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An automatic integrated microfluidic system for allergy microarray chips†‡

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Billions of people suffer from allergies, though in many cases, the source allergen is unknown. If one knows which allergens to avoid, this would result in an improved quality of life. Since a rapid, high-throughput, automatic allergen detection method is of great need, an integrated system combining microfluidic techniques and microarray chips has been developed herein to automate the allergen detection process. The developed microfluidic system could automatically carry out the entire procedure such as reagent incubation, hybridization, transport, and washing without any intermediate step. The microarray chip could be easily detached from the microfluidic chip afterwards, enabling it to be read under a fluorescence scanner. The experimental results indicated that the developed microfluidic system can automatically perform all the incubation processes, including hybridization, reagent transportation, and washing. It is worth noting that active mixing has been applied in the present study which is different from our previous study using micro-channels for passive incubation. Comparable results to a conventional benchtop approach were obtained in ~30% less time with ~25% less samples/reagents. Similar results were also demonstrated while detecting immunoglobulin E samples. The developed system could therefore provide a rapid, reliable, and automated approach for detecting allergen-specific antibodies in human serum.

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Introduction

When one's immune system over-responds to a normally harmless substance (*i.e.*, an "allergen"), he/she is said to have an allergy. Allergies are common (30–40% of all humans), especially in well-developed countries; more than 300 million people suffer from asthma and food allergies alone according to the White Book on Allergy published by the World Allergy Organization.¹ Allergies can cause a variety of symptoms, including flu-like symptoms, rashes, headaches, sneezing, runny nose, swelling, and nausea, amongst others. The suite of symptoms depends on (1) the types of allergens, (2) the

organs and tissues affected, and (3) the method of exposure to the allergens (direct contact, injection, inhalation, or ingestion). In severe cases, an allergy can lead to life-threatening complications, such as anaphylaxis. Therefore, allergy detection and prevention (*i.e.*, avoidance of the respective allergen) are important human health issues.

In those with type I hypersensitivity, B cells are triggered and stimulated to produce immunoglobulin E (IgE) antibodies that are specific to the allergen. In contrast to the response to an infectious disease, the antibodies produced by the B cells are predominantly IgE rather than IgA, IgG, or IgM. During sensitization, the IgE antibodies trigger downstream immune-responses, resulting in a variety of symptoms.^{2–4} The identification of the allergens to which patients are hypersensitized is an important step for diagnosis and treatment of allergies; if the allergen(s) is/are identified, the patient can modify his/her lifestyle to attempt to limit or eliminate exposure.

There are two primary methods for detecting allergies, and both are based on allergen–antibody binding; these include skin prick tests (SPT) and allergy blood tests (ABT).^{5,6} In the SPT, a minute quantity of the suspected allergen is introduced beneath the patients' skin with a needle or lancet; if localized swelling and redness are observed at the site of introduction, then the allergy is verified.^{1,2} The SPT is rapid (20–30 min) and can be carried out on all age groups, including babies.

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However, it may frighten those who are averse to needles, and it is life-threatening in some cases.⁷ Furthermore, the SPT is not high-throughput, sensitive, or particularly reliable; patients oftentimes need follow-up tests to confirm the results. Alternatively, the ABT detects the presence of allergen-specific antibodies in the patients' serum *in vitro*. Generally, it is relatively safe when compared to the SPT, and the quantities of allergen-specific antibodies can be measured relatively accurately. The concentrations of these antibodies are regarded as proportional to the severity of the allergy.⁸ To detect the allergens or antibodies, enzyme-linked immunosorbent assays (ELISA) or ELISA-like assays are commonly used in clinics.^{9–12} However, these methods are not high-throughput and rely on well trained personnel; they also require considerable sample and reagent volumes.

Microarray technology is a mature technique to accelerate detection with high throughput, which allows the simultaneous detection of a large number of interactions between the bio-molecules in a small area. For instance, protein microarray chips have been utilized for measuring antibody concentrations in human serum, and the sensitivity of this method is higher than that of conventional protein coating processes.¹³ Based on colorimetric analyses of the microarray chips, the levels of human IgE can be accurately measured, and, as mentioned above, IgE levels can be used to gauge the severity of an allergy.^{9,14,15} Although microarrays are high-throughput and require only a small amount of samples and reagents, they are still relatively labor-intensive and require bulky equipment. As such, there is still a great need for an automated system for diagnosing allergies accurately in a high-throughput manner.

Recently, microfluidic technologies have become popular for performing a number of biological applications, such as sample transport, mixing, and incubation; not only can these chip-based systems be automated, but they only require small quantities of biological samples and reagents.^{16–19} They are also more compact and portable than their large-scale counterparts, and many are highly sensitive. For instance, a LabCD platform was used to automate the ELISA process for allergy detection.²⁰ Rat IgG was used and the entire process could be automated by using centrifugal forces. However, only a very limited number of allergens could be detected since it was not compatible with existing allergy microarrays.

Several microfluidics-based systems have further been integrated with allergy microarrays; for instance, microfluidic cartridges were used to detect the presence of allergen-specific antibodies in serum.^{21–24} However, in those studies, a uni-directional liquid transport was performed with syringe pumps or large-scale actuators; there was no vigorous mixing during liquid transport, and this could lead to weak signals due to insufficient sample + probe mixing. Besides, vigorous mixing could accelerate hybridization and reduce incubation time. Herein, microarray and microfluidic technologies were integrated to form a composite system capable of detecting allergies; this chip comprised a flow control module, a temperature control module, and a packaging module. The present microfluidic chip also featured a pneumatically-driven micro-

pump for both liquid transportation and mixing. It was hypothesized that such a microfluidic system could outperform not only the SPT and ABT, but also the aforementioned microarray-integrated microfluidic chips.

The advantages of the developed approach over the existing methods for microarrays include (1) reduction of reaction time including less incubation time and fewer intermediate steps (~30%), (2) reduction of reagents (25%), (3) automation of the entire process and (4) compatibility of the existing microarray techniques such that they could be adopted with ease for the subsequent fluorescence detection using commercially available scanners. Most importantly, the simple assembling with currently available microarray slides, without any additional modification or bonding processes, could allow us to greatly benefit from both the advantages of microfluidics and microarray technologies.

Materials and methods

Experimental procedure

To detect the anti-allergen antibodies in the serum, the experimental procedures performed on the integrated microfluidic chip, which was composed of a microfluidic control chip and a microarray chip, are schematically shown in Fig. 1. The experiment protocols for allergen detection by using a commercially available allergy microarray chip were according to the manufacturer's instruction with minor modifications (Excelsior Bio-System Inc., Taiwan). Briefly, the allergens on the microarray chip were incubated with the serum sample for hybridization (Fig. 1(a) and (b)) and then washed with buffer solutions (A and B, sequentially) to remove unbound serum samples and non-specifically bound antibodies (Fig. 1(c)). Then, the resulting immune-complexes (allergen–antibody

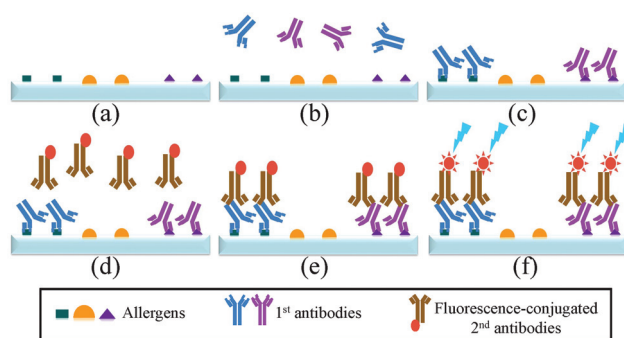


Fig. 1 Illustration of the experimental procedure performed on the integrated microfluidic chip, which was composed of a microfluidic control chip and a microarray control chip. The allergens were spotted on the glass substrate of the microarray chip (a), incubated with primary antibodies present in the serum (b), washed to remove unbound and/or non-specifically bound antibodies (c), hybridized with secondary antibodies conjugated to fluorescent dyes (d), washed again to discard excessive reagents and/or non-specifically bound antibodies (e), and the fluorescence intensity of the spots was measured by using a fluorescence scanner (f).

complexes) were incubated with fluorescence-conjugated anti-immunoglobulin secondary antibodies (2nd antibodies) (Fig. 1(d)). The excessive samples and non-specifically bound fluorescence-conjugated secondary antibodies were washed with buffer solutions again (Fig. 1(e)). The microarray chip was subsequently rinsed with reverse osmosis (RO) water and dried with nitrogen. Finally, the microfluidic chip and the microarray chip were detached and the fluorescence signals on the microarray chip were acquired by using a fluorescence scanner (InnoScan 710, Arrayit Inc., CA, USA). In accordance with the microarray manufacturer's suggestions, spots with signal intensities >2000 (a.u.) were deemed "positive" (*i.e.*, there were allergen-specific IgG antibodies in the serum). This guideline was designed for the on-bench protocols. For parallel comparison, the results obtained by the on-chip protocols also followed the same standard. Notably, there were several intermediate steps during manual operations, while they could be skipped by using the present microfluidic system. The time requirement of on-bench and on-chip methods is listed in ESI Table 1.† Briefly, the time reduction by using our developed system was ~30%.

The integrated microfluidic system

The microfluidic system for allergy detection included a microfluidic control module, a temperature control module, and a packaging module (Fig. 2). The microfluidic control module was used to control the flow of samples and reagents. It was composed of 30 electromagnetic valves (EMVs, VK332V-5G-M5, SMC Inc., Japan) connected to two compact, electro-pneumatic regulators (ITV0010-2 M, SMC Inc., Japan), and it was able to precisely regulate the target gauge pressure. Importantly, two different pressures were used for controlling micropumps and micro-valves; relatively low gauge pressures (−10 kPa) were applied to the micropumps to prevent their membranes from scraping allergen spots off the slides. In contrast, relatively high gauge pressures (−30 kPa) were applied to the micro-valves to prevent liquid leakage.

A custom-made temperature control module composed of one Peltier cooling/heating unit (TEC1-127.15, SMC Inc., Japan) was used throughout the entire hybridization process to maintain the temperature within the range of 37.0 ± 0.5 °C. Because the allergy antigen spots on the microarray chips were potentially vulnerable to traditional microfluidic bonding processes (*e.g.*, oxygen plasma treatment), a packaging module featuring pneumatic swing clamps (MKB12-10LZ-M9BWM and MKB12-10RZ-M9BWM, SMC Inc., Japan) was used to connect the cover and microarray chip to the microfluidic chip, and the composite unit was placed on the temperature control module during experiments (Fig. 2(c) and (d)). The detailed information about the integrated control system is found in the ESI.‡ No leakage of reagents or sera was observed at any time. In order to prevent light from bleaching the fluorescence signals, the entire system was placed in a black box. After the hybridization and washing steps, the microarray chips could be easily unclamped from the microfluidic chip and subsequently scanned. All procedures of this integrated microfluidic

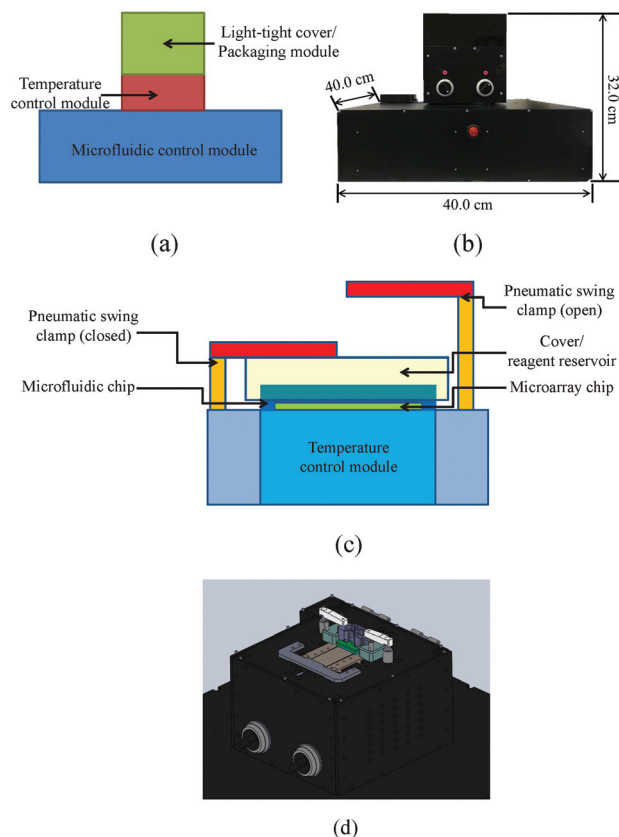


Fig. 2 The integrated microfluidic system coupled with an allergy microarray chip. (a) The composite unit was composed of three major modules, including a microfluidic control module, a temperature controller module, and a packaging module. (b) A photograph of the 40 × 40 × 32 cm (length × width × height) system. (c) A detailed schematic illustration of the packaging module, which was placed on top of the temperature control module. Pneumatic swing clamps were used to clamp the cover, microfluidic chip, and microarray chip together to avoid leakage. (d) An isometric view of the packaging module. EMV, electromagnet valve.

dic system were controlled by a computer and therefore automated such that no human intervention was required (except for the final scanning of the microarray chip).

Chip and loading cover design

The integrated microfluidic chip (Fig. 3) was composed of multiple micro-devices, including six loading chambers, one incubation micropump, and several microvalves. The incubation micropump aided in both mixing and transporting samples and reagents and was located above the microarray chip. Six independent loading chambers were designed to be loaded with (1) serum samples, (2) secondary antibodies, (3) wash buffer A, (4) wash buffer B, and (5) RO water (Fig. 3(a)). One of the six chambers was designed for use as a temporary inlet. The microvalves upstream of the reaction area were used to control the flow of samples and reagents. The entire experimental procedures for on-chip operation (as schematically illustrated in Fig. 1) are shown in Fig. 4. Briefly, all the

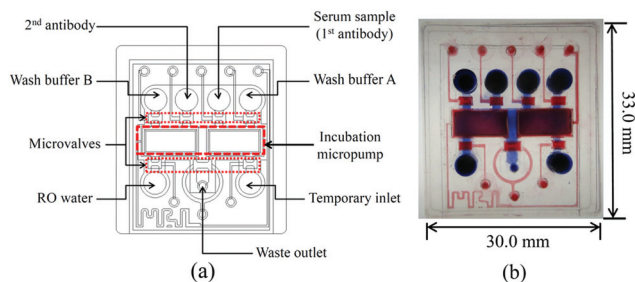


Fig. 3 (a) An integrated microfluidic chip equipped with multiple micro-devices, including six chambers (designed for loading with the antibodies/serum, wash buffers, and RO water prior to the reaction), one incubation micropump, and several microvalves. A photograph of the integrated microfluidic chip with dimensions of 33.0 mm $\times$ 30.0 mm (b).

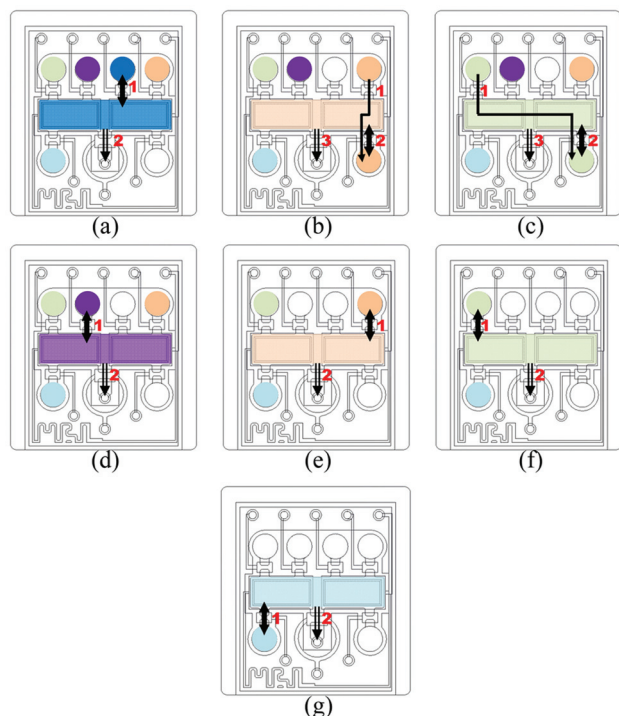


Fig. 4 Illustration of the reagent/sample flow on-chip during experimental procedures. (a) The serum sample was first transferred into the reaction zone, mixed for incubation, and pumped out. (b, c) Then, wash buffer was temporarily transferred to the bottom-right inlet, mixed for washing, and pumped out. (d) Secondary antibodies were subsequently transferred into the reaction zone and mixed for incubation. (e, f) The reaction zone was filled and mixed with wash buffers again. (g) Finally, RO water in the bottom-left chamber was transferred and mixed for rinsing.

samples/reagents preloaded in the corresponding chambers were sequentially transferred into the reaction zone which was aligned with the incubation micropump for mixing and washing in an automatic manner.

In terms of its construction, the 33 $\times$ 30 mm (length $\times$ width) microfluidic chip (Fig. 3(b)) was composed of a loading cover, two polydimethylsiloxane (PDMS) layers, and one micro-

array slide. The detailed information about the microfluidic and microarray chips (exposed views) is found in ESI Fig. 2.† Notably, there were microwalls around the reaction chamber and liquid channels on the backside of the thick PDMS layer that was in contact with the microarray slide (Fig. 5(b)). These microwalls (100 μ m in height) were designed to concentrate the pneumatic forces provided by the pneumatic swing clamps in order to prevent liquid leakage. The width of the chip was slightly longer than that of the microarray slide (Fig. 5(c) and (d)).

A polymethylmethacrylate loading cover was designed to not only seal the unit, but also serve as a reagent reservoir (Fig. 5), which prevented the possible analyte adsorption and also the diffusion of the non-cross-linked biomolecules. Regarding the former function, there were marginal, wing-like flaps on the right- and left-hand sides that acted as contact points for the pneumatic swing clamps (Fig. 2(d)). Because the microfluidic chip was relatively thin (4.5 mm in height), the loading cover was designed to encompass a sufficient volume to contain all samples and reagents: 150 μ L of serum + 150 μ L of secondary antibody + 900 μ L of wash buffer A + 900 μ L of wash buffer B + 150 μ L of RO water = 2.2 mL (minimum volume). The volumes of samples and reagents were 25% less compared with the standard manual operations on large-scale instruments (Excelsior Bio-System Inc., Taiwan). It is note-

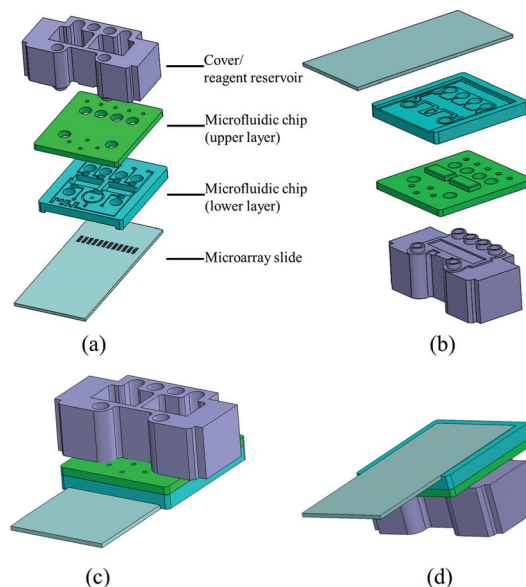


Fig. 5 Exposed views of the microfluidic and microarray chips from the top (a) and bottom (b). The complete unit was composed of a cover/reagent reservoir (grayish purple), two microfluidic chip layers (green and turquoise) and a microarray slide (grayish blue). The schematic illustrations of the assembled chips are also shown (c, d). Briefly, the upper layer and lower layer of the microfluidic chip were firstly bonded together with oxygen plasma treatment. The cover was subsequently aligned together with the microfluidic chip by using the via-hole structures on the cover. Later, the cover and the bonded chip were placed on top of the microarray slide aligned by the U-shape margin on the back side of the chip. Finally, they were placed and bonded on the temperature control module by the pneumatic swings, as shown in Fig. 2.

worthy that the backside of the loading cover embossed surrounding microstructures to the reaction zone, which may function as microwalls for avoiding possible leakage. In addition, six holes were made in the back of the loading cover such that it could be embedded into the loading chambers tightly to avoid any leakage of samples or reagents (Fig. 5(b) and (c)).

Allergy microarray chips, serum samples, and antibodies

A commercially available allergy microarray chip from Excelsior Bio-System Inc. (Taiwan) was integrated into a microfluidic chip. The microarray slides stored at 4 °C were assembled with the cover and microfluidic chips prior to the experiments. The construction of the microarray chip is described in detail below. Regarding the microarray, allergens (*i.e.*, the antigens) were coated on a glass slide, and there were 252 spots in four identical blocks (technical replicates). Each block contained 20 different antigens and mock-spotted PBS only (triplicate spots/antigen) in an 8 × 9 (rows × columns) array. These antigens included common allergens such as Der P and Der F from mites and food allergens (*i.e.*, banana, honey, cashew, peanut, wheat *etc.*) (Fig. S1†). The detailed information about the illustrations of the allergy microarray chip is found in the ESI.† Serum samples were purchased from AbBaltis Ltd (UK). To detect the presence of IgG and IgE, serum samples were diluted 10- and 3-fold, respectively. For detecting IgG, the anti-IgG secondary antibody, Alexa Fluor® 647 goat anti-human IgG, was obtained from Jackson ImmunoResearch Laboratories Inc. (USA) and diluted 1:200 into 1× phosphate buffer saline prior to use (final concentration = 0.1 µg ml⁻¹). To detect IgE, the anti-IgE secondary antibody, mouse monoclonal anti-human IgE, was obtained from Biocheck Inc. (Taiwan), conjugated to the Cy3 with the mono-reactive Dye Pack (GE Healthcare Life Sciences, UK), and diluted 1:10 into 1× phosphate buffer saline prior to use (final concentration = 0.2 µg ml⁻¹).

Statistical analysis

The coefficient of variation (CV [%]) was calculated across triplicate spots averaged over the 21 groups of allergens within the four identical blocks on a microarray slide. The precision across the replicate blocks was calculated as the number of positive spots found in all four blocks/total number of spots. A similar proportion was calculated for the consistently negative spots across the technical replicates.

Results & discussion

Characterization of the incubation micropump

A micropump above the reaction area was used to incubate the serum with the microarray chip spotted with the allergens; this micropump was also responsible for transporting reagents (Fig. 3(a)). While the micropump transported liquids back and forth, as shown in Fig. 4, it performed as a vigorous micro-mixer with high mixing efficiency.^{25,26} In particular, the present pneumatically-driven micropump was equipped with

right and left chambers, which were through the same via-hole with different lengths of routes such that there was a brief delay of PDMS membrane deflection (*i.e.*, uplift) between the right and left sides during the supply of air pressure. It allowed for the pump to transport fluids not only in a vertical orientation across the chip, but also horizontally (Fig. S2†). Therefore, it could perform the incubation process efficiently. The latter property likely aided in the incubation process. There was a 0.033 second delay in fluid influx between the right and left sides of the micropump at the applied pressure (Fig. 6); such horizontal flow may have increased the allergen-antibody hybridization efficiency and therefore led to the relatively high fluorescence signal intensities documented (discussed in more detail below).

In addition to the incubation process, the micropump could also transport samples and reagents. At a driving frequency of 1 Hz, the transport rate was found to increase with increasing applied gauge pressure from -20 to -30 kPa, at which point it reached a plateau (Fig. 7(a)). Notably, the standard deviation of the pumping rates was less than 0.44 µl s⁻¹ (<1% CV), indicating that our integrated microfluidic system precisely controlled the fluid flux.

Optimization of the operating parameters

In order to optimize the operating conditions for antigen array chip plus serum incubation, several parameters were investigated. Here we firstly detected the IgG in the sera because they were dominant and easy to be detected. Also, a standard serum sample which contained relatively high levels of allergic antibodies, which were determined by the on-bench protocols, was used in the present IgG testing. Compared to passive diffusion, pneumatically-driven micropumps can mix liquids vigorously,²⁵⁻²⁷ and these prior studies found that micropump

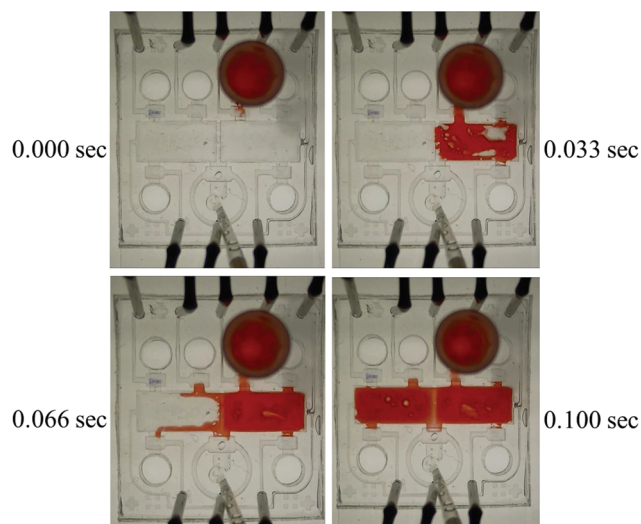


Fig. 6 A series of images of the delayed-action micropump. Water with red ink was injected into the loading chamber. The pneumatically-driven micropump was operated under a negative gauge pressure (*i.e.*, a vacuum), and the time course of liquid influx and flow between the right and left chambers was observed.

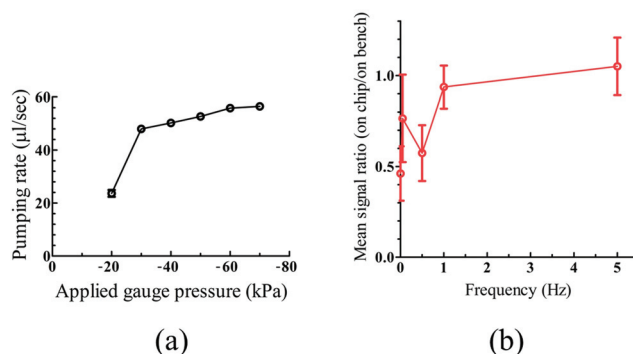


Fig. 7 (a) The pumping rate of the pneumatically-driven micropump under different applied gauge pressures. (b) The mean signal ratios (on-chip/benchtop) under different incubation frequencies. Error bars represent standard deviation ($n = 3$).

driving frequencies directly affect the mixing efficiency. After 30 minutes of hybridization, the signal ratio for the on-chip/benchtop method increased with increasing frequencies except at 0.5 Hz (Fig. 7(b)). This may be due to (1) the fact that this frequency was too low to achieve sufficient mixing, or (2) the short incubation time per pumping cycle (*i.e.*, not enough time for passive diffusion) at this frequency. Based on these results, a driving frequency of 5 Hz was chosen for the following experiments.

Previous studies revealed that pneumatically-driven micropumps provide efficient mixing for biological reactions and are capable of significantly reducing reaction times.^{26–28} As micropumps were used in the system designed herein, incubation times were generally short (<30 min). To explore whether the microarray fluorescence signal intensities were affected by incubation time in the developed system, hybridization times of 15, 20, and 30 min were tested (Fig. 8(a)–(e)). The signal intensities using microfluidic chips with a 30 min hybridization time (Fig. 8(a)) were slightly higher than those of the benchtop method (Fig. 8(f); ratio = 1.16). For parallel comparison and time reduction, the incubation time was shortened to evaluate the efficiency of the developed integrated microfluidic system. When incubated for 20 min, the signal intensities (Fig. 8(b); ratio = 0.95) were statistically similar. However, the signal intensity ratio for the 15 min incubation time (Fig. 8(c)) decreased to only 0.76, indicating that 20 min was the optimal incubation time for the hybridization performed on-chip.

Although higher gauge pressures could provide better liquid transport, such high pressures could also cause damage to the microarray. As shown in Fig. 8(a)–(d), many spots located in the middle of the chip were broken or had disappeared. When the negative gauge pressure for the micropump was lowered to -30 kPa, these spots were still intact (Fig. 8(e)). In addition, the mean signal ratio under this pressure after 20 min hybridization was almost comparable to that under a gauge pressure of -50 kPa after a 30 min incubation (Fig. 9(a)). Therefore, the operating conditions of -30 kPa for 20 min resulted in good signal intensities with few damaged spots.

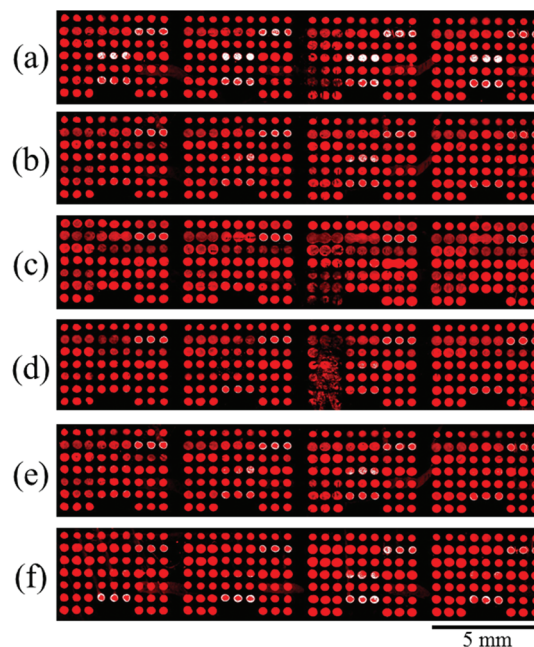


Fig. 8 The fluorescence signals of the microarray chips using on-chip (a–e) and benchtop methods (f). These microarrays were incubated with serum for 30 (a), 20 (b), or 15 (c) min under an applied micropump gauge pressure of -50 kPa, or for 20 min under an applied micropump gauge pressure of -40 (d) or -30 kPa (e). The signal intensities were compared to those obtained using the benchtop methods (30 min incubation/hybridization time (f)).

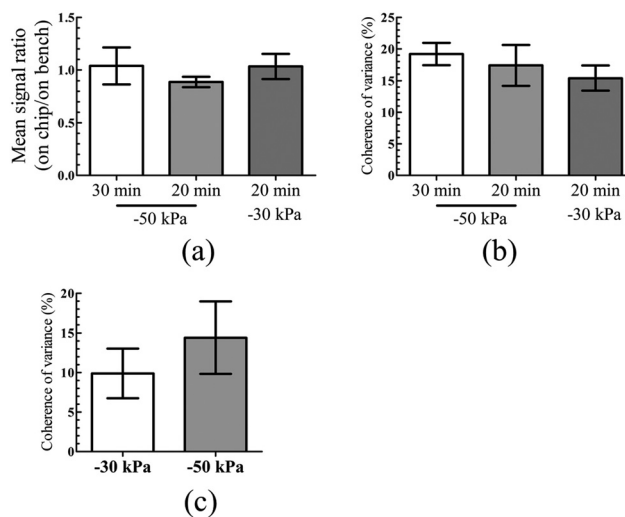


Fig. 9 (a) The mean on-chip/benchtop fluorescence signal intensity ratios under different allergen plus serum/antibody incubation times and applied gauge pressures. (b) The mean coefficients of variation across triplicate spots for samples incubated with allergen microarrays for different times under different gauge pressures. (c) The mean coefficients of variation across triplicate spots on the third microarray block under a variety of different applied gauge pressures ($n = 3$ or 4 for each group). Error bars represent standard deviations.

Table 1 Methods comparison. The cutoff value for a “positive” signal was a fluorescence intensity greater than or equal to 2000 (a.u.). Error terms represent standard deviation ($n = 3$)

Method	# of positive signals	# of negative signals
Benchtop protocol (30 min)	240 ± 0	12 ± 0
On-chip (30 min, −50 kPa)	237 ± 2	15 ± 2
On-chip (20 min, −50 kPa)	237 ± 2	15 ± 2
On-chip (20 min, −30 kPa)	239 ± 0	13 ± 0

Table 1 shows the results of the microarray experiment (from Fig. 8). In general, the results were similar between the on-chip (20 min, −30 kPa) and benchtop methods. It is worth noting that each allergen was spotted in triplicate in one block area (Fig. S1†) to confirm the reproducibility of the results. The CV across triplicate spots ranged from 10 to 20% with the benchtop method (data not shown), similar to that obtained on-chip (~15%; Fig. 9(b)). The CV of the third block (Fig. S1†), which was damaged under gauge pressures of −50 kPa, was only 9.9% at −30 kPa (Fig. 9(c)). Based on these data, the optimal conditions for hybridization were determined to be −30 kPa for 20 min.

IgE testing

The presence of anti-allergen IgE antibodies in the serum was the major indicator to tell whether a person is allergic to a particular substance.^{1–3} Therefore, the presence of IgE antibodies in the serum was also tested herein using the developed integrated microfluidic system under the aforementioned optimized operating parameters. In order to confirm that our on-chip protocols could be practicable for real situations in the clinic, serum samples from four different individuals were tested. Fig. 10 displays the resulting signals of the sera incubated with antigens spotted on the microarray chips using either on-chip or on-bench protocols. The fluorescence signal intensities provide a quantitative indication of how severe a patient's allergy is to a potential allergen, such as Der P and Der F from mites (Fig. S1†). The signal intensities were strong

enough to be visualized for diagnosis (>200 a.u.; Fig. 10); they were 1.29 ± 0.32 -fold higher than those of the benchtop protocol ($n = 12$ repeated experiments), with no obvious difference. The partial raw data of on-bench and on-chip results are shown in ESI Fig. 3.† The total number of positive spots using the on-chip method was almost the same as with the benchtop method ($99.0 \pm 1.1\%$). However, the former method used less sample material, fewer reagents, and required less technical skill and hands-on time compared to the benchtop protocol.

Conclusions

Nowadays, microarray technologies are maturing to overcome the limitations of traditional methods for allergy detection. For instance, ImmunoCAP®^{29,30} is available in the market. This kind of ABT-based method has become a widely used commercialized product. Nevertheless, these systems have limitations regarding sample handling, operation time, bulky equipment, and technician requirement. Although microarray technology provides high-throughput detection with multiple allergen dots on a small slide (*i.e.*, a commercially available kit from Excelsior Bio-System Inc. in Taiwan provides 66 different allergens for detection), manual operations and bulky equipment are still inevitable. This study has demonstrated an integrated microfluidic system to automate the entire process, including hybridization, mixing, reagent transportation, and washing for the detection of allergies by using commercially available microarrays. The developed system could decrease operation time significantly and achieve comparable results when compared to the on-bench method performed by well-trained personnel. Notably, the operation time was reduced by approximately 30%, and the consumption of samples and reagents was decreased by 25%.

Moreover, the detachable approach between the microarray slides and microfluidic chip made it easy to separate for scanning the resulting signals. Indeed, the present system did not integrate the signal detection since the microarray signal detection had been well established and is commercially available. There are several decent papers in the literature demonstrating the automated systems that integrated sample/reagent processing, and even signal detection.^{31–33} This is the first work to use it for microarrays. We also would like to design an “open” system such that all microarrays could be adopted. Therefore, we did not integrate the fluorescence scanning module in this work. The different applications require different automated microfluidic sample/reagent transportation and incubation processes. For microarrays, relatively low efficiency of sample transportation/incubation/washing processes by manual operation is the major bottle neck of existing microarray technology. Herein, we demonstrated that an integrated microfluidic system could perform these tedious processes automatically within a short period of time. Most importantly, the simple assembling with currently available microarray slides, without any additional modification or

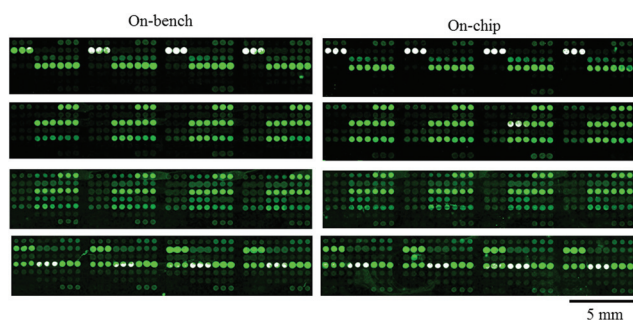


Fig. 10 The IgE fluorescence signals of the microarray chips using benchtop (left panel) and on-chip (right panel) methods. Four different batches of sera were tested, and there were four replicate arrays for each serum sample.

bonding processes, could allow us to greatly benefit from both advantages of microfluidics and microarray technologies. The reduction of reagents and labor by using the developed integrated microfluidic system may push the limitation restrained by manual operations, which provided a comparable evaluation (without misleading results from too high signal levels) of allergy severity for diagnosis. Thus, the present integrated microfluidic system could be easily extended for automating multiplex detection on allergy microarray chips. It would dramatically accelerate the allergy detection and reduce the labor costs with no human bias.

Conflicts of interest

There are no conflicts to declare.

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Supplemental information

An automatic integrated microfluidic system for allergy microarray chips

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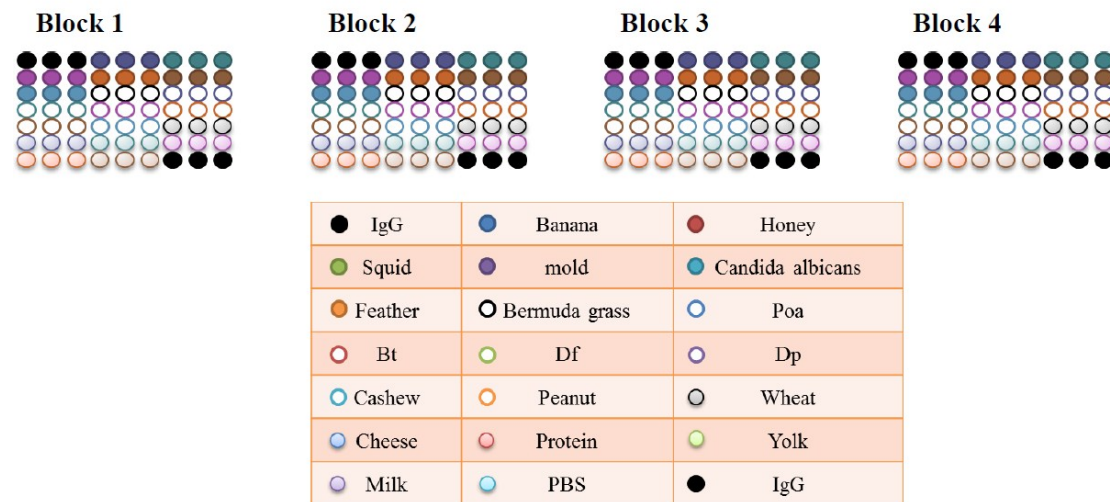


Fig. S1. Illustrations of the allergy microarray chip. There were four identical blocks, each containing twenty kinds of allergens (or control immunoglobulin G [IgG] spots) in triplicate spots. Poa, allergens from *Poa annua*; Bt, allergens from *Bacillus thuringiensis*; Df and Dp, Der p and Der F peptidases from mites; PBS, phosphate buffered saline.

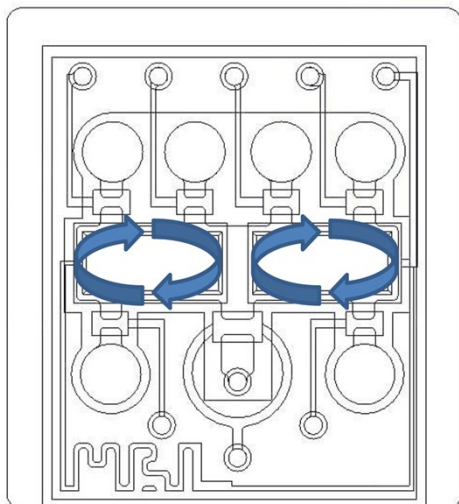


Fig. S2. Illustration of the vortex flows generated in the micropump. The video was available online: <http://xxxxxx>

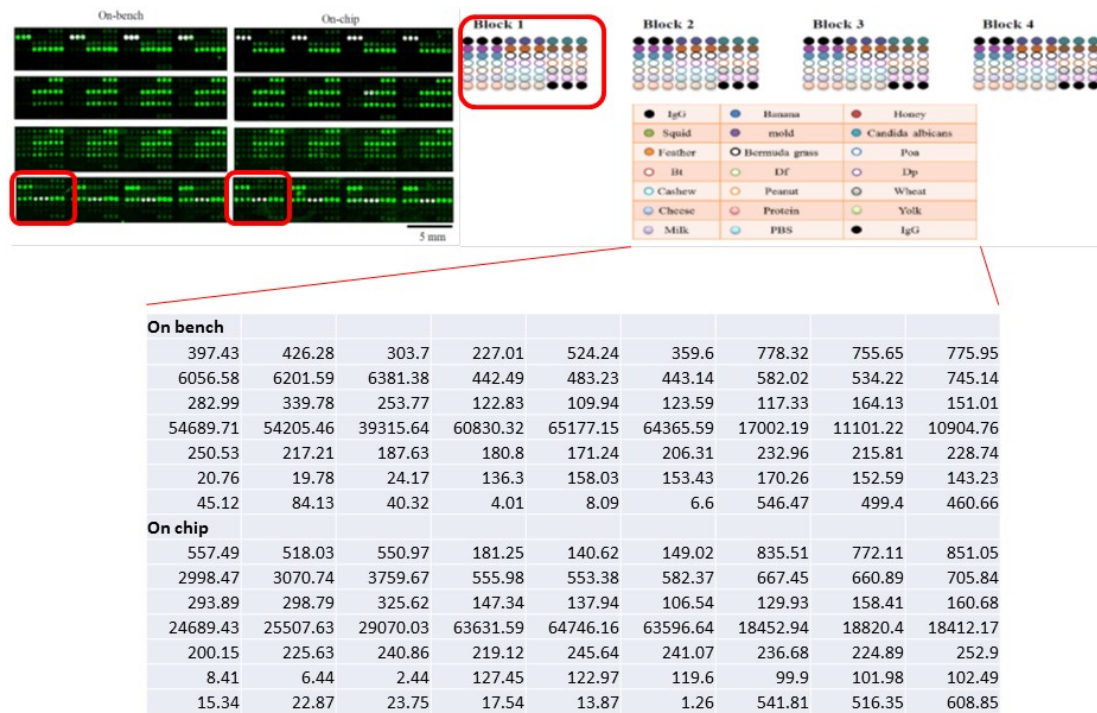


Fig. S3. The fluorescence intensities (a. u.) from Fig. 10. The raw data of spots in block 1 area in the fourth test (red rectangle; bottom row) were shown. The fluorescence intensities (a. u.) were almost comparable between the on-bench and on-chip tests.

Table S1. Methods comparison.

Sequential procedure	On bench (time)	On chip (time)	Note
1. Serum-allergen hybridization	30 min	30, 20, or 15 min	
2. Remove slides from a shaker, briefly dry, put it back, and add reagents	~2 min	N/A	
3. Wash	5 min	5 min	3 times
4. Remove slides from a shaker, briefly dry, put it back, and add reagents	~2 min	N/A	3 times
5. 2 nd antibody hybridization	30 min	30, 20, or 15 min	
6. Remove slides from a shaker, briefly dry, put it back, and add reagents	~2 min	N/A	
7. Wash	5 min	5 min	3 times
8. Remove slides from a shaker, briefly dry, put it back, and add reagents	~ 2 min	N/A	3 times
9. RO rinse	2 min	2 min	2 times
Total	~110 min	94, 74, or 64 min	
Time reduction		~15%, ~33%, or ~42%, respectively	