



Allergen diagnosis microarray with high-density immobilization capacity using diamond-like carbon-coated chips for profiling allergen-specific IgE and other immunoglobulins

Koichi Suzuki^a, Mineyoshi Hiyoshi^a, Hitomi Tada^a, Miwa Bando^a, Takao Ichioka^b, Norio Kamemura^a, Hiroshi Kido^{a,*}

^a Division of Enzyme Chemistry, Institute for Enzyme Research, The University of Tokushima, Tokushima 770-8503, Japan

^b Pediatrics, Health Insurance Naruto Hospital, Tokushima 772-8503, Japan

ARTICLE INFO

Article history:

Received 21 February 2011

Received in revised form 1 August 2011

Accepted 23 August 2011

Available online 1 September 2011

Keywords:

Allergen

Diamond-like carbon

Immunoassay

Microarray technology

ABSTRACT

The diagnosis of antibody-mediated allergic disorders is based on clinical findings, skin prick tests and detection of allergen-specific IgE in serum. Here, we present a new microarray technique of high-density antigen immobilization using carboxylated arms on the surface of a diamond-like carbon (DLC)-coated chip. High immobilization capacity of antigen on DLC chip at $(0.94\text{--}7.82) \times 10^9$ molecules mm^{-2} allowed the analysis of allergen-specific immunoglobulins against not only purified proteins but also natural allergen extracts with wide assay dynamic range. The higher sensitivity of the allergen-specific IgE detection on DLC chip was observed for comparison with the UniCAP system: the DLC chip allowed lowering the limit of dilution rate in UniCAP system to further dilution at 4–8-fold. High correlations ($\rho > 0.9\text{--}0.85$) of allergen-specific IgE values determined by the DLC chip and UniCAP were found in most of 20 different allergens tested. The DLC chip was useful to determine allergen-induced antibodies of IgA, IgG, IgG1, and IgG4 in sera, apart from IgE, as well as secretory IgA in saliva against the same series of allergens on the chip in a minimal amount (1–2 μL) of sample.

© 2011 Elsevier B.V. All rights reserved.

1. Introduction

The diagnosis of type I allergy is based on clinical presentation, skin prick tests, histamine release tests, and serological analysis of total and allergen-specific immunoglobulin (Ig)E [1]. Skin prick tests can sometimes cause discomfort, risk anaphylaxis [2] and increased sensitivity to allergens [3]. As safer and more quantitative alternative, measurement of allergen-specific IgE in serum by a solid-phase immunoassay, such as the UniCAP (FEIA) system (Phadia AB) [4,5], is widely used worldwide. In recent years, new technologies of protein microarray-based sandwich immunoassay and related immunoassays have been developed for rapid, high sensitivity and multiple allergen determinations using small amounts of serum, such as the immuno solid-phase allergen chip (ISAC), immunoblot allergen screening panel and the ADVIA Centaur microbead systems [6–9]. Compared with the high immobilization doses ($\sim 35 \mu\text{g}$) of antigens on a sponge-like matrix column unit of the UniCAP system [10–13], one limitation of the microarray is the low doses of immobilized antigens,

which sometimes results in detection failure of some allergens particularly in natural allergen extracts.

To improve the microarray by increasing the dose of immobilized antigens, we designed the high-density DNA solidification technique of the carboxylated diamond like carbon (DLC) chip [14] for the allergen microarray technique. The covalently bound oligonucleotide to the carboxylated DLC chip was approximately 42 pmol ($\sim 2.5 \times 10^{13}$ molecules) per 9 mm^2 of the chip surface, the highest density DNA chip reported [14]. By using the carboxylated DLC chip, we achieved high-density allergen immobilization and improved the analysis of allergen-specific immunoglobulins against purified proteins and natural allergen extracts with wide assay dynamic range. The new technique also achieved highly reproducible measurement using small amounts of sera and saliva with high sensitivity.

2. Materials and methods

2.1. Materials

Carboxylated DLC film [14] coated glass slide (Gene slide) was purchased from Toyo Kohan Co. (Tokyo, Japan). Natural allergens were from Allergen (Ängelholm, Sweden) and purified allergens,

* Corresponding author. Tel.: +81 88 633 7423; fax: +81 88 633 7425.

E-mail address: kido@ier.tokushima-u.ac.jp (H. Kido).

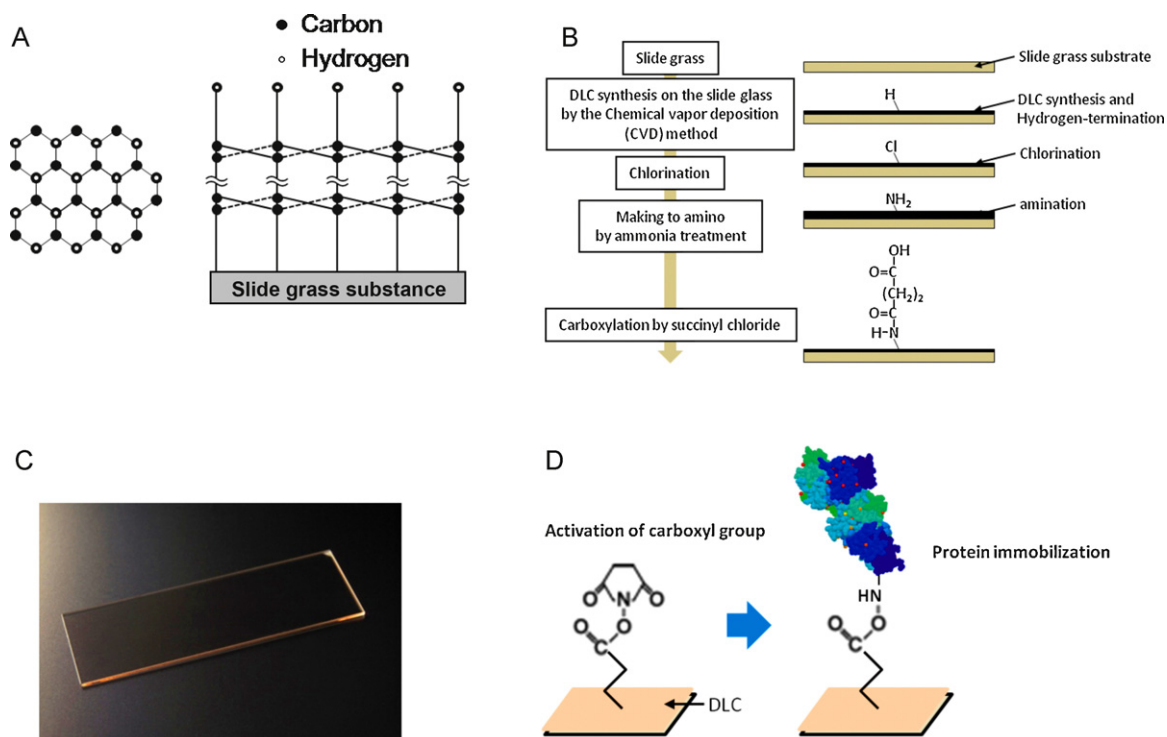


Fig. 1. Schematic illustration of the DLC chip, carboxylation and antigen immobilization. (A) Schematic arrangement of carbon and hydrogen on the DLC chip. (B) Chemical treatment processes for DLC surface carboxylation. (C) The DLC slide. (D) Antigen immobilization processes on the DLC chip.

such as ovomucoid, ovalbumin, conalbumin, α -casein, β -casein, and β -lactalbumin, were from Sigma–Aldrich (St. Louis, MO). Human IgE serum (75/502), IgG, IgA and IgM serum (67/086) were from the National Institute for Biological Standards and Control (Hertfordshire, England). All other chemicals were obtained from Sigma–Aldrich and Wako Pure Chemicals (Osaka, Japan).

2.2. Activation of carboxylated DLC slides and fabrication of allergen microarray

Fig. 1 provides a schematic illustration of the DLC chip, the carboxylation procedure and antigen immobilization. For activation of the high-density carboxyl group on the surface of DLC film on the GeneSlide®, the slide was incubated with freshly prepared 100 mM 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (Dojindo Co., Kumamoto, Japan) and 20 mM N-hydroxysuccinimide in 0.1 M potassium phosphate buffer, pH 6.0, at 25 °C for 30 min (Fig. 1D). Then the slide was covered with a customized water-repellent seal (Suwascho Techno Co., Tokyo), designed to create 6 mm × 4 mm rectangular areas representing 12 individual reaction holes (Fig. 3A). Each reaction well has a total of 108 spots capacity with no cross-contamination. Allergens were solubilized at optimal concentrations of 0.25–1.0 mg mL⁻¹ in either 20 mM phosphate buffer, pH 7.4, 20 mM Tris–HCl buffer, pH 7.5, 100 mM 4-(2-hydroxyethyl)-1-piperazine ethanesulfonic acid buffer, pH 8.0, or 100 mM N-cyclohexyl-3-aminopropanesulfonic acid buffer, pH 10.0 with or without 0.05% Triton X-100 or 0.5 M NaCl; casein at 0.25 mg mL⁻¹; ovomucoid, eggwhite and soybean at 0.5 mg mL⁻¹; *Dermatophagoides pteronyssinus* (Dp) and *Dermatophagoides farinae* (Df) at 0.75 mg mL⁻¹ and milk at 1 mg mL⁻¹. For Ig standard calibration curves, WHO human serum IgE (10–1000 IU mL⁻¹), IgG, IgA and IgM (0.024–3.12 U mL⁻¹) were used. Spotting of allergens onto DLC glass slides was performed by the pin method using OmniGrid Accent microarrayer (Genomic Solutions, Boston, MA) with pin #SMP9 (tip area = 0.074 mm²,

volume per spot = 3.3 nL), under 50 ± 5% humidity (mean ± SD) at room temperature. Each allergen was spotted in triplicates or tetraplicates onto the surface in each reaction well. After spotting, allergen chips were allowed to stand at 37 °C for 3 h and excess activated carboxyl group were blocked at 4 °C overnight with 0.1 M glycine and 1% bovine serum albumin (BSA) in 20 mM phosphate buffer, pH 7.4, containing 10% polyethylene glycol and BioUltra 6000 (Amicon). After blocking, allergen chips were rinsed twice with deionized water, dried under nitrogen flow and then stored at 4 °C until use.

2.3. Patients

Serum and/or saliva were obtained from allergic patients (112–322 patients aged 0–30 years for each allergen listed in Table 4) and healthy volunteers (20 volunteers aged 0–30 years) at the Health Insurance Naruto Hospital, Tokushima and University of Tokushima. The study was approved by the institutional Ethics Committees of The University of Tokushima and the Health Insurance Naruto Hospital. The diagnosis of type I allergy was based on case history, skin prick tests and IgE serology performed using the UniCAP system. Blood was taken for assay of serum specific IgG and stored –30 °C until use. Saliva was collected by Salivette® (SARSTEDT AG & Co. Nümbrecht, Germany) according to the protocol offered by the manufacturer and was stored –30 °C immediately after preparation until use. Allergen assay was conducted within one year after storage of the samples.

2.4. Allergen chip assay

The individual arrays in Fig. 3A were incubated with 20 µL of 1:10 or more diluted human serum or 1:2 or more diluted saliva with dilution buffer (20 mM phosphate buffer, pH 7.4, containing 1% BSA, 0.3 M KCl and 0.05% Tween20) as the 1st antibody for 60 min at 37 °C. Arrays were then washed twice with TTBS (50 mM Tris–HCl,

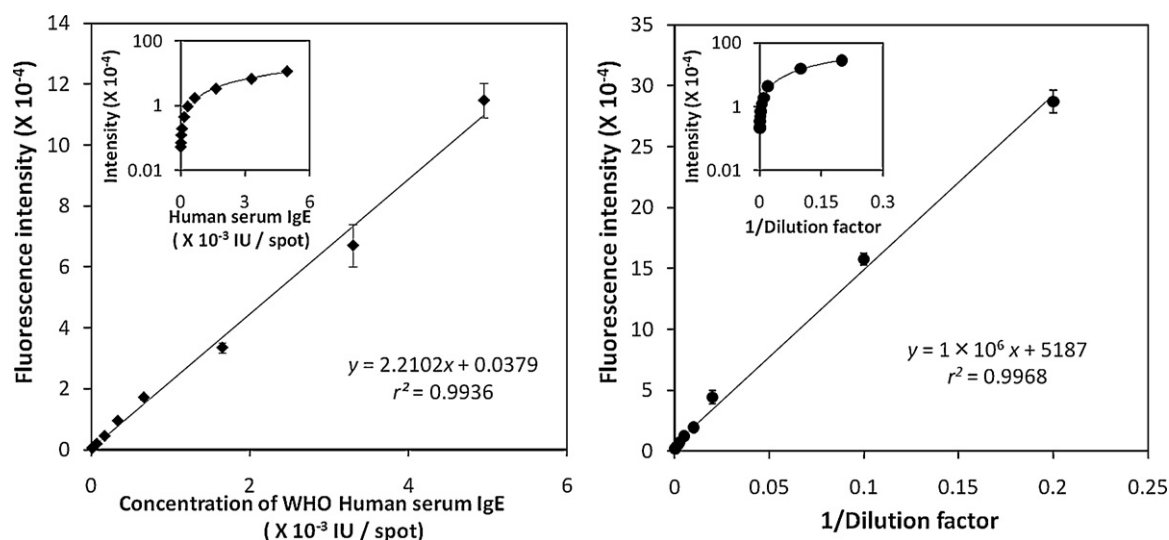


Fig. 2. (A) Immobilization linearity of proteins on the DLC chip. Eight standards with known concentrations of WHO human serum IgE (75/502) were immobilized on the DLC chip covering $(0.02\text{--}4.95) \times 10^{-3}$ IU spot $^{-1}$. Hilyte Fluor 555 labeled anti-human IgE was used for detection. Data are mean $\pm$ SD of tetraplicate spots. The observed values were compared to the expected by linear regression analysis ($y = 2.210x + 0.037$, $r^2 = 0.993$). The inset graph presents the data in semi-logarithmic scale. (B) Linearity of detection of α -casein-specific IgE by sandwich fluorescence immunoassay. Serum of a patient with milk allergy was diluted from 1:5 to 1:9600 and the dilution recovery and the linearity of detection ranges were analyzed. Data are mean $\pm$ SD of tetraplicate spots. $y = 1 \times 10^6 x + 5187$, $r^2 = 0.996$. The inset graph presents the data in semi-logarithmic scale.

pH 7.5, containing 150 mM NaCl and 0.05% Tween20), rinsed with deionized water and dried by centrifugation at $500 \times g$ for 3 min. A HiLyte FluorTM 555 labeled 2nd antibody against human IgE (Mili-pore, Bedford, MA) was adjusted to $6.0 \mu\text{g mL}^{-1}$ by the dilution buffer, and $20 \mu\text{L}$ of the antibody was applied and incubated for 1 h at 25°C . After reaction, arrays were washed twice with TTBS (each 5 min), rinsed with deionized water, dried by centrifugation and then scanned. For detection of antigen-specific IgG, IgG4, IgG1 and IgA in serum and secretory IgA (S-IgA) in saliva, mouse monoclonal antibodies against each human immunoglobulin (Zymed Laboratories Inc., San Francisco, CA) were used at $1.5 \mu\text{g mL}^{-1}$ as the 2nd antibodies and incubated for 1 h at 37°C . The 2nd antibody (anti-human IgA antibody) was almost equally reacted with both IgA in serum and S-IgA in saliva.

2.5. Image data acquisition and data processing

Images were acquired by scanning of allergen DLC chips with a FLA-8000 scanner (Fujifilm Co., Tokyo) with an excitation wavelength of 532 nm and an emission wavelength of 570 nm under 60% impressed voltage. Images were analyzed using FLA-8000 image reader (ver. 1.2) software (Fujifilm Co.) and each spot signal in the images was calculated to numerical fluorescent intensity using Array gauge (ver. 2.10) software (Fujifilm Co.). The fluorescence intensities of each of the tetraplicates spotted allergen had to be above the overall signal cut-off value, which represented the mean $+3\text{SD}$ fluorescence signal determined for control sera from non-allergic volunteers. The difference between the mean and median values of the fluorescence intensity of each spot was less than 30%. The coefficient of variation between the fluorescence intensities of all tetraplicates was less than 13.5%.

On each allergen chip, various concentrations of IgE, IgG or IgA were spotted in tetraplicates, detected by the fluorescence-labeled anti-human IgE, IgG or IgA antibody, respectively, and the mean value of each concentration was calculated. From the cubic equation of IgE, IgG or IgA standard concentrations, the amounts of allergen-specific antibodies bound to allergen on the chips were calculated and expressed as Binding Unit (BU). The BU of IgE was reported as BUe.

2.6. Statistical analysis

The coefficient of variance (CV) was calculated as the standard deviation divided by the mean of replicates. Paired correlations were calculated by the Pearson's correlation test. Statistical analysis was performed using The Statistical Package for Social Sciences SPSS software (SPSS, Chicago, IL).

3. Results

3.1. Immobilization levels of antigens on DLC chip

We first analyzed the immobilization capacities of various antigens and proteins, such as ovalbumin, ovomucoid, conalbumin, α -casein, β -casein, α -lactalbumin, β -lactoglobulin and protein A, covalently bound on the DLC chip, using escalating doses of each fluorescein isothiocyanate (FITC)-labeled allergen on the carboxylated DLC chip. After immobilization reactions followed by double washing to remove the unbound antigens, we analyzed the fluorescence intensities on the chip to determine the doses of immobilized antigens. The immobilized FITC-labeled antigens tested were $2.60\text{--}38.6 \text{ pg}$ per 0.074 mm^2 spot ($35.10\text{--}521.6 \text{ pg mm}^{-2}$) and were also expressed as number of moles per mm^2 to compare the values of immobilized antigens on ELISA plastic plate, BLACK MAX-ISORT (NUNC) (Table 1). As shown in Table 1, the numbers of

Table 1
Comparison of immobilized allergens on DLC chip and plastic ELISA plate.

	Number of molecules per mm^2		
	DLC chip	ELISA	DLC chip/ELISA ^a
Ovalbumin	2.49×10^9	3.61×10^8	6.90
Ovomucoid	1.00×10^9	1.80×10^8	5.50
Conalbumin	3.31×10^9	7.77×10^8	4.30
α -Casein	3.41×10^9	0.24×10^8	142
β -Casein	0.94×10^9	0.12×10^8	80.6
α -Lactalbumin	4.19×10^9	0.65×10^8	64.8
β -Lactoglobulin	6.59×10^9	8.33×10^8	7.90
Protein A	7.82×10^9	1.14×10^8	68.7

^a The ratio of antigen immobilization doses between DLC chip and ELISA.

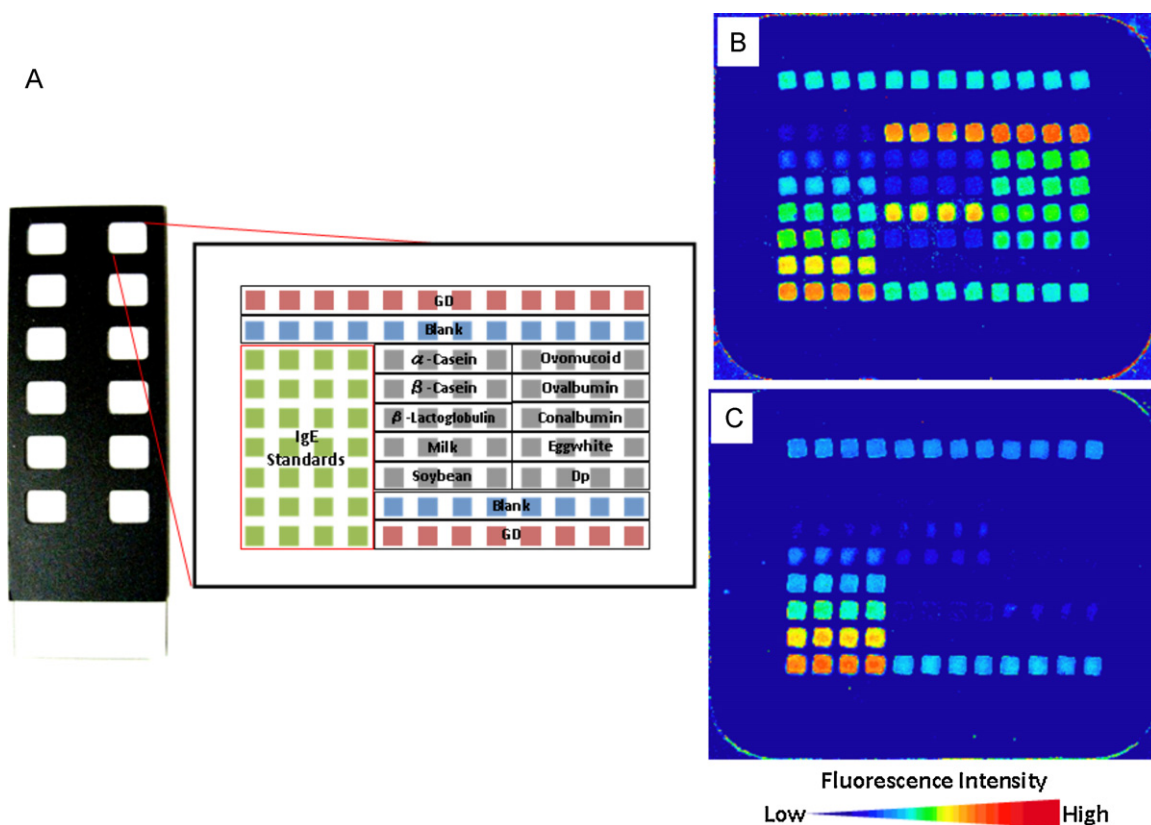


Fig. 3. IgE profiling on the DLC chip. (A) General layout of the allergens on the DLC chip. GD: guideline dot by IgE standard. Rainbow displays of fluorescence images of allergen-specific IgE binding on the DLC chip probed with serum from a patient with eggwhite and milk allergy (B), a non-allergic individual (control) (C).

immobilized antigen molecules per mm^2 on the DLC chip tested were $(0.94\text{--}7.82) \times 10^9$ molecules and were 4.30–142-fold higher than those of immobilized antigens on the plastic ELISA plate.

3.2. Sandwich immunoassay of IgE determination using DLC chip

To measure the allergen specific IgE quantitatively, various doses of WHO human serum IgE (75/502; NIBSC), corresponding to $(0.02\text{--}4.95) \times 10^{-3}$ IU per spot, were spotted in tetraplicates and visualized with $6.0 \mu\text{g mL}^{-1}$ HiLyte FluorTM 555 labeled anti-human IgE for calibration (Fig. 2A). The linear regression parameters were observed in working assay ranges between 0.02 and 4.95×10^{-3} IU spot⁻¹.

Linearity of allergen-specific IgE quantification and dilution recovery were analyzed (Fig. 2B). The pooled sera of 4 patients with α -casein-specific IgE were diluted 1:5, 1:10, 1:50, 1:100, 1:200, 1:400, 1:600, 1:1200, 1:2400, 1:4800 and 1:9600 and the antigen-specific IgE levels were analyzed on DLC-chip. The data showed a linear fit across the wide assay range from 0.2 to 0.2×10^{-3} of 1/dilution ratios.

To analyze the reproducibility of IgE determination, inter-assay precision was evaluated on five replicate microarray chips using pooled sera of allergy patients positive for allergen-specific IgE against eggwhite, ovomucoid, milk, α -casein, soybean and Dp (Table 2). Within-slide precision and batch-to-batch precision with five different batches of slides on 4 consecutive days were also analyzed. The accuracy of the numerical value was represented by the % coefficient of variation (CV: standard deviation in % of mean value). The inter-assay CV was 5.70–13.5%, within-slide CV 7.70–25.2% and batch-to-batch CV was 2.7–24.4%.

To assess the analytical sensitivity of DLC chip for the allergen-specific IgE, each serum pool [UniCAP(FEIA) class 6; $>100 \text{ UA mL}^{-1}$]

of five patients containing high levels of IgE against milk, α -casein, eggwhite or ovomucoid was tested in 11-fold geometric dilutions starting from 1:5 to 1:9600. The values obtained from the DLC chip were compared with the measurements obtained by UniCAP(FEIA) system at the same dilutions (Table 3). The DLC chip assay was more sensitive than the UniCAP system: the antigen-specific IgE detection on DLC chip allowed lowering the limit of dilution rate in UniCAP system to further dilution at 4–8-fold.

Fig. 3 shows typical allergen-specific IgE analyses on the DLC chip of a patient allergic to milk and eggs (Fig. 3B) and non-allergic person (Fig. 3C). Ten different allergens including natural allergen extracts, egg white, milk, Dp and soybean and purified allergen proteins, α -casein, β -casein, β -lactoglobulin, ovomucoid, ovalbumin and conalbumin were spotted in tetraplicates. The patient serum sample reacted strongly with α -casein, ovomucoid and milk and mildly with ovalbumin, conalbumin and egg white, but did not react with soybean, Dp, β -casein and β -lactoglobulin.

3.3. Correlation of allergen-specific IgE values determined by DLC chip system and UniCAP system

The correlation of allergen-specific IgE values determined by the DLC chip and UniCAP was analyzed in 20 different typical natural allergen extracts and purified allergen proteins (Table 4). The

Table 2
Reproducibility of DLC chip testing allergen-specific IgE against eggwhite, ovomucoid, milk, α -casein, soybean and Dp.

Reproducibility	Eggwhite	Ovomucoid	Milk	α -Casein	Soybean	Dp
Inter-assay	10.1	5.70	12.4	13.5	12.0	5.80
Within-slide	14.9	25.2	8.90	8.60	14.7	7.70
Batch-to-batch	24.4	24.4	17.8	13.7	2.70	22.3

Table 3Comparison of assay sensitivity in detecting antigen-specific IgE against milk, α -casein, eggwhite and ovomucoid using the DLC chip system and UniCAP system.

Serum dilution ^a	DLC chip (fluorescence units)				UniCAP (PRU)			
	Milk	α -Casein	Eggwhite	Ovomucoid	Milk	α -Casein	Eggwhite	Ovomucoid
1:5	>1500	>1500	>1500	>1500	36.1	49.2	33.3	33.9
1:10	>1500	>1500	1366	1382	17.3	26.3	16.5	17.0
1:50	445.6	633.2	527.5	295.6	3.96	5.55	3.49	3.17
1:100	191.0	306.2	280.7	121.2	1.96	2.69	1.81	1.67
1:200	99.76	161.6	144.1	76.16	1.06	1.42	0.940	0.860
1:400	61.71	94.22	71.33	38.17	0.540	0.710	0.530	0.440
1:600	38.88	66.67	48.64	23.62	0.390	0.500	ND [#]	ND [#]
1:1200	21.80	41.56	25.06	12.59	ND [#]	ND [#]	ND [#]	ND [#]
1:2400	10.63	25.17	19.07	ND [*]	ND [#]	ND [#]	ND [#]	ND [#]
1:4800	ND [*]	24.44	ND [*]	ND [*]	ND [#]	ND [#]	ND [#]	ND [#]
1:9600	ND [*]	ND [*]	ND [*]	ND [*]	ND [#]	ND [#]	ND [#]	ND [#]

Individual allergens were tested by the DLC chip and UniCAP. ND^{*} of DLC chip assay: <mean + 3.5SD of control sera. ND[#] of UniCAP assay: <0.35.^a Serum was pooled from three patients allergic to milk and eggwhite.

coefficient of correlation (ρ) values ($P < 0.01$) for 14 allergens was >0.900 , 3 allergens >0.850 , 2 allergens >0.800 and one at the lowest of 0.744 of house dust. Fig. 4 shows the correlations between IgE values determined by the DLC chip system and UniCAP system for milk, cat dander, ovomucoid and α -casein. Good correlations between IgE values determined by the DLC chip and UniCAP were found for natural allergen extracts and purified allergens.

3.4. Determination of allergen-specific IgA, IgG, IgG1 and IgG4 in serum and S-IgA in saliva by sandwich immunoassay

Allergen-specific IgA, IgG, IgG1 and IgG4 levels in serum and S-IgA levels in saliva were also analyzed on the DLC chip by the same method except dilution of the initial sample at 10–50-fold, because allergen-specific IgA, IgG, IgG1 and IgG4 levels in serum

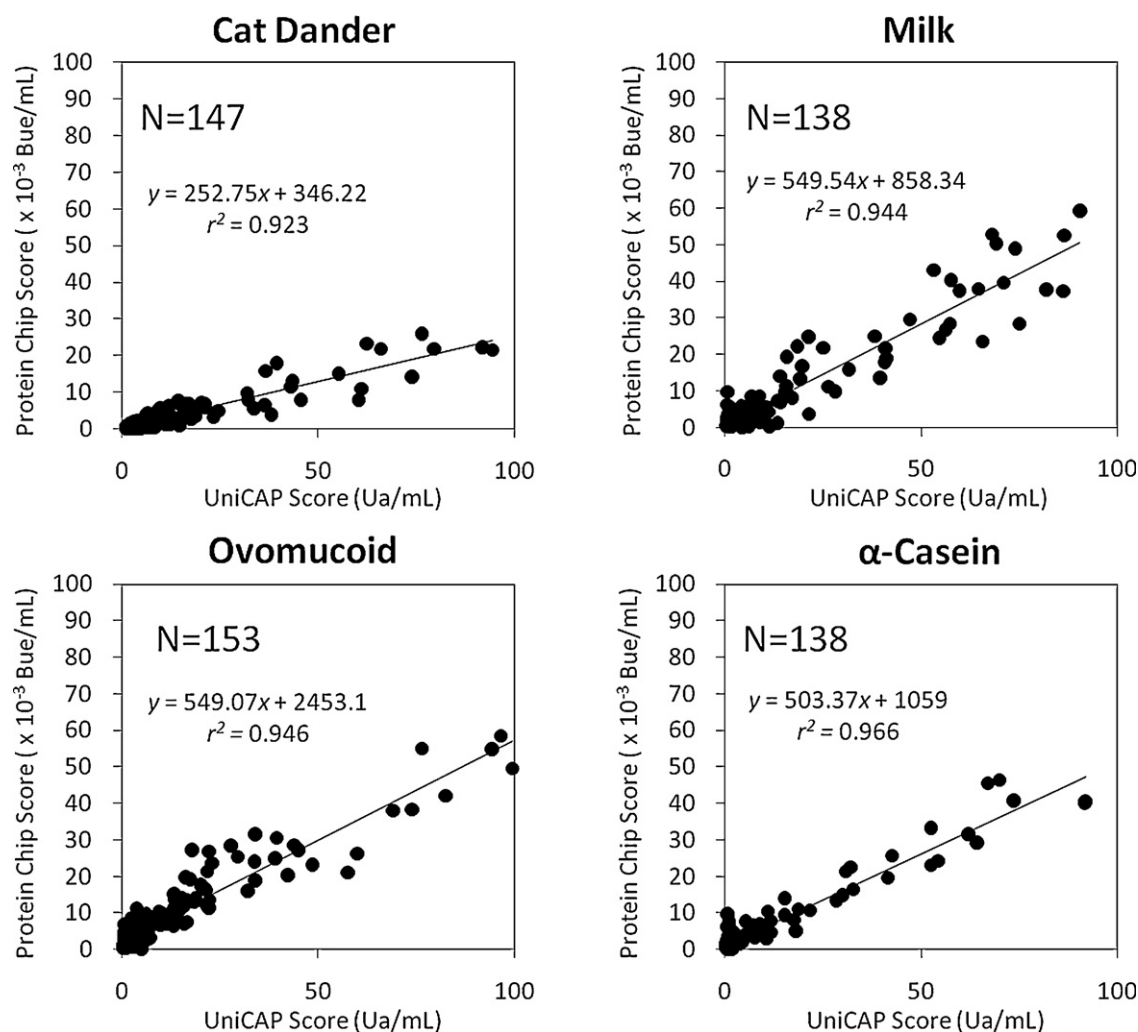


Fig. 4. Correlation of IgE reactivities on the DLC chip with the results obtained by UniCAP system. The reactivity profiles of individual IgE in sera of 147 patients with cat dander allergy, 138 patients with milk allergy, 153 ovomucoid patients and 138 patients with casein allergy were determined by the multi-allergen DLC chip system and UniCAP system. The results were tested by linear regression analysis. Bue: binding unit of IgE. Ua: Arbitrary Unit.

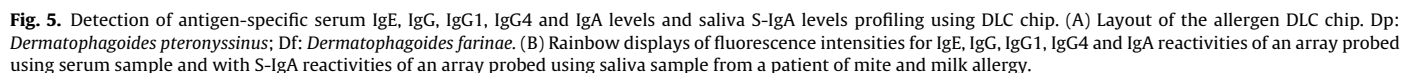


Table 4
Correlation of IgE reactivities between DLC chip and UniCAP on various allergens.

4. Discussion

By using carboxylated DLC chip technology, we developed a protein microarray for determination of allergen-specific immunoglobulins against immobilized multi-allergen using a minimal amount of sera and saliva (1–2 μ L) in a single test. The DLC chip

has the capacity of 108 spots per 24 mm² of single chip window. A special feature of the carboxylated DLC chip is the high allergen immobilization capacity ($\sim 2.60\text{--}38.6\text{ pg spot}^{-1}$) with stable covalent binding, and the levels of immobilized allergens were higher at 4.30–142-fold than the values on the ELISA plastic plate in all allergens tested (Table 1).

This high immobilization capacity of antigens on the DLC chip is advantageous for determination of allergen-specific antibodies: (1) Wide dynamic range for measurement of antigen-specific IgE as shown in Fig. 2. Although the antigen-specific IgE levels are several magnitudes lower than the antigen-specific IgG and IgA levels in serum, the good dilution recovery in Fig. 2B suggests that there was no obvious interference of IgE binding to antigens by the other abundant immunoglobulins. (2) Improved detection sensitivity of antigen-specific IgE in the DLC chip system compared with the sensitivity of the UniCAP system (Table 3). The antigen-specific IgE in the sera could be detected by the DLC chip even by further 4–8-fold dilution of the limit of dilution in UniCAP system. (3) The DLC chip was a useful tool to determine allergen-specific immunoglobulins against not only purified proteins but also natural allergen extracts with good correlation to the UniCAP system (Table 4). Many of the microarrays reported so far are useful for determination of the allergen-specific IgE against recombinant proteins, purified proteins and peptides, but not for natural allergen extracts [7,20,21], probably because of the limitation of immobilized antigen levels on microarrays.

Another advantage of the DLC chip is the determination of allergen-specific IgA, IgG, IgG1 and IgG4 in serum and S-IgA in saliva (other than IgE in serum) by using a series of the same or similar DLC chip system. It is possible that the allergen recognition patterns and the levels of antigen-specific IgE, IgA, IgG and IgG4 in serum and S-IgA in saliva against each allergen might change during the course of allergy progression and regression. The DLC chip is a useful tool for monitoring these values and might be also helpful in the clinical setting.

Determination of allergen-specific immunoglobulins on the DLC chip was highly reproducible (Table 2) and the inter-assay, within-slide and batch-to-batch CV values were ranged between 2.70 and 25.4, although the CV values of batch-to-batch and within-slide for some natural extracts were slightly higher, probably because of the inaccuracy of microarray spotting. This problem might be overcome by improvement of spotting conditions, particularly the strict regulation of humidity and temperature during spotting.

In the present allergen DLC chip, we used sandwich immunoassay with fluorescently labeled antibodies for detection. The high immobilization capacity of proteins on the DLC chip might be advantageous for other detection methods in protein microarray experiments, such as enzyme-linked immunosorbent assay [22], radio isotope-labeled analyte assay [16], surface plasmon resonance assay [23], electro-chemical assay by conductivity measurement [24,25] and mass spectrometry assay [26,27].

5. Conclusions

The present study showed that the DLC chip microarray system with high immobilization capacity allows the analysis of

antigen-specific immunoglobulins against both natural allergen extracts and purified allergen proteins with wide assay dynamic range. The higher sensitivity of the DLC system was observed for comparison with the UniCAP system. Simultaneous measurement of the levels of antigen-specific IgE and other immunoglobulin parameters, such as IgA, IgG1, IgG4 and IgG in serum and S-IgA in saliva against multiple allergens might be useful for the diagnosis and immunotherapy monitoring of Type I allergy.

Acknowledgments

This work was supported, in part, by Grant-in-Aid 18659293, 21659245 and 19659253 from the Ministry of Education, Culture, Sports, Science, and Technology of Japan and by supporting program for creating university ventures from Japan Science and Technology Agency.

References

- [1] A.B. Kay, L. Barata, Q. Mengs, S.R. Durham, S. Ying, *Int. Arch. Allergy Immunol.* 113 (1997) 196–199.
- [2] G. Liccardi, G. D'Amato, G.W. Canonica, A. Salzillo, A. Piccolo, G. Passalacqua, J. Invest. Allergol. Clin. Immunol. 16 (2006) 75–78.
- [3] B. Jahn-Schmid, C. Harwanegg, R. Hiller, B. Bohle, C. Ebner, O. Scheiner, M.W. Mueller, *Clin. Exp. Allergy* 33 (2003) 1443–1449.
- [4] P.W. Ewan, D. Coote, *Allergy* 45 (1990) 22–29.
- [5] A. Leimgruber, B. Mosimann, M. Claeys, M. Seppey, Y. Jaccard, V. Aubert, R. Peitrequin, M-P. Nisoli, A. Pecoud, *Clin. Exp. Allergy* 21 (1991) 127–131.
- [6] B. Jahn-Schmid, C. Harwanegg, R. Hiller, B. Bohle, C. Ebner, O. Scheiner, M.W. Mueller, *Clin. Exp. Allergy* 33 (2003) 1443–1449.
- [7] K. Deinhofer, H. Sevcik, N. Balic, C. Harwanegg, R. Hiller, H. Rumpold, M.W. Mueller, *Methods* 32 (2004) 249–254.
- [8] A.B. Petersen, P. Gudmann, P. Milvang-Grønager, R. Mørkeberg, S. Bøgestrand, A. Linneberg, N. Johansen, *Clin. Biochem.* 37 (2004) 882–892.
- [9] I. Herzum, N. Blümer, W. Kersten, H. Renz, *Clin. Chem. Lab. Med.* 43 (2005) 963–966.
- [10] L. Wide, H. Bennich, S.G.O. Johansson, *Lancet* ii (1967) 1105–1107.
- [11] S.G.O. Johansson, *Expert Rev. Mol. Diagn.* 4 (2004) 89–95.
- [12] L. Yman, in: D. Wild (Ed.), *The Immunoassay Handbook*, Nature Publishing Group, London, UK, 2001, pp. 664–680.
- [13] D. Wild, in: D.G. Wild (Ed.), *The Immunoassay Handbook*, Elsevier, London, Amsterdam, New York, 2005, pp. 358–361.
- [14] K. Takahashi, M. Tange, O. Takai, H. Okamura, *Diamond Relat. Mater.* 12 (2003) 572–576.
- [15] H. Zu, M. Snyder, *Curr. Opin. Chem. Biol.* 7 (2003) 55–63.
- [16] G. MacBeath, S.L. Schreiber, *Science* 289 (2000) 1760–1763.
- [17] H. Zhu, J.F. Klemic, S. Chang, P. Bertone, A. Casamayor, K.G. Klemic, D. Smith, M. Gerstein, M.A. Reed, M. Snyder, *Nat. Genet.* 26 (2000) 283–289.
- [18] C. Bieri, O.P. Ernst, S. Heyse, K.P. Hofmann, H. Vogel, *Nat. Biotechnol.* 17 (1999) 8105–8110.
- [19] Y-H. Rogers, P. Jiang-Baucom, Z.-J. Huang, V. Bogdanov, S. Anderson, M.T. Boyce-Jacino, *Anal. Biochem.* 266 (1999) 23–30.
- [20] M. Cretich, G.D. Carlo, R. Longhi, C. Gotti, N. Spinella, S. Coffa, C. Galati, L. Renna, M. Chiari, *Anal. Chem.* 81 (2009) 5197–5203.
- [21] K.A. Vigh-Conrad, D.F. Conald, D. Preuss, *PLoS ONE* 5 (2010) 1–11 (e10174).
- [22] T.O. Joos, M. Schrenk, P. Hopfl, K. Kroger, U. Chowdhury, D. Stoll, D. Schorner, M. Durr, K. Herick, S. Rupp, K. Sohn, H. Hämmerle, *Electrophoresis* 21 (2000) 2641–2650.
- [23] J.M. McDonnell, *Curr. Opin. Chem. Biol.* 5 (2001) 572–577.
- [24] M. Pohanka, O. Pavliš, P. Skládal, *Talanta* 71 (2007) 981–985.
- [25] H. Ogi, T. Yanagida, M. Hirao, M. Nishiyama, *Biosens. Bioelectron.* 26 (2011) 4819–4822.
- [26] P.M. Lizardi, X. Huang, Z. Zhu, P. Bray-Ward, D.C. Thomas, D.C. Ward, *Nat. Genet.* 19 (1998) 225–232.
- [27] W. Liu, S. Liu, H. Li, F. Song, Z. Liu, S. Liu, *Rapid Commun. Mass Spectrom.* 25 (2011) 973–978.