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Magnetophoretic Immunoassay of Allergen-Specific IgE in an Enhanced Magnetic Field Gradient

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We demonstrate a novel magnetophoretic immunoassay of allergen-specific immunoglobulin E (IgE) based on the magnetophoretic deflection velocity of a microbead that is proportional to the associated magnetic nanoparticles under enhanced magnetic field gradient in a microchannel. In this detection scheme, two types of house dust mites, *Dermatophagoides farinae* (*D. farinae*) and *Dermatophagoides pteronyssinus* (*D. pteronyssinus*), were used as the model allergens. Polystyrene microbeads were conjugated with each of the mite extracts followed by incubation with serum samples. The resulting mixture was then reacted with magnetic nanoparticle-conjugated anti-human IgE for detection of allergen-specific IgE by using sandwich immuno-reactions. A ferromagnetic microstructure combined with a permanent magnet was employed to increase the magnetic field gradient (~10^4 T/m) in a microfluidic device. The magnetophoretic velocities of microbeads were measured in a microchannel under applied magnetic field, and the averaged velocity was well correlated with the concentration of allergen-specific IgE in serum. From the analysis of pooled sera obtained from 44 patients, the detection limits of the allergen-specific human IgEs for *D. farinae* and *D. pteronyssinus* were determined to be 565 (0.045 IU/mL) and 268 fM (0.021 IU/mL), respectively. These values are 1 order of magnitude lower than those by a conventional CAP system. For evaluation of reproducibility and accuracy, unknown sera were subjected to a blind test by using the developed assay system, and they were compared with the CAP system. As a result, coefficient of variance was less than 10%, and the developed method enabled a fast assay with a tiny amount of serum (~10 μL).

Immunoglobulin E (IgE)-mediated type I allergies are one of the most common diseases in the populations of industrialized countries. In the last few decades, the prevalence of allergy has increased dramatically and resulted in the high cost for diagnosis and treatment, representing a major burden for the health care systems.[1−3] For diagnosis of allergy, a number of in vivo and in vitro methods have been developed. Since the IgE level in serum is very low (less than 1/40000 IgG level), a highly sensitive method is prerequisite for accurate diagnosis of allergy.[4,5] Typically, conventional in vivo testing method, including skin prick test with allergens, is known to be sensitive and reliable. However, this method has a potential risk of causing adverse reactions, such as systemic reactions and anaphylactic shocks.[6] In an effort to overcome the drawbacks of in vivo test, in vitro serological analysis of allergen-specific IgE has been developed and employed along with the in vivo provocation test for allergy diagnosis, especially when standardized allergen extracts are not available.[7,8]

Since the radioallergosorbent test (RAST)[9] was reported in 1967, the original radioisotope assay in RAST has been replaced with chromogenic enzyme immunoassay or fluorescent enzyme immunoassay.[10] Currently, a commercialized CAP system is most widely used for analysis of total IgEs and allergen-specific IgEs.

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Despite the availability of numerous in vitro methods for diagnosis of allergies, most of the assays are expensive and time-consuming, requiring large amounts of reagent and serum.[11]

Recently, the use of magnetic microparticles has gained much attention in a micro total analysis system (μTAS).[12−15] Due to their easy separation and conjugation with biomolecules,[16] magnetic microparticles have been widely used to develop various detection methods in microfluidic devices as a solid support for reactions.[17−20] Meanwhile, magnetic nanoparticles were employed as a label in sandwich immunoassay of an analyte.[21−24] In this case, the amount of magnetic nanoparticles that are associated with a target analyte is proportional to the analyte concentration, and the signal generated from the magnetic nanoparticles can be used for quantitative analysis of a target analyte. For example, superconducting quantum interference device (SQUID) and giant magnetoresistive (GMR) sensor have been attempted for immunoassays.[24−26] The SQUID and GMR sensor generally allow a detection of analyte with high sensitivity, but these systems have some drawbacks, such as tedious washing steps, long analysis time, and cost of instrument. In addition, these methods have a serious limitation in multiplexed analysis and improvement of detection limits.

We previously developed a new detection system based on the magnetophoretic mobility of a microbead which is directly proportional to the amount of associated superparamagnetic nanoparticles under applied magnetic field gradient.[27] By measuring the magnetophoretic deflection velocity of microbeads in a microchannel, the multiplexed analysis of analytes (rabbit IgG and mouse IgG) were carried out. In this system, the magnetophoretic deflection velocity was also affected by the magnetic field gradient, and the detection limit could be lowered up to the femtomolar range by introducing the ferromagnetic material like Ni microstructure.[28−31] In this paper, we demonstrate a magnetophoretic immunoassay of allergen-specific IgE in serum. Two types of mite allergens, *Dermatophagoides farinae* (*D. farinae*) and *Dermatophagoides pteronyssinus* (*D. pteronyssinus*), were employed as the model system. The magnetophoretic deflection velocity of a microbead was measured and correlated with the concentration of allergen-specific IgE in serum (Scheme 1). Sera from 44 patients were pooled, analyzed by our system, and the results were compared with those from a conventional test kit (CAP system) in terms of detection limit, reproducibility, and accuracy. Details are reported herein.

EXPERIMENTAL SECTION

Materials. Bovine serum albumin (BSA), sodium azide, and Tween 20 were purchased from Sigma-Aldrich (St. Louis, MO). All solutions were prepared using deionized water. Carboxylated microbeads with 5.6 μm diameter were purchased from Luminex Corporation (Austin, TX). The superparamagnetic Fe_3O_4 nanoparticles with a diameter of 9 nm were synthesized according to the previously reported method.[32] Goat anti-human IgE and purified human IgE were from CHEMICON international, Inc (Temecula, CA). Sera from allergy patients were from Ajou University Hospital (Suwon, Korea), and two lyophilized mite allergens were purchased from Allergopharma (Hamburg, Germany).

Fabrication of a Microfluidic Device. The microfluidic device with a microchannel was fabricated by using a conventional poly(dimethylsiloxane) (PDMS) (Sylgard 184; Dow Corning, Midland, MI) molding process, and a Ni microstructure was electroplated on a Pyrex glass wafer. A multi-exposure method was employed for construction of the microfluidic device mold with different heights. The negative photoresist (PR) SU-8 (Microlithography Chemical Co., MA) was used for molding. After double ultra-violet (UV) exposures, SU-8 was developed by using a SU-8 developer. This SU-8 mold was employed for the microchannel and Ni microstructure. Following the patterning, the prepared mixture of PDMS was poured onto the mold and cured for 2 h at 100 °C on a hot plate. For the Ni microstructure, chrome/gold seed layer was deposited successively on the glass wafer by sputtering. THB-151N negative PR (JSR Corp., 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. The Ni microstructure was about 50 μm in height. After Ni electroplating, PR was stripped by JSR THB-S1 stripper for about 2 h at 60 °C. The PDMS microfluidic device and the Ni microstructure on the glass wafer were aligned and bonded. The dimensions of the microchannel and Ni microstructure are shown in Figure 1.

Conjugation of Mite Lysate to Microbeads. For sandwich immunoassay of the allergen-specific human IgE, the crude extracts of respective mite allergen were conjugated onto carboxylated microbeads. A general conjugation chemistry comprising EDC plus Sulfo-NHS conjugation strategy was employed with slight modifications of a protocol described in elsewhere.[33] Briefly, carboxyl terminated microbeads (6.25×10^5 beads) were activated

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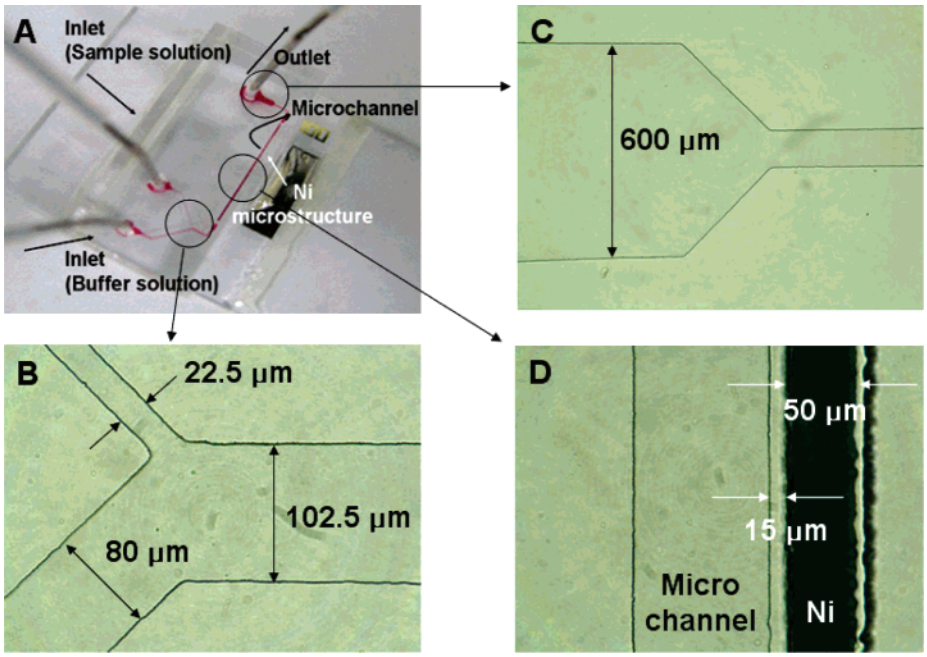
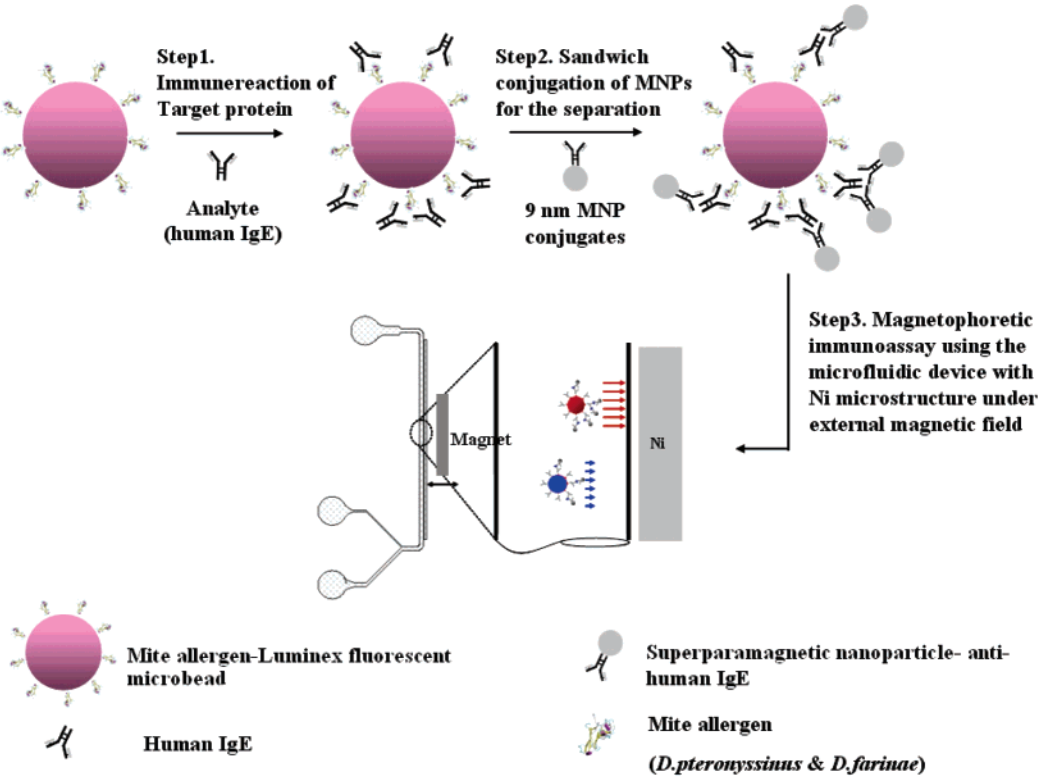


Figure 1. (A) Photograph of the microfluidic device with a Ni microstructure and optical microscope images of the microchannels; (B) inlet part, (C) outlet part (expansion part), and (D) microchannel that is 15 μm apart from the Ni microstructure.

Scheme 1. Schematic Diagram of the Magnetophoretic Immunoassay Procedure for Mite Allergen-Specific Human IgE[a]



[a] MNP: magnetic nanoparticle.

with 20 mM EDC (1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride), and 60 mM sulfo-NHS (*N*-hydroxysulfosuccinimide) in 200 μL of 100 mM sodium phosphate buffer (pH 6.2) for 30 min at room temperature. β-Mercaptoethanol stock solution was added at a final concentration of 200 mM in a reaction mixture to quench free EDC. The resulting microbeads were washed with 500 μL of 100 mM MES (2-(*N*-morpholino)ethanesulfonic acid) buffer (pH 6.0) three times, and then they were collected by centrifugation at 10 000*g* for 1 min at 4 °C. Activated microbeads were incubated with 400 μL of 1.5 mg/mL of clear mite lysate for 3 h at room temperature under gentle mixing followed by centrifugation at 10 000*g* for 1 min at 4 °C. Collected microbeads were incubated with 500 mL 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) under mild stirring at room temperature for 1 h, washed three times with 500 μL of PBS−TBN buffer, and stored in the same buffer. Microbeads were counted by using traditional hematocytometry method under inverted microscope (Nikon, Japan).

Conjugation of Superparamagnetic Nanoparticles with Goat Anti-Human IgE. A mixture of 200 μg of goat anti-human IgE and 440 μg of BSA was reacted with 100 μM of sulfo-SMCC (sulfosuccinimidyl 4-(*N*-maleimidomethyl)cyclohexane-1-carboxylate) cross-linker in 110 μL of 10 mM sodium phosphate buffer (pH 7.2) for 30 min at room temperature. Free linkers were removed by using 5 mL of desalting column presaturated with 10 mM sodium phosphate buffer (pH 7.2). Activated proteins were incubated with 510 μg of 9 nm superparamagnetic nanoparticles in 660 μL of buffer solution under mild stirring at 4 °C for overnight. After blocking the free sulfo-SMCC with 1 mM β-mercaptoethanol for 15 min at room temperature by adding concentrated β-mercaptoethanol stock solution, superparamagnetic nanoparticles-conjugated proteins were collected using superdex-200 column. The resulting conjugates were stored in 10 mM sodium phosphate buffer (pH 7.4) at 4 °C. To estimate the conjugation ratio between proteins (goat anti-human IgE/BSA) and magnetic nanoparticles, the concentrations of proteins and magnetic nanoparticles were determined by using the Bradford assay and the visible spectrophotometric method, respectively. The extinction coefficient of magnetic nanoparticles used was 8.77 L/(g cm) at 450 nm. As a result, about 3 goat anti-human IgE molecules and 15 BSA molecules were observed to be conjugated onto a single magnetic nanoparticle. The number of magnetic nanoparticles in solution was estimated to be 2.6×10^{11} particles/μL based on the assumption that magnetic nanoparticles are spherical.

Pooling of Serum Samples. Sera from 44 patients (age: 2∼62) were obtained from the Ajou University Hospital (Suwon, Korea) and used for assays. It was reported that sera from patients with different allergies are composed of different IgE reactivity profiles to house mite allergens.[34,35] In order to minimize the effect of allergen heterogeneity in IgE reactivity profiles on the standard calibration curve and the sample-to-sample variation in their protein contents, four or five sera from patients were pooled with same volume for *D. farinae* and *D. pteronyssinus*, respectively. Mite allergen-specific IgE level in pooled serum was determined by using a commercial diagnostic kit (CAP system; Pharmacia Diagnostics, Uppsala, Sweden) (Table 1).

Instrumentation. The microfluidic device was mounted on an inverted microscope (Nikon, Japan) with a 50 W mercury lamp as light source. A CCD camera (Nikon, Japan) was integrated on the inverted microscope to capture the images of microbead movement. The microfluidic device was composed of two inlets and one outlet. The tubing was inserted into the holes to connect the 10 μL and 50 μL microsyringes (1700 series gastight syringes; Hamilton Company, NV). The microsyringes were connected with another side of tubing to pump the aqueous medium by a dual syringe pump (Pump 11 Pico Plus; Harvard Apparatus, Inc., MA). The reacted sample solution was injected through one of the inlets, and the PBS−TBN (pH.7.4) buffer solution was injected through another inlet. Consequently, magnetic nanoparticle-associated microbeads were hydrodynamically focused to the left side of the microchannel. Magnetic field was applied by using NdFeB 35 permanent magnet (Magtopia, Korea) with a dimension of $50 \times 25 \times 10$ mm^3 and Br = 12 000 G. The permanent magnet was placed 2 mm apart from the microchannel. The movement of microbeads was traced with a CCD camera, and the magnetophoretic deflection velocity of a microbead was determined by using a commercial measurement program, i-Solution (IMT; Image and Microscope Technology, Korea).

Table 1. List of Pooled Sera Used in the Experiments and Classification

pool	IgE (pM)	IgE (IU/mL)	no. of included serum	class[a] of CAP system
D. farinae 1	6.11	0.48	5	1
D. farinae 2	27.4	2.17	5	2
D. farinae 3	105.8	8.36	4	3
D. farinae 4	551.6	43.7	4	4
D. farinae 5	1,025	81.1	5	5
D. pteronyssinus 1	7.95	0.63	5	1
D. pteronyssinus 2	22.6	1.79	5	2
D. pteronyssinus 3	56.8	4.50	4	3
D. pteronyssinus 4	281	22.3	4	4
D. pteronyssinus 5	956	75.7	5	5

[a] 1 IU/mL equals 12.6 pM of IgE.

Procedure of Magnetophoretic Immunoassay. Pooled serum samples were serially diluted 2-fold with a reaction buffer, and 10 μL of the sample solution was added to a reaction mixture containing allergen-conjugated microbeads in 70 μL of the reaction buffer. The numbers of microbeads for magnetophoretic immunoassay were adjusted to 5649 for *D. farinae* and 5130 for *D. pteronyssinus* in 70 μL of reaction buffer (1 × PBS, 1 M NaCl, 0.02% Tween 20, pH 7.4), respectively. The resulting solution was incubated for 10 min at 4 °C under mild mixing, and microbeads were separated by centrifugation at 13 000*g* for 5 min at 4 °C. The collected microbeads were resuspended in PBS−TBN followed by addition of 5 μL of the solution containing anti-human IgE-conjugated magnetic nanoparticles. The number of magnetic nanoparticles in solution was estimated to be 1.31×10^{10} particles/mL. The reaction mixture was again incubated for 10 min at 4 °C under mild stirring, and the resulting solution was injected into the microchannel using a syringe pump. The flow rate of a solution was 3 μL/h, and the deflection velocity of a microbead in the direction of *x*-axis was determined as described above. To test the background level, 10 μL of a reaction buffer was added to a reaction mixture instead of a serum sample solution. As the negative control experiments to check the cross-reactivities of specific mite allergens toward other IgEs in serum, the magnetophoretic immunoassays were carried out in the presence of 190, 475, and 4750 μM of the purified human IgE.

Blind Test. In an effort to evaluate the reproducibility and accuracy of the developed magnetophoretic immunoassay system, unknown sera were subjected to blind test, and their concentrations were compared with those by a conventional test kit. Unknown sera from allergy patients were also obtained from Ajou University Hospital and used without pooling. For comparison,

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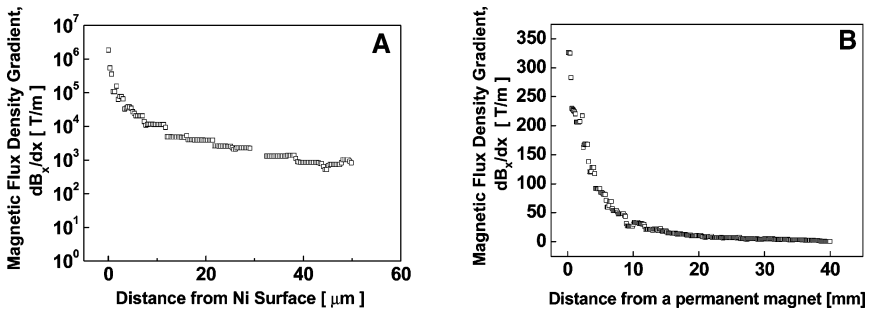

Figure 2. Comparison of the simulated results between a permanent magnet and a Ni microstructure under external magnetic field: profiles of simulated magnetic field gradient (A) with a Ni microstructure under external magnetic field and (B) with a permanent magnet only.

the concentration of mite allergen-specific IgEs was also determined by using a CAP system.

RESULTS AND DISCUSSION

Effect of a Ni Microstructure on the Magnetic Field Gradient. In the magnetophoretic assay system, movement of superparamagnetic nanoparticle-conjugated microbeads is affected by the gradient of magnetic field. Since the magnetophoretic drag velocity is proportional to the analyte concentration and magnetic field gradient, detection limit can be improved by increasing the velocity under the higher magnetic field gradient. For this, we introduced a Ni microstructure as the ferromagnetic material into the microfluidic device based on the fact that the ferromagnetic material concentrates the magnetic flux density under the external magnetic field. To confirm this effect, the magnetic flux density gradient of a permanent magnet and that of Ni microstructure under the external magnetic field were simulated by using a FEMM program (finite element method magnetics; Aladdin Enterprises, Menlo Park, CA). All simulation conditions were identical with experimental ones. The height, width, and length of the NdFeB 35 permanent magnet were 10, 25, and 50 mm, respectively. The height and width of the Ni microstructure were 50 and 50 μm, respectively. The distance between the permanent magnet and the Ni microstructure were 2 mm. The properties of permanent magnet were as follows: relative recoil permeability, 1.05; density, 7.4 g/cm^3; specific resistivity, 144 $\mu\Omega$ cm.

Parts A and B of Figure 2 show the simulated results with a Ni microstructure under the external magnetic field and only a permanent magnet, respectively. The simulated magnetic field gradient of a permanent magnet was about 200 T/m in a concerned region that is about 2 mm apart from the permanent magnet. On the other hand, Ni microstructure under the external magnetic field exhibited about 50-fold enhanced gradient ($\sim 10^4$ T/m) compared to the permanent magnet, confirming that the ferromagnetic material concentrates the magnetic flux density. On the basis of the simulation results under various conditions, we determined the optimal dimension of microfluidic device with a Ni microstructure. The gap between the microchannel and Ni microstructure, and the height of a Ni microstructure are 15 and 50 μm, respectively. In this case, the calculated magnetic field gradient (dB_x/dx) is $\sim 10^4$ T/m.

Magnetophoretic Immunoassay of Mite Allergen-Specific Human IgE. With the optimized Ni microstructure, magnetophoretic immunoassay of mite allergen-specific human IgE was carried out. Two types of mite allergens, *D. farinae* and *D. pteronyssinus*, were conjugated onto microbeads (5.6 μm in diameter), and the resulting beads were used for immunoassay of allergen-specific IgE in serum. The deflection velocity of a superparamagnetic nanoparticle-associated microbead was revealed to be proportional to the number of associated magnetic nanoparticles as expected. In the background test, the velocity of a microbead for allergen of *D. farinae* was determined to be about 0.3 ± 0.16 μm/s (Figure 3A). For the negative control experiment, 190 μM of the purified human IgE containing no mite allergen-specific human IgE was tested. The mean velocity of microbeads was 0.65 ± 0.36 μm/s (Figure 3B). The deflection velocities of microbeads in the negative control experiments were generally within the range of background level, even though the concentration of added IgE was much higher than the maximum concentration of allergen-specific IgE (data not shown). This result strongly implies that cross-reactivities of the allergen extracts toward other human IgEs in serum are negligible.

We determined the concentrations of the mite allergen-specific human IgEs in serum by using the developed system for the pooled sera. As a result, the velocity of a microbead conjugated with allergen from *D. farinae* increased with increasing concentration of *D. farinae* allergen-specific human IgE ranging from 547 fM to 102.5 pM. The lowest concentration of human IgE measured above the background level was about 547 fM. The mean velocity at the lowest concentration (547 fM) of human IgE was 1.78 ± 0.36 μm/s as shown in Figure 3C. For 102.5 pM of human IgE, the velocity was estimated to be 14.58 ± 0.81 μm/s (Figure 3D).

A similar experiment was conducted for *D. pteronyssinus* allergen. The velocity of a microbead was measured for the concentration of *D. pteronyssinus* allergen-specific human IgE ranging from 795 fM to 56.2 pM. The lowest concentration of human IgE measured above the background was about 795 fM. The mean velocity at the lowest concentration (795 fM) of human IgE was 1.0 ± 0.48 μm/s. As for 56.2 pM of human IgE, the velocity was 10.0 ± 0.33 μm/s. The velocity in the background test was determined to be about 0.11 ± 0.09 μm/s. The effect of added IgE was also revealed to be insignificant as observed for the allergen of *D. farinae*.

From the measured velocities, the standard calibration curves for two allergen-specific human IgEs are shown in Figure 4. In this work, the same concentration of proteins from each mite lysate was used for conjugation onto microbeads. However, we observed discrepancy in the calibration curves for *D. farinae* and *D. pteronyssinus* allergens. The velocities of microbeads in the assay system were also different even for the same serum, depending on the conjugated allergen. It was reported that

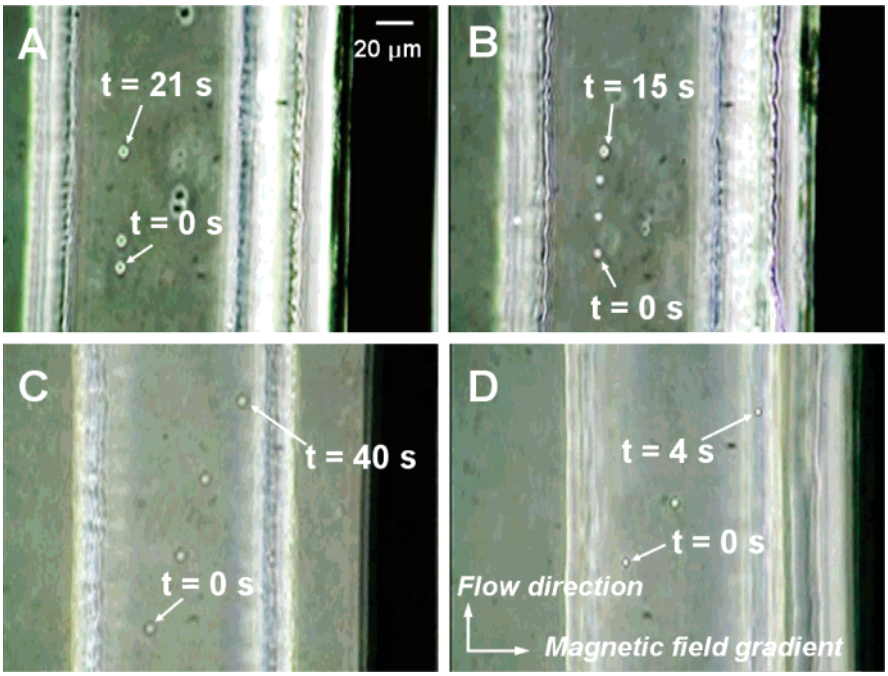

Figure 3. CCD images showing the movement of *D. farinae* allergen-conjugated microbeads at different time intervals for various concentrations of analyte. (A) The analyte was 10 μL of PBS (for a background control), and the mean velocity was calculated to be about 0.3 $\pm$ 0.16 μm/s. (B) As a negative control, 10 μL of 190 μM of the purified human IgE containing no mite allergen-specific human IgE was injected. The mean velocity was estimated to be 0.65 $\pm$ 0.36 μm/s. (C) Injection of 547 fM *D. farinae* allergen-specific human IgE. The mean velocity was about 1.78 $\pm$ 0.36 μm/s. (D) Loading of 102.5 pM *D. farinae* allergen-specific human IgE. The mean velocity was calculated to be about 14.58 $\pm$ 0.81 μm/s.

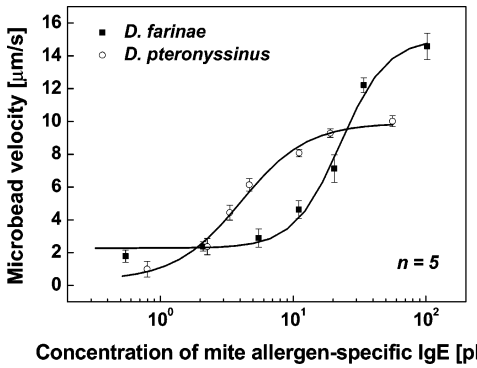

Figure 4. Deflection velocities of microbeads with respect to the concentrations of *D. farinae* and *D. pteronyssinus* allergen-specific human IgEs, respectively. The velocities of five microbeads were independently determined. Error bars indicate the means and standard deviations of measurements.

D. farinae and *D. pteronyssinus* have extensive cross-allergenicity, but they also have unique allergenicity in each side.[36] Thus, the allergenicity of the conjugated lysates seems to be different, which might cause the discrepancy in binding of allergen-specific IgE.

To check the utility of the developed system for allergy diagnosis, sera from 40 patients were pooled and subjected to analysis. For this, the concentrations of mite allergen-specific IgEs in pooled sera were first determined by using a CAP system, and their concentrations were found to be 3.98 pM and 4.90 pM for *D. farinae* and *D. pteronyssinus* allergen-specific IgEs, respectively. And then, the velocities were measured for the sera with known concentration of allergen-specific IgE. When the pooled serum with 3.98 pM of *D. farinae* allergen-specific IgE was tested, the resulting velocity of microbeads was averaged to be 2.82 $\pm$ 0.61 μm/s. The measured velocity is comparable to the value of 2.44 μm/s derived from the calibration curve in Figure 4. The velocity for the serum with 4.90 pM of *D. pteronyssinus* allergen-specific IgE was measured to be 5.83 $\pm$ 0.77 μm/s, which is also comparable to the velocity from the calibration curve (5.90 μm/s). These results support the reliability of the developed assay system for allergy diagnosis.

Diagnosis of Mite Allergy. It was reported that Omalizumab has been widely used to be administered to allergy patients in clinics. Omalizumab is known to specifically bind F_c region of IgEs in human blood, which might raise a concern that excess Omalizumab in human blood has a possibility to decrease the accuracy of the assay of IgE.[37] Even though the effect of Omalizumab was not evaluated, it is expected that the effect might be insignificant because polyclonal anti-human IgEs were used in this work.

For evaluation of the developed magnetophoretic immunoassay system, unknown sera were subjected to blind test, and their concentrations were compared with those by a conventional test kit (Table 2). Fourteen sera from patients were used for the diagnosis of *D. farinae* and *D. pteronyssinus* allergens. From the measured velocity of a microbead using the same procedure, the concentration of mite allergen-specific human IgE in serum was determined from the respective calibration curve shown in Figure 4. The concentrations of respective IgE were compared with those

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Table 2. Blind Test of Unknown Sera and Comparison with a CAP System

patient number	microbead velocity[a] (μm/s)	concn of IgE from the standard curve (pM)	concn of IgE from the standard curve (IU/mL)	concn of IgE from the CAP system (IU/mL)
		D. farinae		
B-1	2.531 ± 0.33	4.89 ± 0.95	0.388	0.37
B-2	2.64 ± 0.34	5.63 ± 2.42	0.446	0.5
B-3	8.215 ± 0.79	21.4 ± 2.16	1.7	1.74
B-4	14.488 ± 0.948	76.3 ± 13.4	6.17	6.33
B-5	3.28 ± 0.47	8.27 ± 1.79	0.656	0.55
B-6	11.69 ± 0.54	34.68 ± 3.04	2.75	2.88
B-7	4.45 ± 0.22	12.05 ± 0.59	0.956	1.01
		D. pteronyssinus		
B-8	1.947 ± 0.35	1.66 ± 0.237	0.131	<0.35
B-9	6.01 ± 0.49	5.0 ± 0.579	0.396	0.38
B-10	7.815 ± 0.82	8.26 ± 2.53	0.65	0.85
B-11	9.683 ± 0.42	33.8 ± 8.05	2.68	2.83
B-12	5.15 ± 0.46	4.12 ± 0.45	0.327	0.37
B-13	0.92 ± 0.35	0.75 ± 0.16	0.06	<0.35
B-14	9.48 ± 0.35	36.13 ± 19.85	2.87	3.17

[a] Five microbeads were used for determination of the average velocity.

by using a CAP system. As shown in Table 2, blind test using magnetophoretic immunoassay (y) revealed a good correlation with the CAP system (x). The correlation is expressed with a linear equation of $y = 0.9644x - 0.02211$, resulting in the R^2 value of 0.9976. The coefficient of variance (CV) of magnetophoretic immunoassay system was calculated to be 9.9% (range; 3.69−20.25%). This value seems to be comparable to a CAP system having 10.3% (range; 6−14%) of CV.[38] Based on this result, it is plausible that magnetophoretic immunoassay system can be effectively used for diagnosis of mite allergy with a good reliability. In addition, the detection limits of *D. farinae* and *D. pteronyssinus* were estimated to be 565 fM (0.045 IU/mL) and 268 fM (0.021 IU/mL), respectively. These values are about 1 order of magnitude lower than those by a conventional CAP system (0.35 IU/mL). A commercial test kit like a CAP system usually requires a relatively large amount of serum (40 μL/assay) and a long analysis time (more than about 2∼3 h). On the other hand, the developed assay method needs a smaller amount of serum (10 μL) and a short analysis time (∼30 min).

The dynamic range of the developed system was shifted downward, retaining a similar interval to that of the CAP system (0.35∼100 IU/mL). It is anticipated that the dynamic range of this system can be tuned up by modulating the size of microbeads and magnetic nanoparticles or increasing the magnetic field gradient in a wider channel. Optimization of the amount of allergen conjugated onto microbeads and the number of functionalized magnetic nanoparticles would also improve the dynamic range. The magnetic field gradient was enhanced remarkably by introducing the ferromagnetic material like a Ni microstructure into the microfluidic device. However, the use of permanent magnet was incongruent for automation, miniaturization, and integration. It was reported that the magnetic field gradient in the microelectromagnetic chip could be significantly enhanced by specific design of materials.[39,40] Thus, some drawbacks caused by the use of permanent magnet might be overcome by introduction of a microelectromagnet into a microfluidic device.

CONCLUSION

We have demonstrated the magnetophoretic immunoassay of mite allergen-specific human IgE in sera under enhanced magnetic field gradient. The velocity of a magnetic nanoparticle-conjugated microbead was well correlated with the concentration of human IgE in serum. The developed system exhibited the detection limits 1 order of magnitude lower than those of conventional test kit (CAP system) for two types of mite alergens, showing a good reliability. In addition, the magnetophoretic immunoassay system enabled a fast analysis with a smaller amount of reagents compared to a conventional method, supporting its utility for analysis of disease biomarkers as well as specific allergens.

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