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Short communication

Translocation of bio-functionalized magnetic beads using smart magnetophoresis

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ABSTRACT

We demonstrate real time on-chip translocation of bio-functionalized superparamagnetic beads on a silicon surface in a solution using a magnetophoresis technique. The superparamagnetic beads act as biomolecule carriers. Fluorescent-labeled Atto-520 biotin was loaded to streptavidin-coated magnetic beads (Dynabead® M-280) by means of ligand–receptor interactions. The magnetic pathways were patterned lithographically such that semi-elliptical $Ni_{80}Fe_{20}$ elements were arranged sequentially for a few hundred micrometers in length. An external rotating magnetic field was used to drive translational forces on the magnetic beads that were proportional to the product of the field strength and its gradient. The translational force at the curving edge of the pathway element of 6 μm diameter was calculated to be ~1.2 pN for an applied field of 7.9 kA m^{-1}. However, the force at the flat edge was calculated to be ~0.16 pN. The translational force was larger than the drag force and thus allowed the magnetic beads to move in a directional way along the curving edge of the pathway. However, the force was not sufficient to move the beads along the flat edge. The top and bottom curving edge semi-elliptical NiFe pathways were obliquely-arranged on the left and right sides of the converging site, respectively. This caused a central translational force that allowed the converging and diverging of the Atto-520 biotin loaded streptavidin magnetic beads at a particular site.

1. Introduction

Controlling specific biomolecules on silicon surfaces in solution is a prerequisite in nano-biotechnology for biomolecular transportation, separation and sensing applications. Since there is no significant magnetic background signal present in most biological samples of interest, magnetic manipulation without affecting biological interactions is a promising technique for both *in vitro* and *in vivo* applications (de Boer et al., 2007; Gijs, 2004; Jansen et al., 2008).

Recently, very highly sensitive magnetoresistance sensors have been developed for the detection of biomolecules by magnetically detecting functionalized superparamagnetic beads specifically positioned near the sensor's surface through ligand–receptor interactions. Also, superparamagnetic beads play an important role in carrying the biomolecules. Applications include enhancing the active hybridization of analyte biomolecules to specific probe molecules on a sensor's surface, and removing non-specifically bound molecules from a sensing area using magnetophoresis devices (Graham et al., 2005; Lagae et al., 2005; Tamanaha et al., 2008; Hall et al., 2010).

Most magnetophoresis devices used for the manipulation and transportation of functionalized superparamagnetic beads consist of on-chip current-carrying wires and coils with millimeter-range dimensions (Deng et al., 2001; Wirix-Speetjens et al., 2005; Ramadan et al., 2006). In general, these devices generate small magnetic fields (≤30 mT) that do not allow the translocation of beads over few tens of micrometer distance regions. In addition, they generate heat on the chip that could damage biological entities.

In contrast, on-chip soft magnetic microstructures provide a convenient scalable method for trapping and transporting superparamagnetic beads. A soft magnetic microstructure has the potential of being more effective than devices containing on-chip current-carrying wires and coils. Microsystems fabricated using soft magnetic microstructures generate no on-chip heat, which is essential to the manipulation process for biological entities. Thus, several groups have demonstrated magnetic bead manipulation systems using soft magnetic structures (Gunnerson et al., 2005; Smistrup et al., 2006; Anandakumar et al., 2009). However, they have been unable to precisely control the magnetic beads in the forward and backward directions, which is crucial in the selective hybridization of biomolecules. Hence, there is a need for new microstructures that can produce directional control of magnetic beads.

We present a new microsystem using lithographically-patterned soft magnetic semi-elliptical NiFe pathways for the directional control of magnetic beads that can carry certain chem-

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ical or biological entities toward a particular sensing site using translational forces on superparamagnetic beads.

2. Materials and methods

2.1. Superparamagnetic beads

We used commercially available Dynabead® M-280 superparamagnetic beads with a diameter of 2.8 μm. The bead surfaces were functionalized with covalently-coupled streptavidin. The beads consisted of superparamagnetic (γFe_2O_3 and Fe_3O_4) particles (6–12 nm in diameter) embedded into a polymer matrix. The low-field magnetic volume susceptibility of the beads, $\chi_{bead} = 0.39$, was estimated by measuring the stray field strength affecting a planar Hall resistance sensor (Thanh et al., 2007). The magnetic beads had a hydrophobic surface area of 4.8×10^3 m^2/kg, and the density of these beads is 1.4×10^3 kg/m^3. Approximately 10 mg of dynabead solution contains 6–7 $\times 10^8$ beads/mL. 1 mg of pre-coated streptavidin magnetic beads has sufficient affinity to bind up to 650–900 pM of free biotin (http://www.dynalbiotech.com).

2.2. Fluorescent-labeled biotin

Atto-520 fluorescent-labeled biotin was loaded onto the magnetic beads with the streptavidin via specific ligand–receptor interactions. The net formula of Atto-520 biotin is $C_{37}H_{53}ClN_6O_8S$ with a molecular weight of 777.37. Atto-520 biotin has a high molecular absorption of 110.000, a quantum yield of 0.90, a sufficient Stokes shift excitation maximum at a wavelength (λ_{ex}) of 520 nm, and maximum emission (λ_{em}) at 542 nm (http://www.sigmaaldrich.com).

2.3. Conjugation of Atto-520 biotin with streptavidin magnetic beads

To load the fluorescent-labeled Atto-520 biotin with streptavidin magnetic beads, we placed 5 μL of streptavidin-coated magnetic bead solution in a clean eppondorf tube. The bead solution was washed three times with phosphate-buffered saline (PBS) at a pH of 7.4 to remove the preservatives. The supernatant of the solution was removed using a micropipette by collecting the magnetic beads at the bottom of the eppondorf tube using a permanent magnet and re-suspending them in 90 μL of 0.1 M PBS buffer.

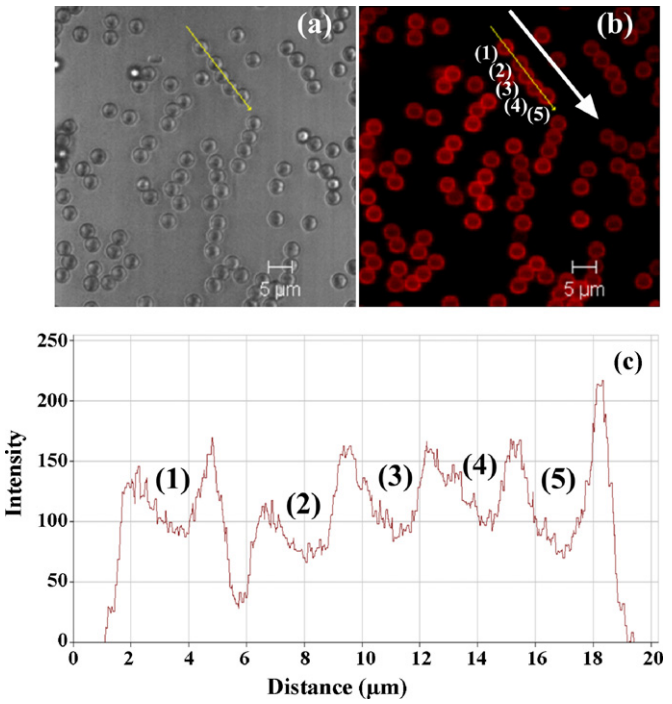

Fig. 1. (a) Optical image of Atto-520 biotin–streptavidin magnetic beads. (b) Confocal image of the magnetic beads exhibiting fluorescent signal. (c) Measured fluorescence intensity of a group of five Atto-520 biotin–streptavidin magnetic beads.

1 mg of Atto-520 biotin was diluted in 200 μL of ethanol. The fluorescent concentration of the Atto-520 biotin was calculated to be 6.4 mM. Then, 5 μL of diluted Atto-520 was mixed with the 90 μL of magnetic bead solution and the solution was continuously stirred for 2 h at room temperature for completion of the streptavidin–biotin conjugation. Finally, the solution was further washed with PBS buffer several times to remove the biotin surplus by means of magnetic separation (Holmberg et al., 2005).

The loading of the fluorescence-labeled biotin on the streptavidin magnetic beads was confirmed using a confocal microscope.

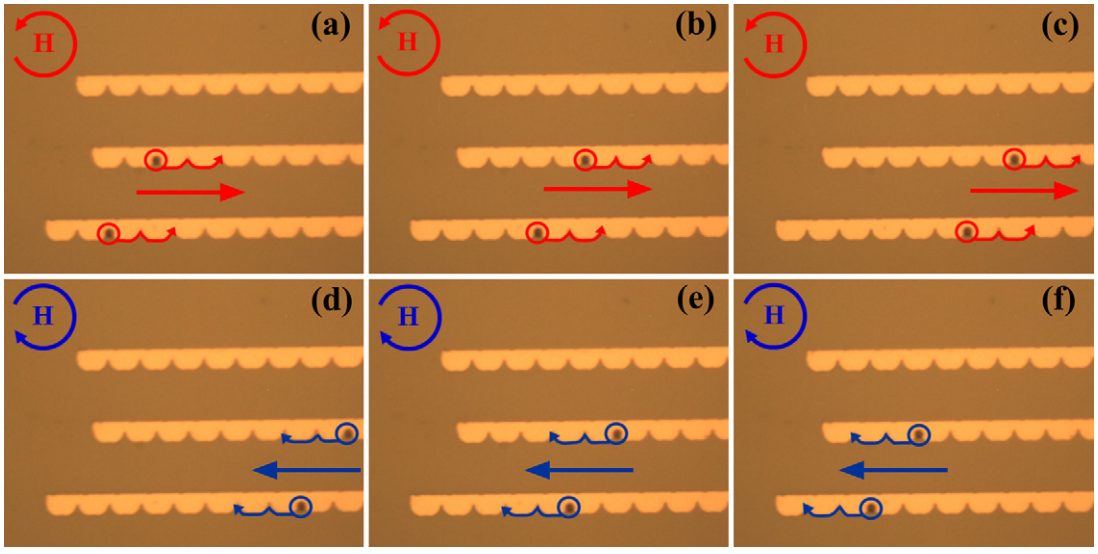

Fig. 2. (a)–(c) Optical images of magnetic beads forward motion on bottom curving edge semi-elliptical NiFe pathways with respect to counter clockwise rotating magnetic field. (d)–(f) Optical images of magnetic beads backward motion on bottom curving edge semi-elliptical NiFe pathways with respect to clockwise rotating magnetic field.