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Influence of Immobilized Biomolecules on Magnetic Bead Plug Formation and Retention in Capillary Electrophoresis

Rachel L. Henken, Rattikan Chantiwas¹, and S. Douglass Gilman

Department of Chemistry, Louisiana State University, Baton Rouge, Louisiana 70803

Abstract

Significant changes in the formation and retention of magnetic bead plugs in a capillary during electrophoresis were studied, and it was demonstrated that these effects were due to the type of biological molecule immobilized on the surface of these beads. Three biological molecules, an antibody, an oligonucleotide and alkaline phosphatase, were attached to otherwise identical streptavidin-coated magnetic beads through biotin-avidin binding in order to isolate differences in bead immobilization in a magnetic field resulting from the type of biological molecule immobilized on the bead surface. Alkaline phosphatase also was attached to the magnetic beads using epoxy groups on the bead surfaces (instead of avidin-biotin binding) to study the impact of immobilization chemistry. The formation and retention of magnetic bead plugs were studied quantitatively using light scattering detection of magnetic particles eluting from the bead plugs and qualitatively using microscopy. Both the type of biomolecule immobilized on the magnetic bead surface and the chemistry used to link the biomolecule to the magnetic bead impacted the formation and retention of the bead plugs.

Keywords

bioreactors; capillary electrophoresis; immobilization; magnetic beads

1 Introduction

Superparamagnetic beads have emerged as essential tools for biochemistry and biotechnology research over the past two decades [1–7]. The rapid and widespread adoption of magnetic beads by researchers is due to the simplicity with which they can be used to separate, immobilize and move biological molecules by application of a magnetic field using simple and inexpensive permanent magnets. Magnetic beads are commercially available with diameters from 0.02–350 μm , and they are commonly used as a separation tool for cell labeling and isolation, and for molecular recognition [1, 2, 4]. Magnetic beads have been utilized for immunoassays and biosensors and for NMR imaging contrast enhancement [1–3]. The versatility of magnetic beads also is based on the wide range of bead surface chemistries available and the ability to easily attach many types of biological molecules to a bead surface.

The advantages of magnetic beads have been employed in microfluidic devices and capillaries [1, 2, 6–8]. Magnetic beads enable researchers to effectively immobilize biological molecules at defined locations within a microfluidic device without performing

Correspondence: S. Douglass Gilman, Department of Chemistry, Louisiana State University, 232 Choppin Hall, Baton Rouge, Louisiana 70803, sdgilman@lsu.edu, Fax: 225-578-3458.

¹Current Address: Department of Chemistry, Mahidol University, Bangkok, Thailand 10400

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covalent immobilization procedures within the confines of a microchannel. Biological molecules can be attached to magnetic beads in relatively large batches outside of the device, and aliquots of these magnetic beads can be immobilized at locations defined by the application of a magnetic field. Beads packed in microchannels offer large surface-to-volume ratios for immobilizing biological molecules and short diffusion distances between packed particles, which increase reagent-bead interactions. Furthermore, magnetic particles are advantageous compared to traditional solid supports because they can be immobilized in the microfluidic channels without the use of frits, difficult packing procedures or coating of the capillary or channel walls, and their immobilization can be reversed by removing the magnetic field and flushing out the beads. Beds of magnetic beads in a microfluidic device can be regenerated by reapplying the magnetic field and adding a new aliquot of magnetic beads after an old bed has been removed. Magnetic beads with a wide range of molecules attached to their surfaces have been used in microfluidic flow streams. Enzymes have been immobilized on bead surfaces for microreactors, tryptic digests and inhibition studies [2, 6, 9]. Nucleic acids have been immobilized on bead surfaces for DNA and RNA hybridization [1, 2]. Antigen and antibody molecules also have been used with magnetic beads for immunoassays and whole cell separations [1–3, 8]. Magnetic beads also have been used to form packed beds in microchannels for chromatographic separations [10, 11].

Most of the current theory to describe the immobilization of superparamagnetic beads in solution focuses on the magnetic interactions. Modeling of magnetic fields and flux is common, as is the determination of the magnetic susceptibility of magnetic beads in bulk and for individual beads [2, 12]. Some recent studies have examined magnetic bead aggregation in the presence of a magnetic field and have suggested that factors other than magnetic forces play a significant role; however, these studies used bare bead surfaces and static flow conditions [13–15]. Recent work has shown that surfactant molecules associated with superparamagnetic particles impact the self assembly of the particles into a chain pattern in the presence of a magnetic field [16, 17]. These studies suggests that the surface groups do have an impact on magnetic bead behavior in a magnetic field; however, most theoretical treatments discount or largely ignore the impact of bead surface chemistry, focusing only on the magnetic dipole-dipole, and magnetic moment interactions [1, 2, 18].

Our laboratory recently applied superparamagnetic beads to capillary electrophoretic studies of enzyme inhibition [9]. Unexpected and unexplained difficulties encountered During that work and other unpublished studies, we experienced difficulties immobilizing beds of magnetic beads that were unexpected and unexplained based on the literature in this area. This led us to pursue basic experimental investigations of the immobilization of magnetic beads in capillaries during electrophoresis. We report here a study of how immobilization of different biological molecules (oligonucleotide vs. antibody vs. enzyme) on otherwise identical superparamagnetic beads impacts the formation and retention of plugs of magnetic beads during electrophoresis. These three biomolecules were chosen to represent common classes of biological molecules attached to magnetic bead surfaces for microfluidic applications. The immobilization experiments were carried out in identical solutions and under identical electrophoresis conditions. An additional objective was to investigate if differences in the chemical linkage used to immobilize biological molecules to the magnetic bead surface would impact plug formation and retention.

2 Materials and Methods

2.1 Chemicals

Boric acid, sodium phosphate, EDTA and sodium hydroxide were purchased from Fisher Scientific (Fair Lawn, NJ). Sodium chloride was from Sigma (St. Louis, MO), and tris(hydroxymethyl)aminomethane was purchased from Acros Organics (Morris Plains, NJ).

A biotinylated oligonucleotide (T7 25mer primer) and biotinylated alkaline phosphatase were purchased from New England BioLabs (Ipswich, MA). Biotinylated goat anti-rabbit IgG was obtained from Chemicon (Billerica, MA). All solutions were prepared in 18 MΩ water from a Modulab water purification system (Palm Desert, CA).

2.2 Bead Preparation

Magnetic beads, M-270 Streptavidin and Dynabead M-270 Epoxy with 2.8 μm diameters and the same interior composition, were obtained from Invitrogen (Carlsbad, California). Aliquots from the same batch of streptavidin-coated superparamagnetic beads were labeled according to manufacturer's specifications, separately through biotin-avidin binding with three different biotinylated biomolecules - an oligonucleotide (T7 25mer primer), an enzyme (alkaline phosphatase), and an antibody (goat anti-rabbit IgG). Additionally, two identical bead types with different initial surface groups, epoxy and streptavidin, were labeled with the same enzyme, alkaline phosphatase. Alkaline phosphatase (not biotinylated) was covalently attached to superparamagnetic beads with an epoxy surface using the manufacturer's instructions. Five bead types were used in total: uncoated streptavidin beads (SA-Bare), streptavidin beads with bound biotinylated antibody (SA-IgG), streptavidin beads with bound biotinylated DNA oligomer (SA-Oligo), streptavidin beads with bound biotinylated alkaline phosphatase (SA-AP), and epoxy beads with covalently bound alkaline phosphatase (E-AP). Drawings representing the chemistry used to link biological molecules to the magnetic bead surfaces in these experiments can be found in Fig. S1 (Supporting Information). Washing and immobilization procedures used 1× PBS, pH 7.40 buffer for SA-IgG and SA-AP, 1× B&W (bind and wash buffer) for SA-Oligo, and 100.0 mM sodium phosphate buffer, pH 7.40, for E-AP. The beads were suspended in 100 μL of their respective buffers in a microcentrifuge tube, and the solutions were mixed by vortexing. The beads were then separated from their wash buffer by placing the centrifuge tube on a NdFeB magnet to immobilize the beads at the bottom of the microcentrifuge tube, and the supernatant was removed by pipet. This procedure was repeated three times. After the wash steps, the beads were resuspended in 30.0 μL of their respective buffers. Next, 30.0 μL of each biomolecule was added at the following concentrations: IgG- 0.2 mg/mL, Oligo- 3.5 mM, and AP- 3.5 mM. The biotinylated biomolecules and the streptavidin beads were mixed for 30 min on a slow tilt platform. The epoxy beads were derivatized by adding 30.0 μL of 14 mM alkaline phosphatase and 30.0 μL of 3.00 M ammonium sulfate. The mixture was incubated at room temperature for 24 hr on a slow tilt platform. After incubation with their respective biomolecules, all beads were washed with 100.0 μL of 20.0 mM borate buffer three times in the manner previously described. The beads were resuspended in 20.0 μL of the same buffer. Beads were stored at 4°C. Each of the magnetic bead stocks were diluted 1.0 μL in 200.0 μL total volume of the running buffer for working solutions. The final bead concentrations for each bead type were SA-IgG (8×10^6 beads/mL), SA-Oligo (8×10^6 beads/mL), SA-AP (8×10^6 beads/mL), and E-AP (6×10^6 beads/mL).

2.3 Capillary Electrophoresis

Identical CE systems, constructed in house, were used for all experiments [19]. A Spellman CZE1000R high voltage power supply (Hauppauge, NY) was used for the application of electrophoretic potential to a Polymicro Technologies (Phoenix, AZ) fused-silica capillary (49 μm id, 366 μm od) with a total length of 57 cm. Capillaries for experiments with light scattering/laser-induced fluorescence detection had a length of 36 cm to the detection window and 16 cm to the immobilization area, while capillaries for imaging experiments had a length of 30 cm to the immobilization/imaged area. A detection window of 5 mm was made by removing the polyimide coating with a window maker from MicroSolv Technology (Eatontown, NJ). New capillaries were conditioned with a manual syringe pump by rinsing with 300 μL each of NaOH (0.1 M in water), water and running buffer, sequentially.

Capillaries were further rinsed with 300 μL of running buffer between runs. A neutral marker, coumarin 334, from Acros Organics (Morris Plains, NJ), was injected just prior to magnetic bead injections at a concentration of 50.0 nM. The neutral marker was injected for 3.0 s, and all bead samples were injected for 10.0 s electrophoretically at 20.0 kV (351 V/cm). All working solutions, including the neutral marker, were prepared in running buffer, 20.0 mM borate buffer at pH 9.00, which was filtered through a 0.2 μm Whatman membrane filter (Hillsboro, OR).

2.4 Magnetic Capture Configuration

All magnets used in this work were NdFeB rare earth magnets from K&J Magnetics (D24, Jamison, PA). The surface field strength of these magnets is reported as 6403 Gauss by the manufacturer. Two magnets were held in place by tubing affixed to a microscope slide at a 20° angle to the vertical axis of the capillary on the same plane as indicated in Fig. 1. Magnets were positioned such that the edge of the magnet was touching the capillary wall. This configuration has previously been described as the most efficient in terms of bead retention and packing [20].

2.5 Electrophoretic Mobility of Magnetic Beads

For electrophoretic mobility measurements, the neutral marker, coumarin 334, and magnetic beads were electrokinetically injected sequentially at 20.0 V for 3.0 s using the CE instrument with light scattering/LIF detection. No magnetic field was applied for these experiments. An applied potential of 20.0 kV (350 V/cm) was used for electrophoretic separation. The magnetic beads formed a distribution of narrow, distinct peaks (data not shown) that were similar in appearance to the bead peaks shown in Fig. 2. To determine an average migration time of the magnetic beads in order to calculate their electrophoretic mobility, a histogram of peak frequency was made with a bin of 0.2 min, and the time period with the highest peak count was used as the migration time. Table 1 lists the electrophoretic mobilities of the bead varieties used. The standard deviation was calculated from the calculated mobilities for 5 consecutive runs.

2.6 Imaging Experiments

The magnetic bead plugs were formed by injecting a solution of beads electrophoretically at 20.0 kV (351 V/cm) for 10.0 s and then immediately reducing the voltage to 5.0 kV (88 V/cm) for 12 min in order to facilitate the transport of the beads to the magnetic capture area. The number of beads injected for imaging experiments, electrophoretic mobility measurements, and light scattering experiments (Table 1) was calculated using the electrophoretic mobilities measured for different bead types (Table 1) and the bead concentrations supplied by the manufacturer. The 20 $\times$ objective of a Nikon ECLIPSE TE 300 inverted microscope (Melville, NY) was used to image the magnetic capture area. The images were recorded using a Princeton Instruments CCD camera (Trenton, NJ), with 0.035 s exposure and WinView Software Version 32. The applied potential was increased in steps of 5.0 kV (88 V/cm) from 88 to 439 V/cm. After each increase, the potential was held constant for 8.0 min in order for the bead plugs to stabilize at the new applied potential. Images were obtained at the onset of the increase and after the 8.0 min waiting time.

2.7 Light Scattering Experiments

The magnetic bead plugs were formed by injecting a solution of the beads electrophoretically at 20.0 kV (351 V/cm) for 10.0 s and then immediately reducing the voltage to 5.0 kV (88 V/cm) for 18 min. The time that a reduced potential was applied (18 min) was longer than that used for imaging experiments (12 min) because of the longer distance from the injection end of the capillary to the magnetic bead immobilization site.

The total capillary lengths and the applied potentials were identical. The voltage was then increased in steps and held at each step for 8.0 min to determine if any magnetic beads were lost from the capture area. An electropherogram showing the eluting beads overlaid with a plot of electrophoretic current is presented in Fig. 2. The eluting beads were detected using a detection system similar to one described previously that was used to detect smaller individual polystyrene spheres with diameters from 110–1000 nm [21]. The 488-nm line from an Innova 90C-5 argon ion laser (Coherent, Inc, Santa Clara, CA) was used for both scattering detection of beads and fluorescence excitation of the neutral marker, coumarin 334, at a power of 10.0 mW. The beam was directed by an Omega Optical XF2031 dichroic mirror (Brattleboro, VT) and focused onto the capillary by a 20× microscope objective from Edmond Industrial (Barrington, NJ). Fluorescence and scattered light were detected at 180° through the same objective and dichroic mirror. The light was then optically filtered with a 520-nm bandpass filter from Omega Optical and spatially filtered by an 800 μm pin hole from Edmond Optics. Though the bandpass filter and dichroic mirror should, in principle, keep light at the source wavelength (488 nm) from reaching the PMT, the intensity of the scattered light is such that enough passes through for individual beads to be detected by light scattering. The scattered and fluorescent light was detected by a HP9306-04 PMT (Hamamatsu, Bridgewater, NJ) at a potential of 700 V. A 250 Hz low pass RC filter was used after the PMT, and the data were sampled at 200 Hz by a PCI-6229 data acquisition board from National Instruments (Austin, TX). A LabVIEW program (Version 7.1, National Instruments) written in house was used for data acquisition. Data analysis was performed with OriginLab 7.5 (Northampton, MA) and Microsoft Excel 2007 (Redmond, WA).

3 Results and Discussion

3.1 Effects of Different Immobilized Biomolecules

The goal of this work was to test the hypothesis that the type of biological molecule immobilized on a superparamagnetic bead surface will significantly impact its immobilization during capillary electrophoresis. Challenges faced (and overcome) when immobilizing enzyme-coated magnetic beads for the study of enzyme inhibition inspired us to probe this question [9]. In several instances, we experienced difficulty immobilizing magnetic beads under conditions which other publications suggested should be successful. The experiments presented here were designed to isolate any observed differences in magnetic bead plug formation and retention to only the effects of the nature of the biological molecule immobilized on the bead surface. The first three bead types examined in this work were identical except for the nature of the biotinylated biological molecule attached to the avidin-coated magnetic bead surface. A single batch of commercial magnetic beads (2.8 μm dia.) with a streptavidin coated surface was split into several aliquots. Three different biotinylated biological molecules, an oligonucleotide, an antibody (IgG), and an enzyme (alkaline phosphatase), were each added to one aliquot of the magnetic beads. Because identical beads from one batch were used, the bead size distribution, magnetic properties and surface coverage with streptavidin were identical. The same buffered solution was used for bead immobilization in the capillary and for electrophoresis. The same capillary, magnets, bead injection and electrophoresis conditions were used for all experiments. These steps ensured that any differences in behavior of the magnetic beads observed were due only to the different biotinylated biological molecule attached to the bead surface by avidin-biotin binding.

Magnetic bead plugs were formed and retained in a 50 μm id fused-silica capillary using two permanent magnets as shown in Fig. 1 [20]. The formation and retention of these magnetic bead plugs was studied qualitatively by light microscopy and quantitatively by light scattering detection of beads eluting from the immobilized bead plugs. The electrophoretic mobilities of the modified beads were also measured (Table 1). Images of

the bead plugs were taken obtained during electrophoresis at 88 V/cm immediately after formation of the magnetic bead plugs (Fig. 3), which was accomplished by electrophoretic injection of the beads at 351 V/cm followed by a reduction in the applied potential (88 V/cm) during which the beads migrated to the immobilization area. The plugs also were imaged as the applied potential was increased stepwise from 88 to 439 V/cm. Images were at the onset of a voltage increase and after the ongoing application of that potential for 8 min. The retention of the different bead types also was studied using light scattering detection of beads as they eluted from the bead plug. A light scattering detection system was used to detect bead loss and was similar to one previously reported for detection of individual nonmagnetic polystyrene beads as small as 110 nm in diameter [21]. The applied potential was varied as described above for imaging studies, with 88 V/cm increases in the potential followed by an 8 min equilibration time.

Imaging experiments showed that the three bead types produced significant differences in the formation of the initial bead plug at 88 V/cm as shown in Fig. 3. The initial bead plug of SA-Oligo beads (Fig. 3A) stood out due to the relatively small number of beads immobilized although the calculated total number of beads injected was similar for the four bead types (Table 1). The bead plugs showed reproducible lengths and shape between replicate runs of each bead type at the initial (88 V/cm) and lower voltages. As voltages were increased there was a greater variation in the size and shape of the bead plugs between replicate runs. It was observed that at the lowest applied voltage (88 V/cm) these beads were slow to reach the imaged portion of the capillary. Instead, they were sometimes immobilized upstream from the imaged area. A recent publication by Gassner et al. indicates similar immobilization behavior under static flow conditions, but this study used unlabeled magnetic beads and did not consider the effect of bead surface chemistry [12].

Bead loss profiles were generated by counting the number of peaks detected after the application of an 88 V/cm increase in applied potential and during the 8 min equilibration time (Fig. 4). Raw data for this experiment for SA-Oligo beads are shown in Fig. 2. No peaks were observed before beads were injected or had time to migrate to the detection window, indicating that only eluting beads produced peaks. These bead loss profiles were similar for SA-AP and SA-Oligo beads as the applied potential was increased stepwise. Both bead types showed the largest loss of beads after the potential was increased from 219 to 263 V/cm. Although SA-AP and SA-Oligo labeled beads showed similar bead loss profiles (Fig. 4), the imaging studies revealed that they do exhibit different retention characteristics. Bead plugs at the end of the experiment when the applied potential was 439 V/cm are shown in Fig. 5. The chain structures, which are clearly visible in Fig. 5, are often reported when similar magnetic beads are in the presence of a magnetic field [1, 2, 5, 16–17]. A comparison of Fig. 5A and 5C shows obvious differences in the bead plugs for SA-AP and SA-Oligo. Alkaline phosphatase coated beads were better retained than those coated with an oligonucleotide according to these images. The bead loss profiles and retention images for SA-IgG were markedly different from those for SA-Oligo and SA-AP (Figs. 4 and 5B). A large loss of SA-IgG beads occurred when the potential was increased from 175 to 219 V/cm, while smaller losses occurred at higher potential fields. It is also interesting and important to note that none of the three bead types with biological molecules attached were retained by the magnets as well as the unmodified streptavidin beads (SA-Bare) as shown in Fig. 4. This quantitative result for unmodified SA beads is supported by the qualitative imaging experiments (Supporting Information, Figs. S2 and S3). Images of beads plugs for SA-Bare at both low applied potentials and after a final potential of 263 V/cm was sustained for 8 min show relatively little bead loss compared to SA-Oligo, SA-AP or SA-IgG. Alone, neither microscopic imaging of bead plugs nor detection of the eluted beads by light scattering provided a complete view of these experiments, but combined these two methods

clearly showed that there are significant differences in plug formation and retention of magnetic beads labeled with different biological molecules.

These results clearly demonstrate that the nature of the biological molecules immobilized on the magnetic bead surface significantly influences the formation and retention of magnetic bead plugs during electrophoresis. Because the same batch of avidin-coated magnetic beads was labeled with different biotinylated biological molecules, the size and fundamental magnetic properties of the bead types studied were identical. The bead surface coverage with avidin was identical, and all immobilization of biological molecules was based on avidin-biotin binding. Any differences in bead surface coverage were due to steric differences between the biological molecules and are consistent with the nature of the immobilized biological molecule significantly impacting magnetic bead behavior. Identical capillary diameters, electrophoresis buffers, applied magnetic fields and applied electrophoretic potentials were used to ensure that observed differences were due solely to the biological molecules coated on the bead surface.

The experiments presented here do not clearly define the mechanism by which the immobilized biological molecules influence magnetic bead behavior. One obvious hypothesis to test is that the electrophoretic behavior of the beads determines the observed differences in their immobilization and retention in the magnetic field. The electrophoretic mobilities of SA-Bare, SA-Oligo, and SA-AP were determined and differed by less than 5% (Table 1), but the immobilization and retention behavior of these bead types was quite different as discussed above. This indicates that the experimental results presented in Figs. 3–5 cannot be explained based solely on electrophoretic mobility or zeta potential. Differences in the sizes of the magnetic beads with different biological molecules attached seems unlikely as all of the molecules attached to the bead surfaces are orders of magnitude smaller than the beads themselves. The molecular weights of the attached biological molecules are listed in Table 1, and these values are all similar with the exception of the oligonucleotide. Another plausible explanation for these observations is that self association of the molecules on the bead surface greatly impacts the behavior of the magnetic beads in a magnetic field during electrophoresis.[16, 17, 22] The experiments presented here were designed to show that different classes of biological molecules (oligonucleotides, antibodies and enzymes) on the bead surface influenced their immobilization during electrophoresis. A study targeting this self-association hypothesis in detail could provide a better mechanistic explanation for the results presented here.

3.1 Effect of Immobilization Chemistry

We also carried out experiments designed to explore how the chemistry used to attach a biological molecule to the magnetic bead surface impacts bead plug formation and retention. Beads with streptavidin or epoxy surfaces were labeled with alkaline phosphatase, through avidin-biotin binding or covalent bonding [9], respectively. Images of the initial bead plugs (Fig. 3C and 3D) showed clear differences between the two types of AP labeled beads. The SA-AP beads had a shorter plug length than the E-AP beads. Comparison of alkaline phosphatase labeled bead images in Fig. 5C and 5D showed that the final plugs were similar in size and appearance; however, the imaging studies only presented a small length of the capillary. Further inspection of the bead plugs revealed that the E-AP beads extended downstream in the capillary beyond the field of view in Fig. 5. The SA-AP beads were more tightly packed, and no beads were found immobilized outside the imaged area. Light scattering detection showed interesting differences in bead retention. The E-AP beads showed smaller bead losses compared to the SA-AP beads as presented in Fig. 6. After the application of 351 V/cm, the E-AP beads continued a minimal bead loss with each increase, while the SA-AP beads underwent a larger loss compared to those at lower applied potentials.

4 Concluding Remarks

These results show clearly that the type of biological molecule attached to the surface of an otherwise identical magnetic bead significantly impacts the behavior of magnetic beads during CE. Clearly the surface chemistry of magnetic beads is an important factor affecting their behavior under electrophoretic conditions in capillaries and microfluidic channels in addition to the beads' magnetic properties. Measurement and comparison of bead electrophoretic mobilities indicate that the zeta potential is not the dominant factor causing these observed differences. These results demonstrate that magnetic bead surface chemistry and the biological molecules attached to the bead surface must be considered during experimental design, when magnetic beads are immobilized in microfluidic devices. A researcher cannot safely assume that a method which works well with magnetic beads coated with one biological molecule will perform equally well when the bead surface chemistry is changed significantly. Until the mechanism of these effects is better defined, it can be assumed that immobilization protocols should be tested if the bead surface chemistry is altered. Future work will focus on more detailed experiments aimed at understanding the mechanism of the effects demonstrated in this paper. This will lead to improved models for magnetic bead immobilization and retention that take into account and examine the impact of the surface chemistry in addition to the magnetic properties of these beads. In summary, use of two experimental techniques to study the capture and retention of magnetic beads labeled with different biological molecules showed that the surface chemistry of the beads is critical for bead immobilization.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations used

AP	alkaline phosphatase
SA	streptavidin
E	epoxy
Oligo	oligonucleotide

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Fig. 1.

Diagram of magnet and capillary placement. Two NdFeB magnets with a surface field strength of 6403 Gauss were abutted to the capillary ($360\ \mu\text{m}$ od) at a 20° angle. This arrangement is based on the work of Slovakova et al. [20]. The dotted circle indicates the approximate field of view of the microscope. The microscope was located above (perpendicular to) the plane represented in the diagram.

Fig. 2.

Electropherogram of streptavidin-coated magnetic beads with a biotinylated oligonucleotide bound to them (SA-Oligo). The neutral marker, coumarin, and beads were injected electrokinetically for 3.0 s and 10.0 s at 20.0 kV (351 V/cm), respectively. The voltage was then immediately reduced to 5.0 kV (88 V/cm) for transport to the magnetic immobilization area over 18 min. The applied potential was increased in steps, as indicated by the labeled, step-shaped plot above (electrophoretic current). The neutral marker elutes at 8.5 min and was detected by fluorescence. The beads eluted after increases in applied potential and were detected downstream of the magnetic immobilization area by light scattering.

Fig. 3.

Images of initial bead plugs formed by a 10.0 s, 351 V/cm injection of 2.8 μm diameter beads, transported by 88 V/cm through a 50 μm id capillary and held in place by two NdFeB magnets as indicated in Fig. 1. The images were collected during electrophoresis at 88 V/cm. SA-Streptavidin, E-Epoxy, Oligo-Oligonucleotide, IgG-antibody, AP-Alkaline phosphatase.

Fig. 4. Magnetic bead elution with increasing electrophoretic potential for identical beads with different biological molecules immobilized on their surface. Beads were injected for 10.0 s at 351 V/cm and transported to the magnetic capture area by an applied potential of 88 V/cm. The applied potential was increased in steps of 88 V/cm (5.0 kV). Error bars are the standard deviation of $n=3$ experiments. Bead type abbreviations are defined in Fig. 3.

Fig. 5. Final bead plugs at 8.0 min during application of 439 V/cm. Bead type abbreviations are defined in Fig. 3.

Fig. 6. Comparison of the effect of immobilization chemistry on bead plug retention. Beads have alkaline phosphatase immobilized to either streptavidin (SA-AP) or epoxy (E-AP) surfaces. Beads were injected for 10.0 s at 351 V/cm and transported to the magnetic capture area by an applied potential of 88 V/cm. The applied potential was increased in steps of 88 V/cm (5.0 kV). Error bars are the standard deviation of $n=3$ experiments.

Table 1

Electrophoretic mobility (μ_e), calculated number of beads injected, and immobilized biomolecule molecular weight for different magnetic bead types

Bead Type	$\mu_e \pm SD$ (n=5) ($\times 10^{-4} \text{ cm}^2 \text{V}^{-1} \text{s}^{-1}$)	Beads Injected	M.W. (Da) of Biomolecule
SA-Bare	-2.2 ± 0.2	3.8×10^2	N/A
SA-IgG	-1.1 ± 0.3	4.7×10^2	~150,000
SA-Oligo	-2.33 ± 0.02	3.6×10^2	7728
SA-AP	-2.18 ± 0.06	3.1×10^2	~140,000
E-AP	-1.9 ± 0.2	2.8×10^2	~140,000