

Microfluidic methods for precision diagnostics in food allergy

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ABSTRACT

Food allergy has reached epidemic proportions and has become a significant source of healthcare burden. Oral food challenge, the gold standard for food allergy assessment, often is not performed because it places the patient at risk of developing anaphylaxis. However, conventional alternative food allergy tests lack a sufficient predictive value. Therefore, there is a critical need for better diagnostic tests that are both accurate and safe. Microfluidic methods have the potential of helping one to address such needs and to personalize the diagnostics. This article first reviews conventional diagnostic approaches used in food allergy. Second, it reviews recent efforts to develop novel biomarkers and *in vitro* diagnostics. Third, it summarizes the microfluidic methods developed thus far for food allergy diagnosis. The article concludes with a discussion of future opportunities for using microfluidic methods for achieving precision diagnostics in food allergy, including multiplexing the detection of multiple biomarkers, sampling of tissue-resident cytokines and immune cells, and multi-organ-on-a-chip technology.

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I. INTRODUCTION

A. Food allergy is increasingly prevalent

Food allergy has reached epidemic proportions and has become a serious public health concern. In the USA, the prevalence is estimated to be 8% in children and 11% in adults.^{1–3} Prevalence data in other countries are not always available. However, from studies carried out so far, food allergy prevalence ranges from 0.1% to 6% in Europe,⁴ 11% and 4% at ages 1 and 4, respectively, in Australia,⁵ and 1%–6% in children in certain Asian countries.⁶ Despite the lack of fully confirmed data, the trend is clear that food allergy is increasingly common worldwide. In the USA, more than 170 foods have been reported to be allergenic. However, eight foods are responsible for 90% of food allergies: milk, egg, peanut, tree nuts, wheat, soy, fish, and crustacean shellfish.^{7,8} Sesame is now emerging as the ninth most common allergen that causes food allergy.⁹ Allergy to multiple foods is also common: 40% of the patients are allergic to more than one food.¹⁰

Food allergy develops as early as infancy and early childhood¹¹ and, depending in part on the food, can last a lifetime. Symptoms range from mild-to-moderate (e.g., angioedema of the lips, eyes, or

face; eczema; hives; and vomiting) to severe and life-threatening (e.g., severe trouble breathing, wheezing, and anaphylaxis).^{1,12} These reactions can be triggered by the ingestion of the offending food, inhalation, or skin contact.¹³ In the USA, 40% of children with food allergy have experienced severe reactions.¹ Food induced anaphylaxis has increased emergency room visits by 214% between 2005 and 2014, with the highest rates in children between 0 and 2 years of age,¹⁴ and is estimated to result in 100–200 deaths a year.¹⁵ Food allergy not only influences the health and quality of life of the patients but also that of their caregivers. The corresponding cost is significant: the overall economic burden of caring for food allergy in children alone is estimated to be \$24.8 billion/year in the USA alone.³

B. What is food allergy?

According to the 2010 Expert Panel Report sponsored by the National Institute of Allergy and Infectious Diseases (NIAID), food allergy is defined as an adverse health effect arising from a specific immune response that occurs reproducibly on exposure to a given food.⁸ It is classified into IgE-mediated, non-IgE-mediated, and

mixed (IgE- and non-IgE-mediated). IgE-mediated food allergy is substantially more common and can result in anaphylaxis.¹⁶ The rest of this review will, thus, focus on IgE-mediated food allergy.

For IgE-mediated food allergy, contact with, or ingestion of, the allergen triggers the production of IgE antibodies specific to the allergen and the binding of these IgEs to FcεRI (high-affinity IgE receptors) on the surface of mast cells and basophils during allergic sensitization. In allergic patients, the subsequent exposure to the allergen causes the cross-linking of FcεRI receptor-bound IgEs and leads to the degranulation of mast cells and basophils with the rapid release of stored mediators and other molecules to initiate an allergic reaction. The etiology of the disease is an active area of research. For details, the readers are referred to previous reviews.^{12,17,18}

Currently, there is no clinically proven way to prevent food allergy in all allergic subjects. However, recent studies have shown that early exposure to diverse foods during infancy may lower the risk of allergies to some foods.^{19–22} There is also no definitive treatment for food allergy. The current standard of care is food avoidance. Pharmaceuticals such as anti-histamines, glucocorticoids, and epinephrine treat the symptoms of food allergy but do not treat the underlying immune disorder.¹⁸ With increasing understanding of the mechanisms underlying food allergy, much progress has been made to develop immunotherapy strategies for treatment. Desensitizing immunotherapy involves administering the offending allergen to the patient in small dosages, which are gradually increased over a period of days, weeks, or months. The desensitizing antigens are typically delivered orally (OIT), sublingually, or epicutaneously (EPIT). On January 31, 2020, Palforzia (or AR101), a standardized peanut powder, became the first OIT biologic to be approved by the FDA to treat peanut allergy.^{23,24} Monoclonal antibodies also have been developed as therapeutic agents in recent years. Omalizumab, for example, binds to the Fc region of IgE and blocks its binding to FcεRI to prevent the degranulation of mast cells and basophils.¹² Promising results have been obtained by combining omalizumab with OIT in accelerating the desensitization to food allergens.¹² For more details of such immunotherapeutic strategies, readers are directed to previous reviews.¹⁸

C. Microfluidic methods for disease diagnostics, management, and treatment

A wide range of methods based on microfluidics and micro-nanotechnology have been developed to facilitate disease diagnostics (e.g., biomarker discovery and validation, sampling, and detection of biomarkers), management (e.g., detection of offending agents that would trigger the disease or symptoms), and treatment (e.g., drug screening, delivery of drugs for treatment of symptoms or disease). For food allergy, most microfluidic devices developed thus far have focused on the detection of known biomarkers to facilitate a diagnosis and the detection of offending allergens in food. The latter is important, since food avoidance is the current standard of care. Detecting allergens in foods in a timely manner at the point of ingestion is thus critical, especially when there are uncertainties about the ingredients and/or preparation methods. A number of microfluidic methods have been developed for rapid allergen detection, which is covered by a recent review²⁵ and will not be repeated here. The remainder of this article will focus on diagnostic approaches in food

allergy, reviewing both conventional and microfluidic methods developed thus far, challenges, and opportunities ahead.

II. OVERVIEW OF CONVENTIONAL FOOD ALLERGY DIAGNOSTIC APPROACHES

Just like in any other disease, the accurate and timely diagnosis of food allergy is critical. However, unlike in other diseases, the accurate and rapid diagnosis of food allergy can literally be life-saving. An ideal, precision diagnostic for food allergy would allow (1) accurate identification of clinical subtypes of the disease, (2) precise determination of the offending allergens (so as to avoid unnecessary dietary elimination of safe foods and to avoid re-exposure to the true culprit), (3) definition of the severity of an allergic reaction upon exposure to the offending allergen, and (4) assessment of the patient's likelihood to respond to different treatment options and the associated risks of adverse effects to such interventions.²⁶ Such an ideal diagnostic requires (1) a set of biomarkers that can accurately reflect the disease risk, status, and a need for intervention, and (2) a way to measure these biomarkers in a manner that is accurate, non-invasive, rapid, and cost-effective.

Unfortunately, conventional food allergy diagnostics are far from ideal. Indeed, there is no simple single test for food allergy. The first-line approach is clinical history and physical examination. A careful assessment of dietary history and associated symptoms can establish the likelihood of a food allergy diagnosis, determine whether an IgE or a non-IgE mechanism is involved, and identify potential offending foods. Additional diagnostic tools include skin prick test (SPT), serum IgE (sIgE) blood test, and oral food challenge (OFC). [Table 1](#) summarizes aspects of SPT, sIgE, and OFC. We have also included the Medicare reimbursement costs of these tests for reference. We note that these costs will differ depending on the state, country, and the local insurance or payment policy.

The gold-standard for food allergy diagnosis is a double-blind placebo-controlled, oral food challenge. A food challenge involves administering the suspected food allergen to the subject orally and monitoring whether an allergic reaction occurs. However, it places the subject at risk for developing anaphylaxis.^{27–30} In one study, it was found that 28% of patients developed severe reactions, including lower respiratory or cardiovascular symptoms, in failed food challenges.²⁹ OFC, therefore, requires supervision by experienced allergists and is both time- and resource-intensive. As such, SPT and sIgE have been used as surrogate tests to predict the likelihood of food allergy and to determine whether OFC is necessary. However, a positive result from either SPT or sIgE is insufficient to diagnose food allergy, as the subject may be sensitized (i.e., possess allergen-specific IgE) but may not display clinical symptoms after exposure to the foods. SPT and sIgE tests, thus, should always be combined with the assessment of the clinical history. A number of alternative tests have also emerged that are minimally invasive, but many are unproven scientifically or are non-standardized and thus are not recommended, including hair analysis, facial thermography, and electrodermal testing.³¹ Consequently, current food allergy tests can be represented qualitatively along the diagonal of a graph plotting test accuracy vs potential risk of an adverse reaction during the test ([Fig. 1](#)).

TABLE I. Comparison of aspects of skin prick test, serum IgE test, and oral food challenge.^{105,106} The Medicare reimbursement costs listed are the unadjusted national schedule fees for Medicare as of February 2020. The actual cost may vary based on state and facility.¹⁰⁷

	Skin prick test (SPT)	Serum IgE (sIgE)	Oral food challenge (OFC)
Principle of the test	Allergen introduced to outer skin activates mast cells to cause wheal-and-flare reactions if the person is allergic	Measures the amount of circulating allergen-specific IgE antibodies in serum using immunoassays	Increasing doses of a potential food allergen are ingested over fixed time intervals, until either an allergic reaction is observed or a maximum dose is reached
Sample tested	<i>In vivo</i> skin test	<i>In vitro</i> blood test (~10–40 μ l of serum per allergen tested) ¹⁰⁸	<i>In vivo</i> oral ingestion of food allergen
Time to result	10–20 min	~5 min (blood draw); generally, 1–3 days (to send the sample to the lab and receive the results)	3–5 h per allergen tested. Patients need to remain in the clinic for 2 h after the challenge to monitor if any delayed adverse clinical reaction occurs. For multiple allergens, a period of at least 2 h should separate oral food challenges. ¹⁰⁹
Accuracy	• A positive SPT result produces a wheal-and-flare reaction within 15 min after introducing the allergen, with wheal diameter > ~3 mm. • Highly sensitive (i.e., low rate of false-negative); moderately specific (but there is a high rate of false-positive) for most foods. • For peanut: sensitivity ~95% and specificity ~61%.¹⁰⁵ A positive SPT result only reflects the response of cutaneous mast cells; SPT alone cannot confirm a diagnosis.	• The threshold for a positive result varies depending on food and patient demographics. • Highly sensitive (i.e., low rate of false-negative); moderately specific (relatively high rate of false-positive) for most foods. • For peanut: sensitivity ~96% and specificity ~59%.¹⁰⁵ • This test indicates the presence of IgE sensitization to a specific food, but this alone is not always linked to clinical reaction to the food. Thus, IgE test alone cannot confirm a food allergy diagnosis.	Double-blind, placebo-controlled, OFC is considered the gold standard of food allergy diagnosis.
Risk of adverse reaction	Relatively safe	Risk level identical to that of a blood draw	Risk of developing a severe allergic reaction, including anaphylaxis
Medicare reimbursement costs	Physician fee (non-facility): \$4.33 per allergen (CPT code: 95004)	Lab fee schedule: \$5.22 per allergen (CPT code: 86003)	Physician fee (non-facility): \$121.62 per allergen (CPT code: 95076)

Figure 1 illustrates the critical need for a better food allergy diagnostic that is both accurate and safe. Meeting such a need requires both innovations in biomarkers that can predict the disease state accurately and methods to detect these biomarkers accurately, rapidly and non-invasively. In the following sections, we will review the recent work on emerging biomarkers (Sec. III) and microfluidic methods to detect existing food allergy biomarkers (Sec. IV).

III. EMERGING BIOMARKERS AND *IN VITRO* DIAGNOSTICS OF FOOD ALLERGY

Much progress has been made recently to identify new biomarkers for food allergy diagnostics, especially for *in vitro* tests that minimize adverse reactions during the test. In particular,

Component Resolved Diagnosis (CRD) and Basophil Activation Tests (BATs) have shown promising results and have been increasingly adopted in research and in some clinical settings.

CRD is a sIgE test that measures the binding of serum IgE to purified or recombinant allergenic proteins (e.g., Ara h 2) instead of to natural food extract (e.g., peanut powder). The use of specific food proteins of known quantities can provide more precise diagnostic information than using a complex mixture of proteins with unknown quantities in food extracts, as it can differentiate allergenic, non-allergenic, and cross-reactive proteins in standardized amounts. Although CRD still has to be validated in a wider range of foods, it has shown particular promise for peanut allergy. It was found that Ara h 2 is the most common peanut protein associated with clinical reactivity.² sIgE to Ara h 2 has 95% specificity and

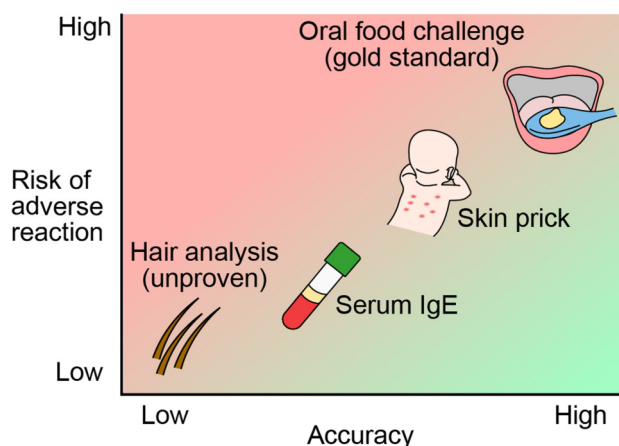


FIG. 1. Common methods for diagnosing food allergy present a trade-off between accuracy and the risk of adverse reaction. The double-blind placebo-controlled oral food challenge is considered the gold standard for food allergy assessment, but it places patients at the greatest risk of developing an adverse reaction. Both skin prick tests and allergen-specific sIgE tests are used as surrogates, but these can only indicate sensitivity to an allergen and have low predictive value for a true allergic reaction. Alternative methods such as hair analysis are minimally invasive but are largely unproven and are not recommended.

73% sensitivity [at a threshold level of 0.46 kU/l, where 1 U (unit) equals 2.42 ng of IgE], whereas sIgE to peanut has 95% specificity but only 44% sensitivity (at a threshold level of 6.2 kU/l).³³ However, similar to conventional sIgE tests, the threshold for a positive result varies depending on patient demographics and other confounding factors.

BAT is a cell-based assay that measures the activation or degranulation of basophils in whole blood upon stimulation with allergens *ex vivo*.^{34,35} It is a functional assay and relies not only on the level of sIgE and the binding of IgE to allergen, but also on IgE epitope specificity, affinity, and clonality.³⁶ Upon stimulation with allergen, basophils from an allergic patient degranulate, thereby upregulating the surface levels of activation markers such as CD63 and CD203c. The extent of increase in upregulation has been found to correlate with the severity of allergic reactions.^{37–40} In contrast, a non-allergic subject has a much lower expression of such markers after stimulation with allergen. Recently, it was found that the binding of avidin, a positively charged molecule, was also predictive of basophil activation, as the degranulation process exposes proteoglycans that cause a large increase in negative charge on the cell surface.^{41,42} Studies carried out thus far have shown that the BAT has high accuracy, with specificity of 75%–100% and sensitivity of 77%–98% for a range of foods including peanut, cow's milk, and egg.^{12,36,39,43–48} In a study that used a stepwise diagnostic approach in patients considered for OFC after SPT and sIgE tests, a positive BAT result confirmed the diagnosis of food allergy and reduced the number of OFCs needed by 66%.⁴³ A note of caution about the BAT is that 10%–15% of OFC-proven food allergic patients are “non-responders,”⁴⁵ who have negative BAT results with no upregulation of CD63 or CD203c toward allergens that cause reactions by OFC.^{40,49} Basophils from non-responders usually

respond to non-IgE-mediated stimulants, however.⁵⁰ To identify such non-responders and prevent false negatives, subjects with a negative BAT result should be tested further using non-IgE-mediated stimulants (e.g., fMLP or ionomycin). Finally, some groups have exploited a type of “indirect” BAT, employing basophils from non-allergic donors, tested either with⁵¹ or without⁵² stripping of native IgE from the basophils' surface.

Recently, a mast cell activation test has been developed, where mast cells (from LAD2 cell line⁵³ or derived from primary human blood using CD117⁺CD34⁺ peripheral blood precursors⁵⁴) are cultured, sensitized with patient serum, and then incubated with allergen. Activation is measured by the percent of cells that are CD63⁺ or CD107a⁺, or by the release of β -hexosaminidase or prostaglandin D₂. In a pilot study (n = 42), a sensitivity of 97% and a specificity of 92% were obtained.⁵⁴ While these results are promising, it is necessary to recognize that the activation of immune cells is tightly regulated and can be modified by cytokines and other molecules in the microenvironment. Cultured mast cells might mimic the behavior of *in vivo* tested mast cells in some assays, but they are distinct from human mast cells *in vivo*. The biological responses (e.g., degranulation ability) of *in vitro*-maintained human mast cells can also vary depending on culture conditions.^{55,56}

In addition to the above assays, a significant number of new biomarkers are being developed, including T-cell and B-cell activity and “-omics” markers. Increasingly, bioinformatics and machine learning approaches are also used to assess multiple markers and identify correlations for improved diagnostics. Readers are directed to previous reviews for details.^{57,58}

IV. MICROFLUIDIC METHODS FOR *IN VITRO* DIAGNOSTICS OF FOOD ALLERGY

In addition to identifying better biomarkers for food allergy diagnostics, it is equally important to be able to detect these biomarkers accurately, rapidly, and non-invasively. Microfluidics is particularly well-suited for this purpose. The microfluidic implementation of sIgE tests is the most established, with many systems already commercialized. A number of them are also compatible with rapid point-of-care diagnosis, with the time to result from 5 min to tens of minutes, as detailed in Sec. IV A and Table II. Other schemes of detection are still at the research phase and have yet to be validated clinically. There has also been some work to develop dissolvable microneedles for skin prick tests to facilitate the delivery of antigens and reduce pain.⁵⁹ However, the readout still relies on measuring wheal size *in vivo*, and such methods have similar limitations as those of conventional skin prick tests. In this section, we will focus on microfluidic methods used for *in vitro* diagnostics of food allergy.

A. Microfluidic sIgE assays

Most microfluidic applications for food allergy diagnostics have focused on miniaturizing and multiplexing sIgE tests against multiple allergens. The methods basically perform the immunocapture of sIgE onto channel surfaces or particles that are functionalized with allergens. To detect the immobilized sIgE, ELISA (enzyme-linked immunosorbent assay) is the most common method, where the readout signals can be fluorescent, chemiluminescent, colorimetric, or electrical [Fig. 2(a)].

TABLE II. Comparison of various microfluidic methods and commercial products for sIgE detection.

Reference	Principle of operation	Number of allergens tested	Limit of detection	Time to result	Sample volume
Sinurat <i>et al.</i> ¹¹⁰ Tai <i>et al.</i> ¹¹¹ Shyur <i>et al.</i> ¹¹²	Microfluidic ELISA array with chemiluminescent readout	Parallelizable up to 40 allergens	2.4 ng/ml	30 min to read, 2–3 h to prepare sample and analyze	100 μ l of serum
Cretich <i>et al.</i> ⁶¹ Huang <i>et al.</i> ¹¹³	sIgE immobilization by allergen and direct fluorescent labelling sIgE immobilization by coated allergen and direct fluorescent labelling	Six recombinant allergens 20		25 min	25 μ l of serum 150 μ l of serum
Heyries <i>et al.</i> ⁶⁰	sIgE immobilization by coated allergen and chemiluminescence	5	33 pM, 6.6 pM, and 66 pM for anti-peanut lectin, anti- β -lactoglobulin, and anti-human IgG, respectively	~1 h total	5 μ l of serum diluted 10 times in buffer
Hiller <i>et al.</i> ³²	Allergen microarray on glass chips with fluorescently labelled anti-human IgE antibodies	78 recombinant and 16 natural allergen molecules		3.5 h plus chip scanning time	200 μ l of serum for all 94 allergens in triplicates (~2.1 μ l/allergen)
Proczek <i>et al.</i> ⁶³	Magnetic bead immunocapture and sandwich ELISA with electrochemical detection	1	17.5 ng/ml	<1 h	10 μ l serum per sample. Capable of measuring eight samples per run
Ohashi <i>et al.</i> ⁶²	Stop-flow ELISA with allergen immobilization on polystyrene beads		2 ng/ml	12 min	5 μ l serum per allergen
<i>Commercial devices</i>					
Phadia TM ImmunoCAP ⁶⁴ Goikoetxea <i>et al.</i> ¹¹⁴ Shyur <i>et al.</i> ¹¹²	Automated ELISA using β -galactosidase/methylumbelliferyl- β -D-galactoside	>650 allergens and 90 allergen components ⁶⁴	0.24–240 ng/ml	3 h	40 μ l serum per allergen ⁶⁴
Phadia TM ImmunoCAP Rapid ⁶⁷	Lateral flow based sIgE immobilization and colorimetric readout	10 allergens	>2.4 ng/ml	20 min	110 μ l blood total
Hycor TM HYTEC 288 ¹¹⁵	Automated ELISA plate reader using alkaline phosphate/p-nitrophenyl phosphate	288 allergens, parallelizable up to 50 patient samples	7 IU/ml (16.8 ng/ml)	65 min	
Siemens TM IMMULITE 200 XPi ¹¹⁶ Abionic TM ⁶⁸	Quantitative chemiluminescent enzyme immunoassay for sIgE Lateral flow based sIgE immobilization with an integrated filter separating the sample introduction and IgE capture regions.	>450 allergens and up to 200 tests/h Fel d 1 (cat dander), Can f 1 (dog dander), Bet v 1 (birch pollen), Phl p 5 (timothy grass pollen), and Der p 1 (house dust mite)	0.1 kU/l 1.4 ng/ml	65 min (200 tests/h) 5 min	50 μ l serum per allergen ^{65,116} 50 μ l of blood per allergen

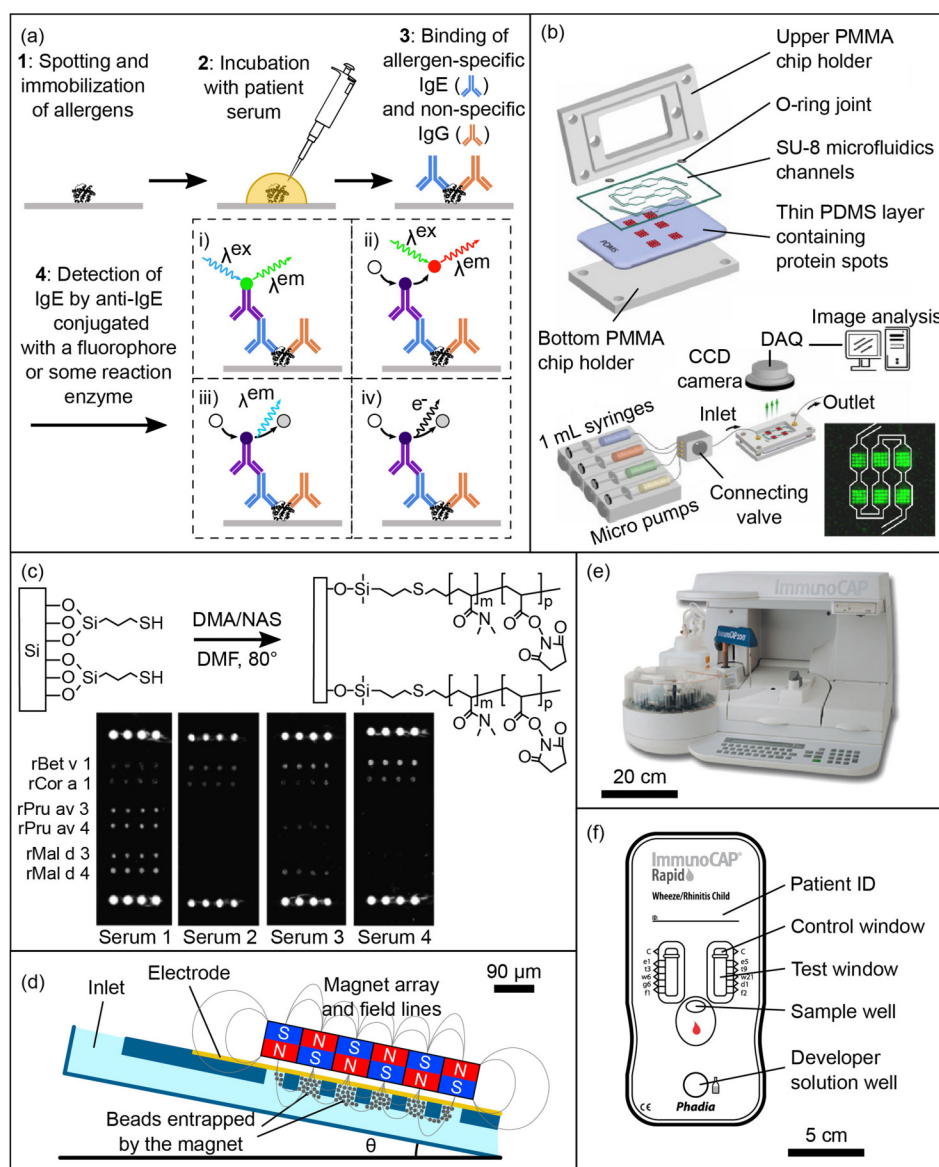


FIG. 2. Examples of sIgE tests. (a) Overall scheme of sIgE capture and detection. Various forms of generating a detectable signal from bound anti-IgE include: (i) measurement of direct fluorescence, (ii) enzyme-catalyzed generation of fluorescent or colorimetric molecules, (iii) enzyme-catalyzed reaction producing chemiluminescence, and (iv) enzyme-catalyzed redox reaction. (b) A microarray-based method to capture sIgE onto antigens, which are transferred to a polydimethylsiloxane (PDMS) layer from pre-spotted antigens on a glass substrate. Serum is injected into a microfluidic channel aligned on top of the antigen arrays held together with poly(methyl methacrylate) (PMMA) supports. Reproduced with permission from Heyries *et al.*, Biosens. Bioelectron. **23**(12), 1812–1818 (2008). Copyright 2008 Elsevier B.V. An ELISA reaction involving HRP-conjugated anti-IgE sandwich antibodies and luminol produces a measurable chemiluminescent signal. (c) Another implementation of the microarray method uses a novel MPS-poly[DMA-co-NAS] copolymer to immobilize allergens which, in turn, capture sIgE from serum for direct fluorescent labelling with Cy3 conjugated anti-IgE. The top and bottom rows show reference controls for IgG capture. Negative controls (not shown) are included by using PBS instead of a capture allergen. Reproduced with permission from Cretich *et al.*, Proteomics **9**(8), 2098–2107 (2009). Copyright 2009 John Wiley and Sons. (d) Magnetic beads are functionalized with a specific allergen that captures sIgE. These beads are injected into a channel tilted by some angle θ . Beads are concentrated by an applied magnetic field gradient and are pulled against an electrode. Measured electrochemical potential changes due to the reaction between alkaline phosphatase (conjugated to the sandwich antibody) and para-aminophenyl phosphate correlate to the amount of sIgE bound to magnetic beads. (e) The ImmunoCAP system uses a sandwich ELISA to measure total IgE and sIgE. Capture allergens or anti-IgE are covalently attached to cellulose solid substrate coated test tubes. Reproduced from Phadia.com with the permission of Thermo Fisher Scientific. (f) The ImmunoCAP Rapid system uses a lateral flow assay that provides a qualitative measurement of up to 10 types of allergen-specific sIgE from $\sim 110 \mu\text{L}$ of blood placed directly onto the sample well. Reproduced from Phadia.com with the permission of Thermo Fisher Scientific.

For methods employing a microarray format, microfluidic channels or some other solid substrates are coated with allergens to capture sIgE. For example, Heyries *et al.* used a micro-dispensing system to deposit arrays of 1.3 nl drops each containing a solution of allergen protein at 1 mg/ml onto a glass substrate.⁶⁰ The spots are dried and then used to capture sIgE from the sample. The detection is then performed using a peroxidase conjugated anti-IgE to oxidize luminol to provide a chemiluminescent readout [Fig. 2(b)]. Cretich *et al.* used a similar strategy, but they employed a novel MPS-poly[DMA-co-NAS] copolymer to immobilize allergens onto a glass slide in order to keep the allergens in their native conformation.⁶¹ The captured sIgEs are detected using direct fluorescent labelling with anti-IgE antibodies [Fig. 2(c)].

For methods employing particles, both polystyrene microbeads and magnetic beads have been used to concentrate and immobilize sIgE for detection. For example, Ohashi *et al.* coated polystyrene microbeads with BSA-biotin-avidin linkers to bind biotinylated water-soluble allergens.⁶² Beads are physically stopped in the flow channels to capture sIgE on the bead surface for subsequent ELISA-based detection of the captured sIgE. Proczek *et al.* used magnetic microbeads with covalently bound anti-IgE, which serve to immobilize sIgE.⁶³ Magnetic beads are then pulled from the carrier fluid and onto electrodes using a magnet, where labelling anti-IgE antibodies conjugated with alkaline phosphatase are added to sandwich the immobilized sIgE. sIgE detection is performed by measuring the electrochemical potential produced by the reaction between alkaline phosphatase and its substrate, para-aminophenyl phosphate [Fig. 2(d)].

The FDA-approved ImmunoCAP system (Phadia Inc.) measures total sIgE and allergen-specific sIgE from 40 μ l of serum per allergen, for up to 19 allergens.^{64,65} The system performs an automated sandwich ELISA where sIgE is captured by allergens (or anti-IgE in the case of total IgE detection) covalently coupled to a cellulose sponge within test tubes.⁶⁶ Non-specific enzyme-labelled anti-IgE is then introduced to bind to the captured sIgE to catalyze the formation of a detectable fluorescent product [Fig. 2(e)]. Another FDA-approved ImmunoCAP Rapid system (Phadia Inc.) detects the presence of sIgE from ~110 μ l of blood added directly into a handheld device using a lateral flow assay.⁶⁷ Serum is drawn into the internal test strip by capillary action. sIgE is captured at specific locations of the test strip corresponding to different allergens. After 5 min, a developer solution is added to wick into the readout portion of the device. After 15 min, colored bands appearing next to the labelled allergens and control locations indicate positive results [Fig. 2(f)]. Abionic produces an FDA-approved device for measuring allergen-specific IgE from a drop of blood using the same principle of capturing IgE on immobilized allergens.⁶⁸ Their approach uses capillary forces to drive blood pre-mixed with fluorescent anti-IgE through a filter and over a region of immobilized allergens that is hundreds of nanometers wide. Devices are loaded onto their abioSCOPE platform to measure fluorescent signals.

Table II summarizes some features of these assays. In the above examples, the patient serum typically is obtained by centrifugal separation of blood cells, with the exception of ImmunoCAP Rapid and Abionic, which use capillary action to draw serum into the test region and separate it from blood cells. Recently, it was reported that sIgE to more than 170 allergens can be determined

accurately from whole blood or serum that is air-dried on paper.⁶⁹ Even after storage for 1 week at 37 °C, 22 °C, 4 °C, or -20 °C, elution with PBS allows recovery of sIgE from the paper-dried blood spots.

B. Microfluidic secretion assays

A key advantage of measuring secretion from cells using microfluidics is the small reaction volume, which concentrates cytokines and facilitates their detection. This approach can be particularly useful for cytokines secreted at low levels. A few microfluidic approaches have been developed to measure secretion from activated mast cells or basophils cultured on a chip using fluorescence microscopy. For example, Liu *et al.* measured the release of β -hexosaminidase from ~1000 activated RBL-2H3 cells cultured on a microfluidic chip using a fluorogenic substrate, 4-methylumbelliferyl-*N*-acetyl- β -D-glucosaminide dehydrate (MUG).⁷⁰ β -Hexosaminidase converts non-fluorescent MUG into a fluorescent product methylumbelliferone (MU). The fluorescence intensity of MU then is used to quantify the amount of β -hexosaminidase released by the cells. As another example, fluorescent dyes such as quinacrine and acridine orange were incubated with RBL-2H3 cells⁷¹ or the human KU812 basophil cell line^{71,72} cultured on a chip. The cells accumulate these dyes into their cytoplasmic granules. Upon stimulation (e.g., with ionomycin, fMLP, anti-IgE, or allergen house dust mite protein Der p 1), the cells degranulate and the accumulated dyes are secreted along with other allergic mediators [Fig. 3(a)]. The released dyes are detected separately using fluorescence microscopy. To assist the mixing of reagents and the separation of released dyes for detection, flow-through microfluidics [Fig. 3(b)]⁷¹ and centrifugal microfluidics (lab-on-a-CD) are used [Fig. 3(c)].^{72,73}

By integrating with microfluidic single-cell trapping methods, it is also possible to monitor the secretion profile from single cells. For example, Han *et al.* employed an array of microwells to trap single T-cells and track the dynamics of secretion of multiple cytokines, including interferon gamma (IFN- γ), interleukin (IL-2), and tumor necrosis factor (TNF- α) [Fig. 3(d)].^{74,75} A glass slide coated with capture antibodies is placed on top of the microwell array facing the cells.⁷⁵ After incubation, the glass slide bearing captured molecules is removed, washed with additional fluorescent antibodies, and then analyzed separately using microscopy. At the same time, a new glass slide with capture antibodies is placed on the microwell array. The process of incubation and analysis is repeated every hour to obtain the secretion dynamics of these cytokines. An *et al.* demonstrated a dynamic single-cell cytokine profiling method for monitoring the secretion of interferon gamma (IFN- γ) from individual natural killer (NK) cells upon stimulation with phorbol 12-myristate 13-acetate (PMA) and ionomycin.⁷⁶ Single NK cells placed in individual microwells could be identified as CD56^{dim}CD16⁺. Each well also contains an anti-IFN- γ functionalized microbead [Fig. 3(e)]. As it is secreted, IFN- γ is captured on the surface of the microbead and is continuously labelled with a secondary fluorescent antibody in solution for a time-resolved measurement of IFN- γ secretion. We note that while these two examples focused on T cells and NK cells, the approach should be

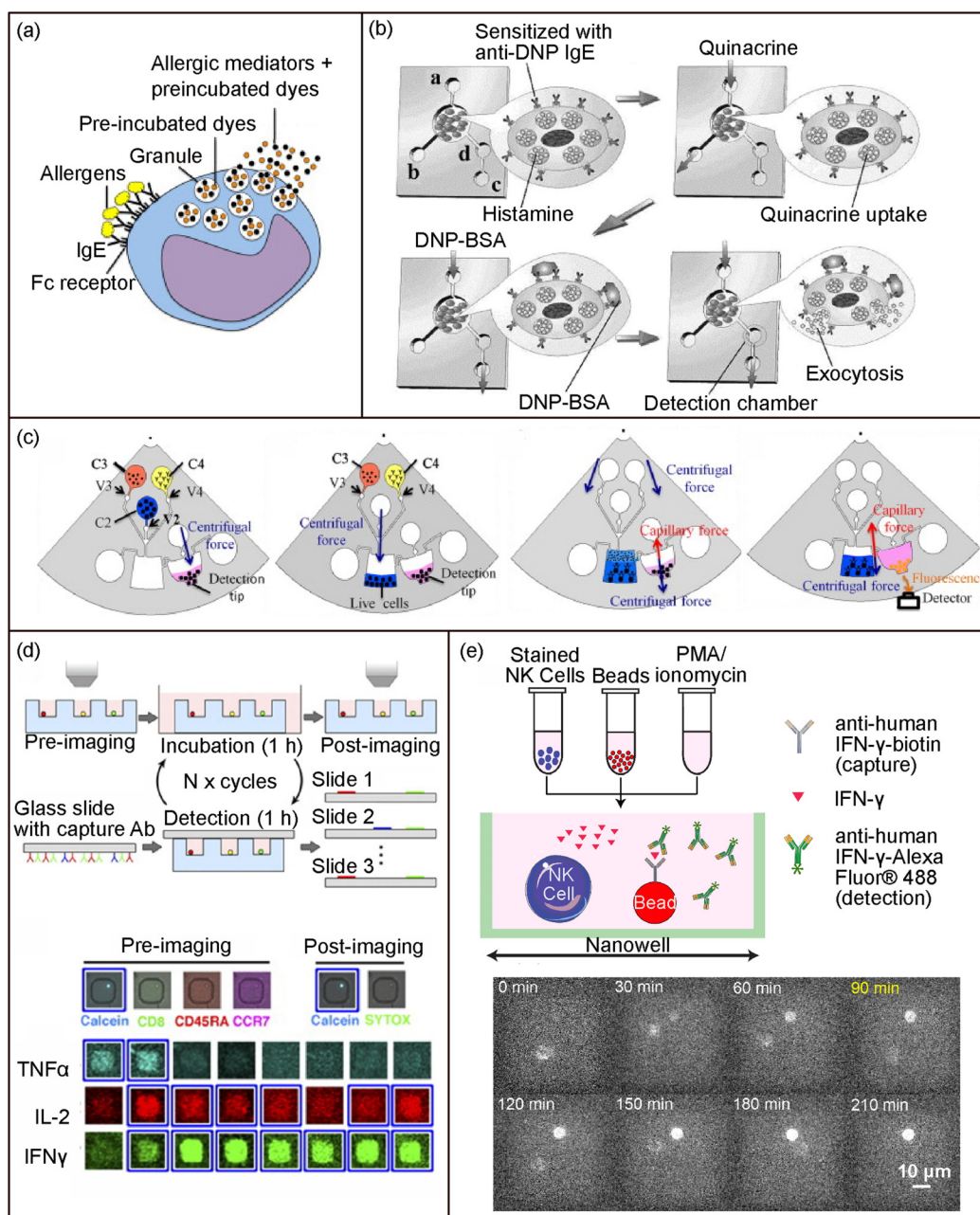


FIG. 3. (a) Schematic diagram of the degranulation process releasing pre-accumulated dyes from cytoplasmic granules. (b) Schematic diagram of the microfluidic cell to measure degranulation from the rat mast cell line. Reproduced with permission from Matsubara *et al.*, *Biosens. Bioelectron.* **19**(7), 741–747 (2004). Copyright 2003 Elsevier B.V. (c) Schematic diagrams showing steps of CD-on-a-chip to stimulate and detect the degranulation of basophils. In this assay, patient serum or PBS (released from C4) and a chemical inducer (i.e., anti-IgE, fMLP) or an allergen (C3) are mixed and incubated with a basophil cell line (C2) whose granules are pre-stained with a dye. Patients with serum IgE specific to the allergen induce the degranulation of basophils, along with the release of the dye that has accumulated in the granules. The released dye is then siphoned into a separate chamber for detection. Reproduced with permission from Chen *et al.*, *Talanta* **97**, 48–54 (2012). Copyright 2012 Elsevier B.V. (d) Cytokine secretion dynamics for individual T cells upon activation. Top: Illustration of serial microengraving to monitor cytokine secretion by viable single T cells in time. Bottom: Representative micrographs of data evaluating viability (Calcein and SYTOX); phenotype (CD8, CD45RA, and CCR7); and TNF α (blue), IL-2 (red), and IFN- γ (green) secretion. Blue squares outline positive events. Reproduced with permission from Han *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **109**(5), 1607–1612 (2012). Copyright 2012 National Academy of Sciences. (e) Dynamic cytokine secretion profiling of individual natural killer cells by using cytokine immobilization beads. Reproduced with permission from An *et al.*, *PLoS ONE* **12**(8), e0181904 (2017). Copyright 2017 Author(s), licensed under a Creative Commons Attribution (CC BY 4.0) License.

applicable for research involving other cell types relevant for allergic diseases.

C. Surface plasmon resonance measurements of activation

Surface plasmon resonance (SPR) allows real-time measurements of live cells and subcellular processes without labelling. The surface plasmon resonance of metallic surfaces (e.g., gold) is sensitive to small changes in optical properties induced by intracellular events, resulting in changes in cell adhesion or morphology close to the metallic surface within the evanescent fields (typically ~500 nm). A series of studies was done by the Hide group to apply SPR for allergy diagnosis, using either isolated human basophils or rat basophil leukemia cell lines expressing human IgE receptors (RBL-48) sensitized with human serum.^{77–80} Basophils were isolated using density gradient separation followed by depletion of non-basophils using magnetic activated cell sorting (MACS) on-chip.⁸⁰ Cells first were seeded on the SPR sensor chip. Antigens (e.g., mite extract, cedar pollen extract, and sweat extract) introduced into the solution above the cells caused the activation and degranulation of the cells [Fig. 4(a)], which in turn led to a change in SPR resonance angle or intensity [Figs. 4(b) and 4(c)]. The intensity started to increase within a few minutes of antigen introduction and continued to increase for more than 30 min. Stimulation by calcium ionophore, ionomycin, or PKC activator, PMA, also caused SPR signal changes.⁷⁸ From donors with different allergic profiles ($n = 4–11$), good correlation was found between the measured SPRI intensity changes and the donors' serum IgE levels (against mite or cedar) or reactivity to sweat antigen.

Interestingly, the authors showed that they could measure an increase in the SPR signal from basophils of “non-responders” (i.e., donors who released no or small amounts of histamine in response to anti-IgE antibody or respective antigens) after stimulation with anti-IgE.⁷⁸ This result could be explained by the fact that SPR is sensitive to changes in molecular mass distribution within the cell (e.g., changes in plasma membrane, protein translocation) as long as the changes are within the evanescent fields, and it does not require whole cell function (e.g., histamine release) or cellular morphology change.

To elucidate the molecular mechanism underlying SPR signal changes upon basophil activation, the authors treated basophils with different inhibitors of intracellular signal transduction (e.g., Syk, Src, PI3 kinases). They were able to measure partial changes in SPR signal after inhibition, suggesting that the SPR signals reflected early events of intracellular transduction instead of exocytosis or morphological changes of the cells.⁷⁹ On the other hand, histamine release was induced only after the entire signaling pathway was complete, including the phosphorylation of tyrosine kinases and increase in intracellular calcium.

D. Impedance measurements

As mentioned before, upon stimulation by allergen, the degranulation process exposes proteoglycans that can cause a large increase in negative charge on the cell surface.^{41,42} This increase should, in principle, be detectable by the impedance measurement of the cell. Irifuku *et al.* showed that it was possible

to measure a change in impedance in RBL-48 cells cultured on comb-shaped electrodes on the bottom of a well plate after stimulating with various antigens [Figs. 4(d) and 4(e)].⁸¹ RBL-48 cells are a human IgE receptor-expressing rat basophilic leukemia cell line that can be sensitized with human serum IgE. This allows them to give responses to antigen recognized by their human IgE. The kinetics of impedance change was found to be similar to that of histamine release and morphological changes caused by actin cytoskeletons.

However, the authors identified that the impedance change was due to morphological changes in the cell rather than degranulation [Fig. 4(e)]. The frequency used (10 kHz) was sensitive to the cell adhesion area on the electrode, rather than the electrical properties of cell membranes.^{82,83} The authors also reported that they could detect a change in impedance upon anti-IgE stimulation of cells sensitized with IgE from “non-responders.” This was attributed to the fact that the impedance measurement actually detected a change in cellular morphology and plasma membrane that was independent of degranulation.

V. CHALLENGES AND OPPORTUNITIES TOWARD PRECISION DIAGNOSTICS FOR FOOD ALLERGY

As we have reviewed, microfluidic devices for detecting sIgE are the most advanced to date, and some have already found commercial use and are also compatible with rapid point-of-care diagnosis. Some assays rely on relatively complex techniques and instrumentation, and more work may be necessary to package these assays into a form that is compatible with personalized or point-of-care diagnostic use. While some cell-based microfluidic assays have shown initial promise, we note that many have used mast cell lines or basophil cell lines in their demonstration of principle. Even if incubated with patient serum, such systems may not fully recapitulate the physiological conditions in the patient. Thus, these assays have yet to be validated with a larger number of patient samples, and it remains to be seen whether they indeed will be clinically useful.

Looking ahead, we believe that microfluidics has the potential to further support food allergy diagnostics and research in the following areas.

A. Multiplexed detection of multiple biomarkers

While ongoing research in biomarkers may reveal a single universal biomarker to diagnose food allergy, until such a goal is realized, the combined analysis of multiple biomarkers may generate a more accurate diagnosis. Indeed, for complex diseases caused by a combination of genetic and environmental factors, multiple biomarkers may be necessary to define the disease state.⁸⁴ It has been proposed that diagnostics based on a set of biomarkers may have better accuracy in identifying the early stages of complex diseases.⁸⁵ Such a concept already has been explored in several diseases including cardiovascular disease.^{86,87} Microfluidics is particularly well-suited for multiplexing the detection of multiple biomarkers, especially if all biomarkers are derived from the same sample (e.g., whole blood). A wide range of microfluidic techniques already exist to detect different types of biomarkers, including nucleic acids,^{88–90} gene methylation,⁹¹ proteins,^{92–94} and metabolites.⁹⁵ It should, thus, be possible

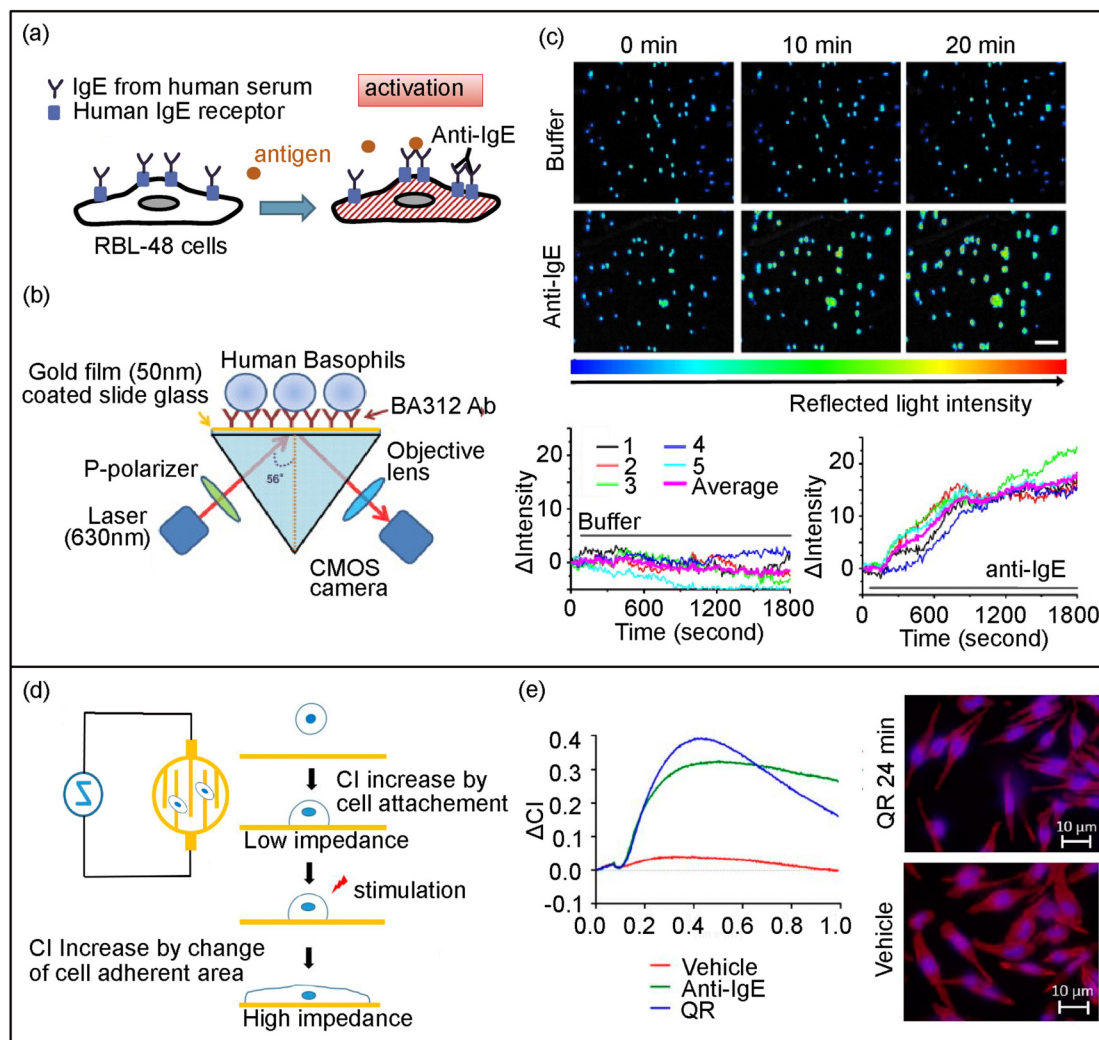


FIG. 4. (a) Scheme for RBL-48 basophil cell line activation. sIgE from patient serum is introduced to RBL-48 cells and binds to the IgE receptors on the cells. Subsequent addition of antigens or anti-IgE crosslink the IgE, which induces cell activation. Reproduced with permission from Yanase *et al.*, *Sens. Biosens. Res.* **2**, 43–48 (2014). Copyright 2014 Author(s), licensed under a Creative Commons Attribution (CC BY 3.0) License. (b) Scheme of the SPRi measurement for activated basophils. A glass prism coated with a gold film (50 nm) is placed between a diode laser with a P-polarizer and an objective lens (2x or 4x) with a CMOS camera. Basophils from peripheral blood were fixed to the sensor surface with BA312 antibodies. The camera captures the change in refractive index of the cells upon stimulation. Reproduced with permission from Yanase *et al.*, *Biosens. Bioelectron.* **32**(1), 62–68 (2012). Copyright 2012 Elsevier B.V. (c) RI in single cells detected by SPRi. 2D images of the RI for anti-IgE stimulation vs buffer at 0, 10, and 20 min (top) time course RI changes in five randomly selected cells and their average (pink bold line), plotted every 10 s. Reproduced with permission from Yanase *et al.*, *Biosens. Bioelectron.* **32**(1), 62–68 (2012). Copyright 2012 Elsevier B.V. (d) Scheme for an impedance-based system for detecting stimulated cells. The impedance sensor can detect a change in attachment and cell morphology typical of activated basophils. Reproduced with permission from Irifuku *et al.*, *Sensors* **17**(11), 2503 (2017). Copyright 2017 Author(s), licensed under a Creative Commons Attribution (CC BY 4.0) License. (e) Real-time change in cell index (CI), an arbitrary unit for impedance, of cells treated with sweat allergic patient serum with sweat antigen QR stimulation, anti-IgE stimulation, or no stimulation. Maximum changes were observed around 24 min after the stimulation (left). Images depicting the horizontal spreading of RBL-48 cells in response to QR stimulation (right). Reproduced with permission from Irifuku *et al.*, *Sensors* **17**(11), 2503 (2017). Copyright 2017 Author(s), licensed under a Creative Commons Attribution (CC BY 4.0) License.

to combine these techniques to detect multiple biomarkers simultaneously to better track the disease state, which will be useful not only for the diagnosis of the disease, but also for monitoring the efficacy of treatment.

B. Sampling of biomarkers from tissues

As blood transports a large variety of cells and signaling molecules to and from all tissues, it contains much information about the disease state and is, therefore, a common source of biomarkers.

Nevertheless, specific tissues may hold information that is not easily accessible by sampling blood only. Leveraging advances in micro-nanotechnology, it is now possible to sample biomarkers from tissues directly. For example, microneedles have been developed to extract interstitial fluids, cytokines, and even tissue-resident immune cells from the skin.^{96,97} Such technology is particularly valuable in the sampling of biomarkers that may have low concentrations in circulating blood.

C. Organ-on-a-chip

Recent advances in organ-on-a-chip technology has made it possible to closely mimic the *in vivo* conditions of human organs. Such technology is especially useful for drug screening without the need for animal testing. For example, Kim *et al.* have demonstrated a human gut-on-a-chip, where the gut microenvironment was reconstructed by applying a continuous flow over the cells and by exerting cyclic strains to mimic physiological peristaltic motions.⁹⁸ Ramadan *et al.* described a “NutriChip” where they cultured a confluent layer of epithelial cells on a permeable membrane above a well containing macrophages.⁹⁹ This system allows them to monitor the response of the macrophages, by measuring the secretion of IL-1 and IL-6, to pro-inflammatory stimuli and anti-inflammatory food.⁹⁹ While these systems used cell lines, there is also increasing effort to use patient-derived organoids to further increase the precision of drug screens.¹⁰⁰ Food allergy is a complex immunological disease and cannot be represented by considering a single organ alone. Some efforts are now directed toward creating immune-system-on-a-chip, which is, of course, a daunting task in its own right.^{101,102} However, if such an endeavor proves successful, it might accelerate the identification of effective treatments for food allergy and may even help understand the influence of allergens and microbiome at a system level.

VI. CONCLUDING REMARKS

To conclude this review, we would like to re-iterate the critical need for a better food allergy diagnostic that is both accurate and safe, as illustrated in Fig. 1. Clinical acceptance of new diagnostic devices not only requires improvements in accuracy, safety, and reliability but also added values such as speed and automation of the test operation and result analysis. In the immediate future, microfluidics can help meet this need by streamlining, miniaturizing, and accelerating assays to detect biomarkers that are already clinically proven.

For example, even though sIgE tests are widely adopted, the separation of patient serum from blood cells in many assays still requires laboratory centrifuges that are not integrated with sIgE detection. As such, the performance of the entire test still requires manual processing steps, which can be prone to human error. A range of microfluidic techniques already exists that can integrate all steps of the test from sample preparation to detection and obtaining a readout. For example, integrated microfluidic chips have been developed to obtain complete blood counts from raw blood¹⁰³ and to perform immunoassays directly from whole blood in a lab-on-a-disk format.¹⁰⁴ Using similar approaches, we believe it should be possible to automate existing food allergy diagnostic tests with sample-in-answer-out capability.

Finally, a fully integrated, standalone test would allow food allergy diagnostics to be performed at the point of care, instead of having to send patient samples to a central laboratory for analysis. This is particularly important if the test requires fresh samples (e.g., for functional cell-based assays). Whether there is a demand for home-based food allergy testing remains to be seen. However, with increasing prevalence of food allergy and new treatment options for food allergy, we believe there will be increasing need for food allergy tests that are accurate, safe, rapid, cost-effective, and simple to use, both for the diagnosis of the disease, and as a companion diagnostic to monitor the efficacy of treatment.

AUTHORS' CONTRIBUTIONS

N.C. and S.C. equally contributed to this work.

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