Supporting Information

Magnetic Nanoclusters for Ultrasensitive Magnetophoretic Assay

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Materials

NHS-PEO_4-Biotin, sulfosuccinimidyl 4-*N*-maleimidomethyl cyclohexane-1-carboxylate (Sulfo-SMCC), NeutrAvidin, and dextran desalting column were obtained from Pierce Chemical (Rockford, IL). NHS-PEO_4-Biotin is a water-soluble and primary amine reactive biotinylation reagent with a 2.9 nm hydrophilic polyethylene oxide spacer arm. Bovine serum albumin (BSA, IgG free), D-biotin, Tween-20, and sodium azide were purchased from Sigma-Aldrich (St Louis, MO). Mouse monoclonal anti-human prostate specific antigen (PSA), goat polyclonal anti-human PSA, and purified human PSA were from Fitzgerald Industries International (Concord, MA). The 9 nm superparamagnetic iron oxide nanoparticles were synthesized according to the previously reported method.[S1] Carboxylate-modified red fluorescent microbeads (FluoSpheres, excitation 580 nm / emission 605 nm) were obtained from Molecular Probes (Eugene, OR). All other reagents used were of analytical grade.

Biotinylation of BSA

To use a biotinylated BSA as a multi-armed linker for formation of magnetic nanoclusters (MNCs), BSA was modified with biotin. Briefly, 125 μL of BSA (20 mg mL^{-1}) in 100 mM sodium phosphate buffer (pH 7.4) was mixed with an equal volume of fresh NHS-PEO_4-Biotin (2 mg mL^{-1}) prepared in the same buffer. The reaction mixture was incubated for 30 min at RT with mild shaking, and loaded onto a 5 mL dextran desalting column to remove free linkers. The eluted solution containing biotinylated BSA was further filtered through a Microcon YM-30 microfilter with the molecular weight cut-off of 30 kD (Millipore, Billerica, MA) five times to ensure complete removal of biotin linkers. Using a biotin binding assay (HABA; Sigma-Aldrich), about six biotin molecules were revealed to be conjugated to each BSA.

Conjugation of monoclonal anti-human PSA onto microbeads

Mouse monoclonal anti-human PSA was conjugated onto carboxylated red microspheres. A general conjugation chemistry comprising 1-ethyl-3-(3-dimethylaminopropyl)cabodiimide (EDC) plus Sulfo-*N*-hydroxysuccinimide (NHS) conjugation strategy was employed. Briefly, 20 μL of red fluorescent microbeads (5.46×10^8 microbeads) were activated with 20 mM EDC and 60 mM Sulfo-NHS in 170 μL of 100 mM MES (2-(*N*-morpholino)ethanesulfonic acid) buffer (pH 6.0) for 30 min at RT. The reaction mixture was added β-mercaptoethanol stock solution at a final concentration of 400 mM, and incubated for 5 min at RT to quench free EDC. The resulting microbeads were collected by centrifugation at 13,000 g for 10 min at 4 ℃, and the supernatant was carefully discarded. Following washing with 50 mM sodium phosphate buffer (pH 7.4) three times, the recovered microbeads were resuspended in 60 μL of 50 mM sodium phosphate buffer (pH 7.4), containing 6.7 mg mL^{-1} of mouse monoclonal anti-human PSA and 0.6 mg mL^{-1} of BSA. After sonication for 20 s, microbeads were incubated at RT for 4 h in an automated mixer (Intelli Mixer, Korea) operating in shaking mode at 80 rpm followed by incubation overnight at

4℃ at the same mixing condition. Collected microbeads were washed five times with 500 μL of PBS (phosphate buffered saline)-TBN buffer (1× PBS, 0.02% Tween 20, 1% BSA, and 0.05% sodium azide (NaN_3), pH 7.4) and stored in the same buffer at 4℃ until further use.

Synthesis of dually-modified magnetic nanoparticles (DMNPs)

For synthesis of DMNPs, 9 nm dimercaptosuccinic acid-coated magnetic nanoparticles were conjugated with neutravidin and goat polyclonal anti-PSA. Briefly, 394 μg of neutravidin and 200 μg of goat polyclonal anti-human PSA were added to buffer solution (10 mM sodium phosphate buffer, pH 7.2), and concentrated to 200 μL using a microfilter with the molecular weight cut-off of 30 kD. Then, 16 μL of freshly prepared sulfo-succinimidyl 4-(*N*-maleimidomethyl) cyclohexane-1-carboxylate (sulfo-SMCC) linker solution (2 mg mL^{-1}, 10 mM sodium phosphate buffer, pH 7.4) was added to the protein solution, and allowed to react for 30 min at RT. After activation by sulfo-SMCC linker, free linkers were removed using dextran desalting column. The eluted solution was concentrated to 300 μL with a membrane filter, and then directly mixed with 280 μL of magnetic nanoparticle solution (1.8 mg mL^{-1}) under constant stirring with a magnetic stirrer, and incubated overnight at 4 ℃ under a mild stirring condition. To block unreacted maleimide groups on proteins, 22 μL of β-mercaptoethanol (27 mM) was directly added to the reaction solution for 15 min at RT. Finally, the reaction solution was passed through superdex G-200 column to remove excess proteins. DMNPs were stored in 10 mM sodium phosphate buffer (pH 7.4) at 4 ℃ until further use. Three antibodies and 15 neutravidin molecules were estimated to be immobilized on a DMNP, when the amount of protein conjugated DMNP was measured by Bradford assay method at 595nm.

Fabrication of a microfluidic device

The microfluidic device to measure the deflection velocity of a microbead consists of a microchannel and a nickel microstructure. The microchannel was fabricated by using conventional poly(dimethylsiloxane) (PDMS) (Sylgard 184; Dow Corning, Midland, MI) molding process, and a Ni microstructure was electroplated on a Pyrex glass wafer. For the Ni microstructure, chrome (500 Å) / gold (3000 Å) seed layer was deposited successively on the glass wafer by sputtering. THB-151N negative photoresist (JSR, Tokyo, Japan) was used to fabricate the mold for Ni electroplating. Following exposure to UV light and development in a THB-D2 developer, Ni was electroplated on the gold pattern. After the electroplating process, a chemical mechanical polishing (CMP) step was followed to make the height of Ni microstructure to be about 40 μm. Then, PR was stripped by a JSR THB-S1 stripper for about 2 h at 60 ℃. The glass wafer around the Ni microstructure was coated with a 40 μm-thick PDMS film fabricated by a thermal-compression method. The height of the resulting PDMS film was as same as that of Ni microstructure. To fabricate the microchannel, negative photoresist SU-8 2050 (MicroChem Corp, MA) was used for PDMS molding. Following the patterning of SU-8 PR, the prepared mixture of PDMS was poured onto the mold and cured for 2 h at 100 ℃ on the hot plate. The resulting microchannel had a width of 100 μm and a height of 80 μm. The peeled-off PDMS microchannel was bonded on the PDMS film-coated Ni microstructure with air plasma treatment.

Instrumentation

The microfluidic device was mounted on an inverted microscope (Nikon, Tokyo, Japan) accompanied with a 50 W mercury lamp as a light source. A charge-coupled device (CCD) camera (Nikon, Tokyo,

Japan) integrated on an inverted microscope was used to capture the images of moving microbeads. The microfluidic device was designed to have two inlets and one outlet. Tube was inserted into the holes to connect the 10 μL and 50 μL microsyringes (1700 series gastight syringes; Hamilton Company, Reno, NV). The microsyringes were connected with two tubes separately inserted in two inlets to pump aqueous medium using a dual syringe pump (Pump 11 Pico Plus; Harvard Apparatus, Boston, MA) into the microfluidic channel. The reaction mixture containing microbeads was injected through one of the inlets, and buffer solution (1x PBS containing 0.5 M NaCl, 0.02% Tween 20, pH 7.4) was flowed into another inlet. By using two inlet channels, microbeads were hydrodynamically focused on the left side of microchannel. NdFeB 35 permanent (50 × 25 × 10 mm^3 and Br = 12,000 gauss (Magtopia, Seoul, Korea)) was located 2 mm apart from the microchannel to provide a magnetic field. The movement of microbead was recorded using a CCD camera, and the captured movement file was separated into series of image frames with commercial measurement program (i-Solution, IMT i-Solution Inc., Korea). The microbead position in the image was used to determine the deflection velocity of a microbead in the microfluidic channel.

Optimization of MNCs for magnetophoretic assay

To optimize the formation of MNCs for application to magnetophoretic assay, different molar ratios of DMNPs to biotinylated BSA were tested in the MNCs formation step. Typically, for preparation of MNCs (1:100), 560 μL of 10 mM sodium phosphate buffer (pH 7.4) was added to 100 μL of 410 nM DMNPs, and well mixed followed by addition of 190 μL of 22 μM biotinylated BSA. The resulting solution was incubated for 30 min with mild shaking at 10 min interval. For blocking of further growth of MNCs, 150 μL of 1 mM D-biotin solution was added to mask free biotin binding sites. The MNCs solution was stored at 4 ℃. To change the ratio, the concentration of DMNP was fixed at a final concentration of 41 nM, and the amount of biotinylated BSA varied for construction of MNCs at the ratios of 1:1, 1:12, 1:25, 1:50, and 1:100. After blocking by addition of excess D-biotin for 2 h, the resulting MNCs were used for magnetophoretic assays. The concentration of PSA was fixed at 10 pg mL^{-1}, and the average deflection velocities of microbeads in a microfluidic channel were determined according to the method described below.

Reference

[S1] Y. W. Jun, Y. M. Huh, J. S. Choi, J. H. Lee, H. T. Song, S. Kim, S. Yoon, K. S. Kim, J. S. Shin, J. S. Suh, J. Cheon, *J Am Chem Soc.* **2005**, 127 (16), 5732.