Separation of λ-phage DNA concatemers at a field of 3.2 V/cm. Other conditions are identical to those in (C).

Suspensions of paramagnetic particles hold many advantages over previous separation media. They have a low viscosity in the absence of a magnetic field, their pore size can be tuned (typically from 1 to 100 μm), and they do not require sophisticated microlithography. They could find a large range of applications for the separation of DNA and other intermediate-size objects such as cells, proteins, organelles, and micro- or nanoparticles.

References and Notes

1. J. Liu *et al.*, *Phys. Rev. Lett.* **74**, 2828 (1995).
2. P. Mayer, J. Bibette, J.-L. Viovy, *Mater. Res. Soc. Symp. Proc.* **463**, 57 (1997).
3. L. Mitnik *et al.*, *Science* **267**, 219 (1995).
4. W. D. Volkmuth, R. H. Austin, *Nature* **358**, 600 (1992).
5. J. Han, H. G. Craighead, *Science* **288**, 1026 (2000).
6. J.-L. Viovy, *Rev. Mod. Phys.* **72**, 813 (2000).
7. Supplementary material is available on *Science* Online at www.sciencemag.org/cgi/content/full/295/5563/2237/DC1.
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