



A Novel Paper-Based Capacitance Mast Cell Sensor for Evaluating Peanut Allergen Protein Ara h 2

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Abstract

A novel paper-based capacitance mast cell sensor has been designed and developed for real-time monitoring of the major peanut allergen Ara h 2. In this study, we created the 3D paper chip printed with carbon electrodes as a non-contact capacitance sensing platform. To improve the conductivity and biocompatibility of the cellulose paper, a polyvinyl alcohol (PVA)-gelatin methacryloyl (GelMA)-nano-hydroxyapatite (nHAP) composite hydrogel (PGHAP gel) was employed to fabricate the prepared paper chip. When rat basophilic leukemia mast cells (RBL-2H3) are immobilized and cultured in the 3D culture system consisting of PGHAP gel and paper fibers, identification signals of Ara h 2 could be specifically monitored non-contact and real-time by capacitance change measurement. This 3D structure combined with time-lapse monitoring completes the capacitance cell sensor. Results indicate that Ara h 2 has given a remarkable decrease to the capacitance in dose-dependent range from 0.1 to 100 ng/mL. Therefore, the real-time cell allergic response could be accurately monitored by this low-cost, disposable cell sensor, which supplies a novel and effective pathway for the rapid and accurate evaluation for food allergens.

Keywords Capacitance monitoring · Peanut Ara h 2 · RBL-2H3 mast cell · Cell-based paper sensor

Introduction

Food allergy produces a serious threat to people's health, affecting up to 5% of the population (Renz et al. 2018). The prevalent use of peanuts in the food processing has turned peanut allergy a serious health problem and often brings severe, widespread, and continuous effects on patients (Almuhsen et al. 2008; Chang et al. 2013). In most people with peanut allergies, serum IgE revealed that the peanut proteins Ara h 1 and h 2 are the major allergens (Kim et al. 2016; Maleki et al. 2000). Peanut Ara h 2 is a 17.5 kDa albumin protein, possesses around 10% of the total peanut protein, and

has been confirmed as the most dangerous peanut allergen, as well as the best predictor of a severe allergic response (Peng et al. 2015). There are no known cures for peanut allergies, and no effective treatment is workable to relieve this severe allergic reaction. Therefore, strict and wide allergy management and control are particularly necessary for manufacturers (Kukkonen et al. 2015). Novel methods for determination and evaluation of peanut allergen are also required for improving the food safety early warning system (O'B 2015; Rong et al. 2012).

There have been several methods developed for the peanut allergens detection. These are primarily based on immunoassay (ELISA) (Anzengruber et al. 2017; Peng et al. 2015) and DNA-based assays (PCR) (Stephan and Vieths 2004). All these approaches are highly specific and sensitive tools that allow high-throughput analysis of samples. Nevertheless, unsatisfied results due to cross reaction, lengthy analysis time, high cost of ELISA kits, and large numbers of sample replications have posed obvious difficulties for the practical applications to complex food matrices (Montiel et al. 2016).

Biosensors have become attractive choices compared with the traditional methods, which offer real-time and high sensitivity detection (Sobhan et al. 2017; Vasilescu et al. 2016; Zhu et al. 2016). As a new evaluating method, mammalian cell biosensor (MCB) is a widespread application in the area of

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in vitro monitoring. They have a unique value in reflecting cellular metabolism not only for quantitative measurements but also for analysis to the physiological dynamics of targets. Numerous examples of combinations of live cell with various sensing devices for the allergens detection have been demonstrated (Campuzano et al. 2016; Jiang et al. 2014; Sun et al. 2013). The surface of RBL cells possesses an abundant of FcεRI family receptors. These receptors can specifically combine with allergen IgE or IgG antibodies, which subsequently cross-link with target allergen and induce cellular degranulation (Graham et al. 2015). Therefore, the mast cells sensitized with Ara h 2 antibody can be the best choice for evaluating the Ara h 2 protein and converting the biological recognition into a visualized and quantifiable electric signal.

Recent reports have demonstrated that the non-Faradaic measurement is becoming one of the most attractive marker-free technologies, representing a strong tool for detecting highly sensitive target analytes in a more amenable manner for point-of-care applications (Arya et al. 2018). Notably, compared with the traditional electrochemical analysis with screen-printed electrodes based on paper devices, the non-contact capacitive sensing technology without using voltage, current, and impedance has shown significant potential in biosensor application and has greatly improved the development of on-site and non-destructive detection (Niyomdech et al. 2018). The principle of capacitive measurement is based on the electrical double layer on the surface of two parallel electrodes. Biosensing element immobilized in the 3D space created by the working electrode and counter electrode produces a stable capacitance response. Recognition of the target analyte by the biosensing element immobilized between the double electrodes would cause the capacitance change (Zeinabad et al. 2018). Therefore, this label-free non-Faradaic technique is widely used in rapid detection and will gradually become a new research hotspot. At present, there is no capacitive cell-based biosensor that has been reported for the detection of allergen.

For cell immobilization, microfluidic paper has attracted public interest due to their easy preparation, high sensitivity, excellent mobility, good disposability, low cost, and sample consumption. Consequently, they are widely employed nowadays in fields such as disease control, environmental monitoring, and food safety (Cai et al. 2018; Hossain and Brennan 2011; Liu et al. 2018; Wang et al. 2009). These microfluidic paper chips can be used not only in real-time analysis without professional instruments but also in the application of simple-to-use, low-cost, and small-size biosensors. For the culture and immobilization of living cells, cellulose paper is an ideal material due to its fibrous porosity, super absorbency, and good biocompatibility, which makes the paper a first-choice candidate for disposable and portable biosensing platforms. Therefore, many paper-based biosensors have been designed and reported. However, up to now, there is no paper-based

biosensor for peanut allergen Ara h 2 determination with complete live cells as sensing elements, especially, based on a low-cost, convenient sensing platform. In addition, the combination of live cells and paper chips, as a new strategy, has a great significance in the field of in vitro detection. Compared with traditional methods, the paper-based cell biosensors require lower preparation cost and less instrument dependency but own faster detection speed (Teengam et al. 2017; Yu et al. 2015). Therefore, it is believed that the paper-based cell biosensor will exhibit a promising development and energetic performance in the field of biosensing detection. Under the continuous stimuli of certain allergen, the shape, structure, and cellular content of mast cell can change over time. Therefore, changes in the time dimension of cells cultured in 3D space can be continuously monitored (Gladman et al. 2016).

Gelatin modified with methacryloyl (GelMA), as a natural product, has recently attracted increasing concern due to its admirable biocompatibility, low cytotoxicity, and strong mechanical characteristics (Klotz et al. 2016). After introducing the biocompatible polyvinyl alcohol (PVA) and nano-hydroxyapatite (nHAP), a composite material PGHAP hydrogel then could be prepared, which possesses 3D porous structure, admirable biocompatibility, good mechanical strength, and excellent conductivity (Zhu et al. 2018). Because of these outstanding properties, the PGHAP gel will greatly promote the biochemical and photoelectric detection performance of paper-based cell sensors.

Herein, we describe a novel and simple paper-based capacitance cell sensor for monitoring the peanut allergen Ara h 2. PGHAP gel was used to modify the wax printing cellulose paper chips with double carbon electrodes and to provide a 3D culture environment for the immobilization of RBL cells on the paper fiber firmly, thus enhancing the efficiency of the sensing platform. The differing capacitive responses induced by the sensitization of peanut allergens were discussed below. Biological evaluation of the cell activity and viability, the measurement of calcium concentration, and the cell apoptosis observed by SEM and TEM were employed to confirm the capacitive evaluation results. These effects could be utilized for the fast identifications and quantification of the peanut Ara h 2 content in the foodstuff, which led to a simple and feasible way to investigate food allergens.

Experimental Procedure

Materials and Apparatus

Ara h 2 standard (ST-AH2) and mouse monoclonal anti-Ara h 2 IgG1 (clone 1C4-G4-C8) antibody were purchased from Indoor Biotechnologies Inc. (Charlottesville, USA). Polyvinyl alcohol and nano-hydroxyapatite were purchased from Sigma-Aldrich Inc. (St. Louis, USA). GelMA hydrogel was purchased from SunP Biotech Co. Ltd. (Beijing, China).

RBL-2H3 cells were purchased from the Chinese Academy of Sciences Cell Bank (Shanghai, China). Cell culture medium and fetal bovine serum were purchased from Gibco (Gaithersburg, USA). All biological kits were obtained from Beyotime Biotechnology Co., Ltd. (Shanghai, China); all solutions and reagents used in the experiment were analytical grade. Cellulose paper was modified by a solid wax printer (Fuji Xerox Phaser 8560DT).

Capacitance was measured by an LCR meter (VC4090C, VICTOR, Xi'an, China). The cell morphology observations were carried out on a Philips transmission electron microscope (Tecnai 12, Netherlands) and a Hitachi scanning electron microscope (S-4800 II, Japan). The infrared analysis was performed on a FT-IR spectrometer (Nicolet Nexus 470, USA). RBL cells were cultured in a Thermo CO₂ incubator (Illinois, USA).

Preparation and Modification of Paper Chip

The paper chips were prepared on a pure cellulose A4 paper, and the processing course (Scheme 1) consists of wax printing, drying, and screen printing and clipping. In brief, firstly, the prototype of paper chip was drawn using Adobe Illustrator CS6 software and wax-printed by a wax printer in bulk. Each printed paper contained a working slice (green one) and an auxiliary slice (blue one) with the same size of 25 mm. A circular hydrophilic working zone with a diameter of 10 mm and a circular hydrophilic auxiliary zone with a diameter of 15.0 mm were reserved on the left and right sides of the chip respectively, with a folding line in the middle. After dried in oven at 150 °C for 100 s, the melted wax on the paper would penetrate into the paper fibers and form a hydrophobic layer. The unprinted area still kept superior hydrophilicity and porosity, and further modifications will not be affected.

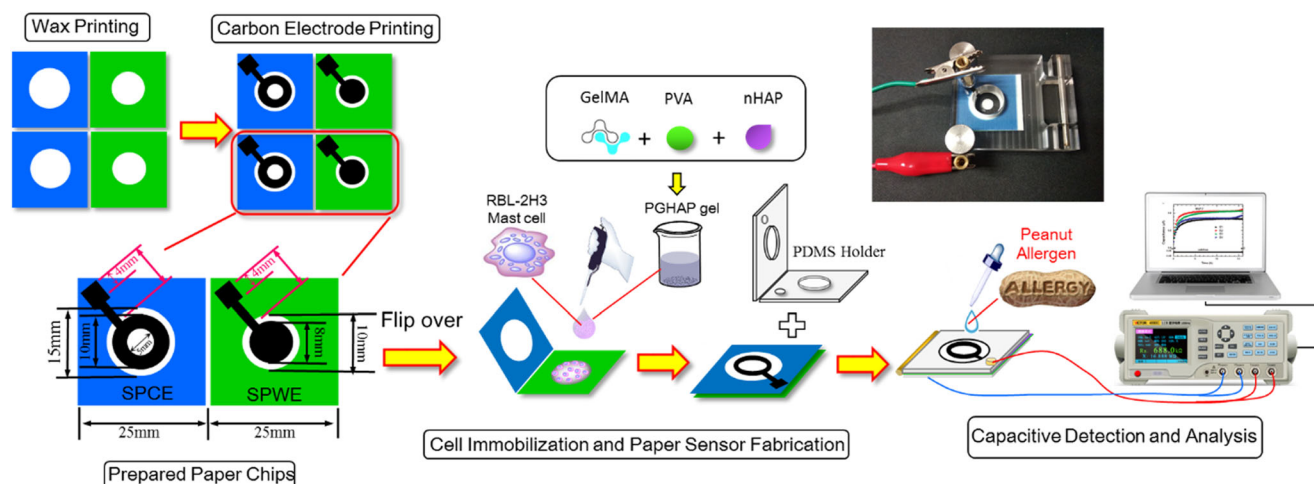
Next, the carbon electrode was quickly screen-printed to the surface of prepared wax-printed paper pieces after cooling

to 20 °C. Carbon ink was selected to print 8 mm working electrode (SPWE) and 10 mm counter electrode on the working and auxiliary slice, respectively. Finally, the capacitive paper sensor with two electrodes can be easily prepared by folding the paper chip along the folding line.

Fabrication of the Capacitance Cell Sensor

The PGHAP hydrogel was fabricated following Zhu's method with some modifications (Y. Zhu et al. 2018). Firstly, 10% PVA (w/v), 10% gelatin (w/v), and 0.5% GelMA hydrogel (w/v) were dissolved in purified water at 90 °C and 60 °C, respectively. Then, the three components were mixed together, meanwhile the 2% nHAP (w/v) were uniformly added into the mixture by stirring at 1200 rpm for 30 min under 50 °C. Finally, the PGHAP hydrogel was successfully obtained by 3–5 times freezing and thawing process. The SEM characterization of the PGHAP hydrogel was showed in Supplementary Fig. S1.

For the immobilization of the RBL mast cells into the capacitive paper-based cell sensor, RBL mast cells in logarithmic phases were chosen and collected, and about 1×10^8 viable cells/mL were resuspended in the complete serum-free medium. The schematic illustration for the preparation of the capacitance cell sensor is shown in Scheme 1. In brief, In order to quantify peanut allergen Ara h 2 specifically and accurately, RBL-2H3 cell was firstly sensitized by three- to five-fold molar excess of anti-Ara h 2 IgG antibody and incubated at 37 °C for 35 min before immobilized into the paper chip. Then activated RBL cells were rinsed thrice with pH 7.4 PBS buffer to wash the excess antibody for subsequent assay utilization. Next, 50 µL PGHAP gel was first dropped into the inner face of the working slice (the green one). After that, 50 µL of RBL cell suspension was dripped gently into the same place and incubated at 37 °C for 5 min. Meanwhile, 50 µL of 1% BSA blocking buffer was used to block the



Scheme 1 Schematic illustration for the preparation of the capacitance cell sensor

nonspecific binding sites on opposite chip (the blue piece); after a rinse with pH 7.4 PBS buffer, the prepared paper chip was maintained at 37 °C for 5 min. Next, the incubated paper chip was carefully placed into the PDMS holder before capacitive measurements. In addition, the blue piece of the paper chip must be kept facing up to the circular hole on the holder which facilitates the addition of allergen samples.

Electrochemical Assay and Capacitance Measurements

Electrochemical characterization was tested on an AUTOLAB electrochemical workstation. The CV (cyclic voltammetry) and DPV (differential pulse voltammetry) signals of the RBL cell immobilization and PGHAP gel modification were monitored, which carried out from -0.2 V to 0.6 V with a pulse amplitude of 50 mV in 1.0 M KCl and 2.5 mM of $\text{Fe}(\text{CN})_6^{3-/4-}$. Capacitance detection was carried out by an LCR meter with a peak-to-peak ac model of 10 mV at frequencies ranging from 100 Hz to 5 kHz. The paper-based cell sensor and LCR meter were connected via a homemade PDMS holder. Every 10 μL of serially diluted Ara h 2 (standard or sample) was dropwise added to the external surface of paper chip and incubated at 37 °C for 6 min. The capacitance change of RBL cells exposed to various Ara h 2 concentrations in the paper chip was measured via a data analysis using a laptop computer connected to the LCR meter. Detection data were collected every 10 min from each sample. RBL cells not exposed to allergen were picked as the control.

Intracellular Ca^{2+} , Degranulation Measurements

The calcium probe fluo-3/AM was used to monitor the cellular Ca^{2+} levels. Dye was dropped into the RBL cells suspension and incubated for 60 min at 37 °C; then the mixture was washed with pH 7.4 PBS buffer and dispersed into the culture plate with different contents of peanut allergen Ara h 2 for 2 h before fluorescence measurement.

Meanwhile, β -hexosaminidase assay was employed to monitor cell degranulation. 50 μL substrate was added to each well of the 96-well plates with both the cells and lysates. After incubating for 60 min at 37 °C, the incubation was terminated by dropping 100 μL of 0.5 M glycine. The absorbance values were obtained by an ELISA reader at 405 nm (Biotek, Winooski, USA); then the cell degranulation conditions were observed through the SEM and TEM Characterization.

Statistical Analysis

All the electrochemical tests and capacitance measurement were conducted in triplicate for each sample. Statistical analysis was performed using SPSS 17.0 software. All data were expressed as the means $\pm$ SD. The Student's t test was used to

determine the significance of difference. Between the control and the test groups, $p < 0.05$ was considered statistically significant.

Results and Discussion

Working Principles of Capacitance Cell Sensors

Figure 1a illustrates the preparation of the paper-based capacitance cell sensor and its working principle of this new method that is used for identifying and monitoring the target allergen. Firstly, anti-Ara h 2 IgG pre-sensitized RBL cells encapsulated in the PGHAP gel were dispensed into the paper fibers. The SEM analysis (Fig. 1b) showed that the RBL cells exhibited complete spherical morphology and emitted filamentous pseudopods from the surface of the cellular membrane, which demonstrated the successful construction of the 3D culture system. Next, after RBL cells were trapped in the electrical double layer consisted by the SPCE and SPWE, they formed a barrier against the electrochemical reaction which led to a sensitive and specific capacitive signal. Finally, when Ara h 2 was used to stimulate the RBL cell, it triggered the cellular degranulation and inflammatory factor release. Hence, continuous changes in capacitance influenced by RBL cells could be real-time monitored rapidly and accurately. Therefore, through the combination of space and time, our capacitance cell sensor for real-time monitoring of allergenic protein Ara h 2 was then successfully developed.

Electrochemical Characteristics of the Capacitance Cell Sensor

The characterization of the prepared capacitance cell sensor was tested with CV, DPV, and impedance in the 2.5 mM $\text{Fe}(\text{CN})_6^{3-/4-}$ (Fig. 2). As seen in Fig. 2a, the CV signal showed a standard redox peak on the surface of bare SPWE (curve a). The current value increased observably when the SPWE was deposited via PGHAP gel (curve b) due to good conductivity of the PGHAP hydrogel. The peak current further decreased when cells were added into the chip (curve c), due to the electrical insulator properties of the cells as the electron transfer of the SPWE decelerated. Similar signal changes are shown in Fig. 2b. The current increased from 2.81 μA (curve a) to (curve b) and a decrease of 47% occurred in the peak current (from 13.94 μA to 7.26 μA) as the cells shielded electronic signals (curve c). The optimization of the amount of PGHAP is discussed in Supplementary Fig. S2.

Due to the addition of RBL cells, the redox reaction on the electrode surface will be blocked which leads to an increase in the impedance (Fig. 2c). The EIS (Electrochemical Impedance Spectroscopy) was used to monitor the immobilization and the physiological change of RBL cells in the paper chip. As the

Fig. 1 **a** The working principle of capacitance cell sensor for allergen monitoring. **b** SEM images of the internal structure of the capacitance cell sensor; RBL cells immobilized on the cellulose fiber encapsulated with PGHAP under $\times 100$ magnification and $\times 400$ magnification

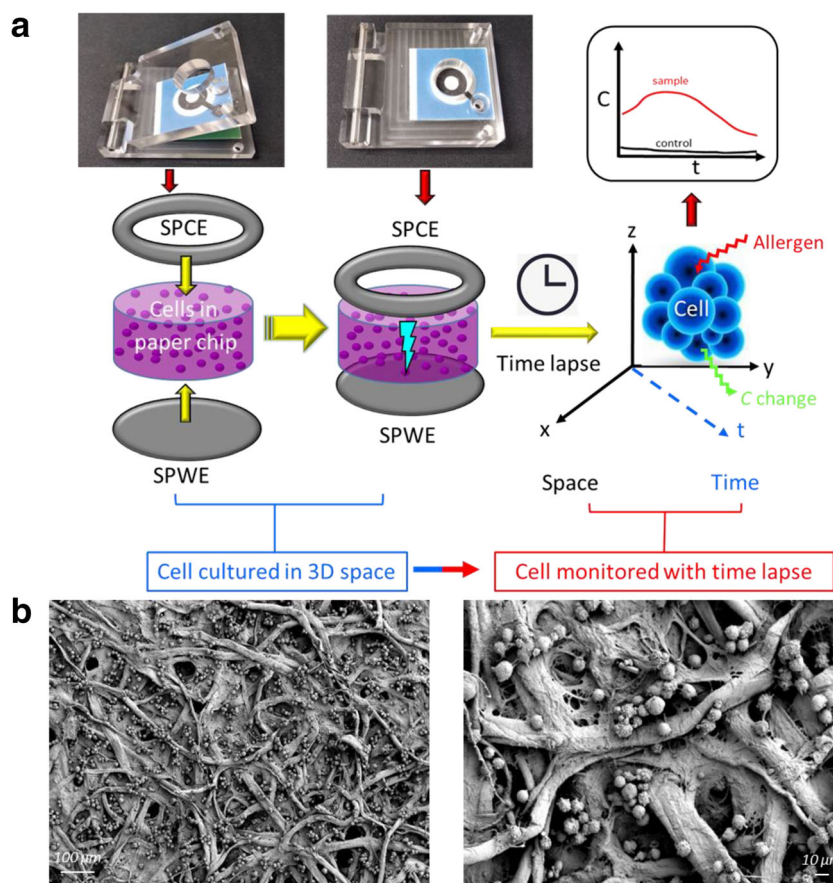
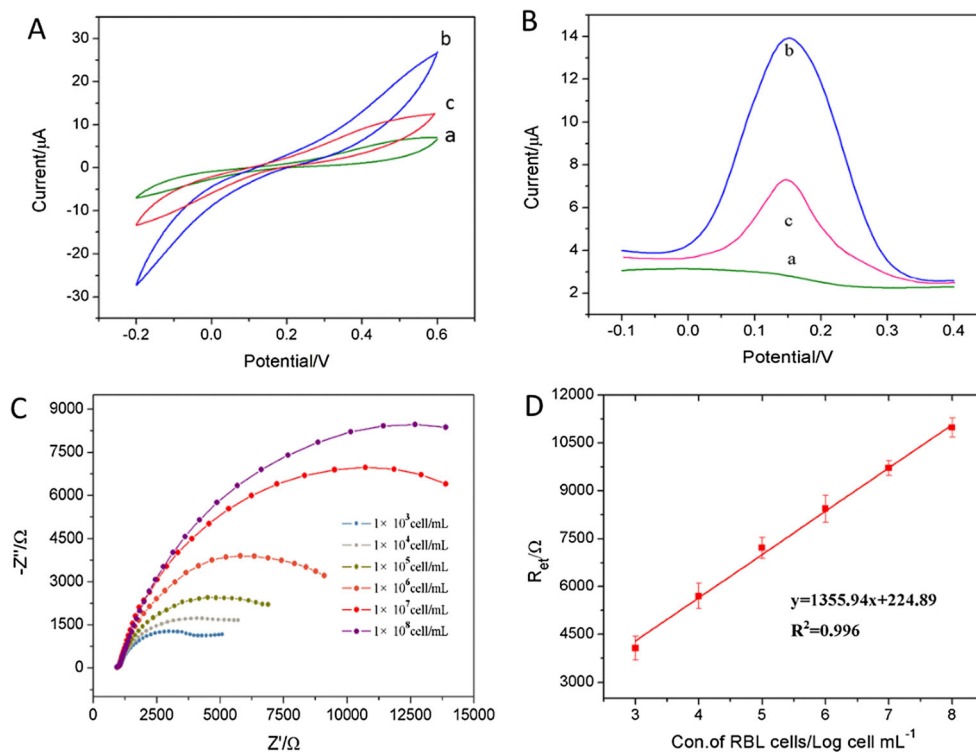


Fig. 2 Electrochemical characterization of the prepared capacitance cell sensor. **A** Cyclic voltammograms and **B** Differential pulse voltammograms of (a) bare SPWE, (b) PGHAP/SPWE, and (c) cell/ PGHAP/SPWE, in $\text{Fe}(\text{CN})_6^{3-/4-}$; scan rate, 100 mV/s. **C** Nyquist diagrams of different cell numbers from 10×10^3 to 1×10^8 cells/mL RBL cells immobilized in the paper chip. **d** Linear regression curve between cell concentration and R_{et} value



cell concentration increased from 1×10^3 to 1×10^8 cells/mL, the impedance value increased, resulting in a gradual increase in the diameter of a semicircle on the Nyquist diagram (Fig. 2c). The impedance of the sensor became stable when the cell number exceeded 1×10^8 cells/mL. Figure 2d shows that any increase in impedance was proportional to the number of cells within a certain concentration range. The impedance value exhibited a linear relationship with cell number from 1×10^3 to 1×10^8 cells/mL. Therefore, the appropriate cell number for capacitance analysis was 1×10^8 cells/mL.

Real-Time Monitoring of RBL Cell Viability

In order to verify whether RBL cell could maintain their activity in the 3D culture system, the developed capacitance cell sensor was employed to monitor the capacitance change of cells in real-time. The capacitance (C) between the SPEs is described by the equation below:

$$C = \varepsilon \frac{S}{d}$$

where ε is the dielectric constant of the medium between two screen-printed electrodes, S is the electrode surface area, and d is the distance between the screen-printed electrodes. To utilize the real-time monitoring of capacitance signal of cells in the 3D culture system, ΔC was applied as measurement parameter to characterize the changes of cell capacitance at specific frequency points; the formula was as follows:

$$\Delta C = \frac{C_{\text{cell}}(t) - C_0}{C}$$

where ΔC means the cell index in t , $C_{\text{cell}}(t)$ means the capacitance value of cells in t time, C_0 means the initial capacitance value, and C implies capacitance values before cell immobilization, normally $C_0 = C$.

Figure 3a shows the frequency dependence of capacitance monitored using the paper sensor for both RBL cell with gels

and cell-free (control) hydrogels. For both samples, the capacitance decreased with the increase of frequency. However, the capacitance of cell with PGHAP gel was higher than that of cell-free gel due to the dielectric constant increase of the cell addition. To investigate the dependent relationship between the capacitance and the concentration of hydrogels, the frequency dependence of cell-free hydrogel capacitance with different amount of PGHAP gel was determined. Results showed that the capacitance values kept stable despite gel changes. Figure 3b shows the time dependence of capacitance monitored at a frequency of 1 kHz for RBL cell with gels and cell-free gels. Both the capacitance of RBL cell with PGHAP gel and cell-free gel kept stable with time lapse, implying that the capacitance could be used as an index for RBL cells monitoring as well as proving the excellent stability of the capacitance cell sensor.

Capacitance Cell Sensor Assays for Allergen Monitoring

In order to monitor allergen reaction, capacitance values were applied to indicate the variation tendency of peanut allergen in real-time with our capacitance cell sensor. Mast cells' surface possess special recognition receptor (FcεRI), these receptors can selectively cross-link with allergen protein to initiate cellular degranulation and inflammatory molecules release. According to this immunity rule, sensitized mast cells could selectively bind with peanut allergen protein within minutes. This specific recognition process causes a series of intracellular activities, including cellular degranulation and cellular content release, such as histamine, tryptase, and β -hexosaminidase. Then, changes in the biochemical properties of these mast cells, which immobilized between the conducting surfaces, may affect their capacitance, allowing for real-time monitoring of mast cell sensitization levels. Therefore, quantitative detection of allergen protein can be indirectly realized

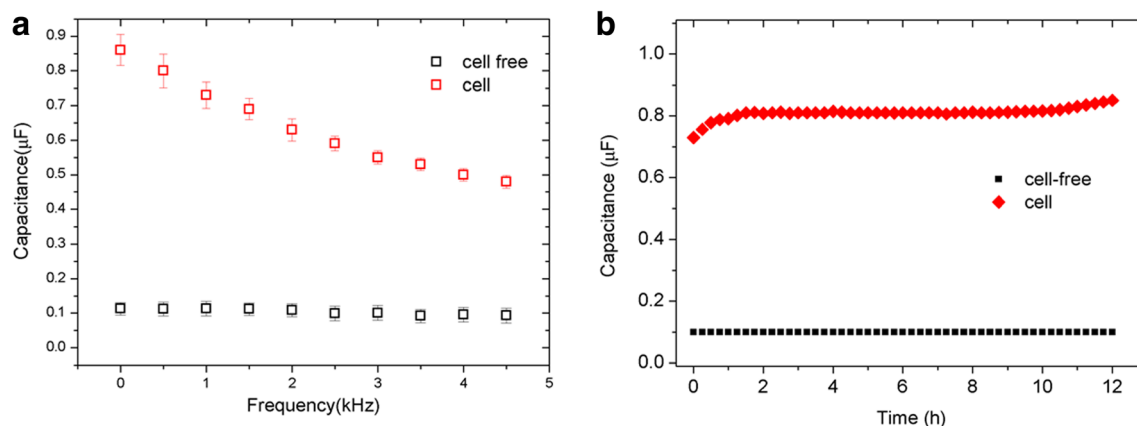


Fig. 3 **a** Frequency dependence of capacitance measured with SPE in cell-free and RBL cell with PGHAP gel. Data represent mean $\pm$ standard deviation ($n = 3$). **b** Real-time capacitance measured with SPE in cell-free and RBL cell-encapsulated PGHAP gel

by evaluating the degree of mast cell degranulation or cellular content release.

Capacitance values were employed to indicate the variation tendency of peanut allergen standards. Figure 4a shows the typical capacitance signals from RBL-2H3 cells that were sensitized by various concentrations of Ara h 2 standards (ranged from 0.1 to 100 ng/mL). Although the concentrations of the groups were different, the monitoring results showed the same trend. For instance, the capacitance value increased slowly when the Ara h 2 standards were just added to the cell culture system. This implied lower concentrations of Ara h 2 stimulated the cell degranulation, allowing the capacitance to increase, due to the release of histamine, tryptase, and β -hexosaminidase. The capacitance value decreased gradually and remained stable as time goes on. This could be because the high allergen concentrations induced cell apoptosis or cytolysis, which led to physiological changes in cells and decreased the capacitance.

Interestingly, it was found that Ara h 2 could be quantitatively identified within a certain concentration range. Therefore, we tried to quantify the peanut allergens by using our capacitive cell sensor. Figure 4b shows the calibration curve for the Ara h 2 that was experimentally obtained. The y-axis showed that the capacitance was estimated using information from Fig. 4a. We measured the accurate Ara h 2

content ranging from 0.1 to 1 ng/mL, with small errors, in less than 1 h. The detection limit was calculated to be 0.028 ng/mL, according to the formula $LOD = 3 s/m$, where s represented the SD value of the blank sample ($n = 3$) and m represented the slope of the standard curve for Ara h 2. These measurements were performed three times to assess the reproducibility and the precision, where an excellent correlation was found between the Ara h 2 concentrations measured with both the ELISA kit (EPC-AH2-1) and the capacitance cell sensor (Supplementary Fig. S7).

The SEM observation of the RBL cells presented a complete structure on the surface of cell plasma membrane (Fig. 4c). There were obvious changes in cell morphology when the cells were stimulated by Ara h 2 at the highest sensitization concentration (Fig. 4d). It was clear that the RBL cells exhibited distinct morphological changes, such as cell deformation, cell shrinkage, secretory granule releasing, incomplete intracellular particles, and vacuolated cytoplasm. This suggested that the cell physical conditions changed as allergen concentration increased, where the obtained capacitance value changed.

Furthermore, the intracellular calcium determination and the β -hexosaminidase assay were also carried out to verify the results of the capacitance measurement that are shown in Supplementary Figs. S4 and S5. TEM scanning of cellular

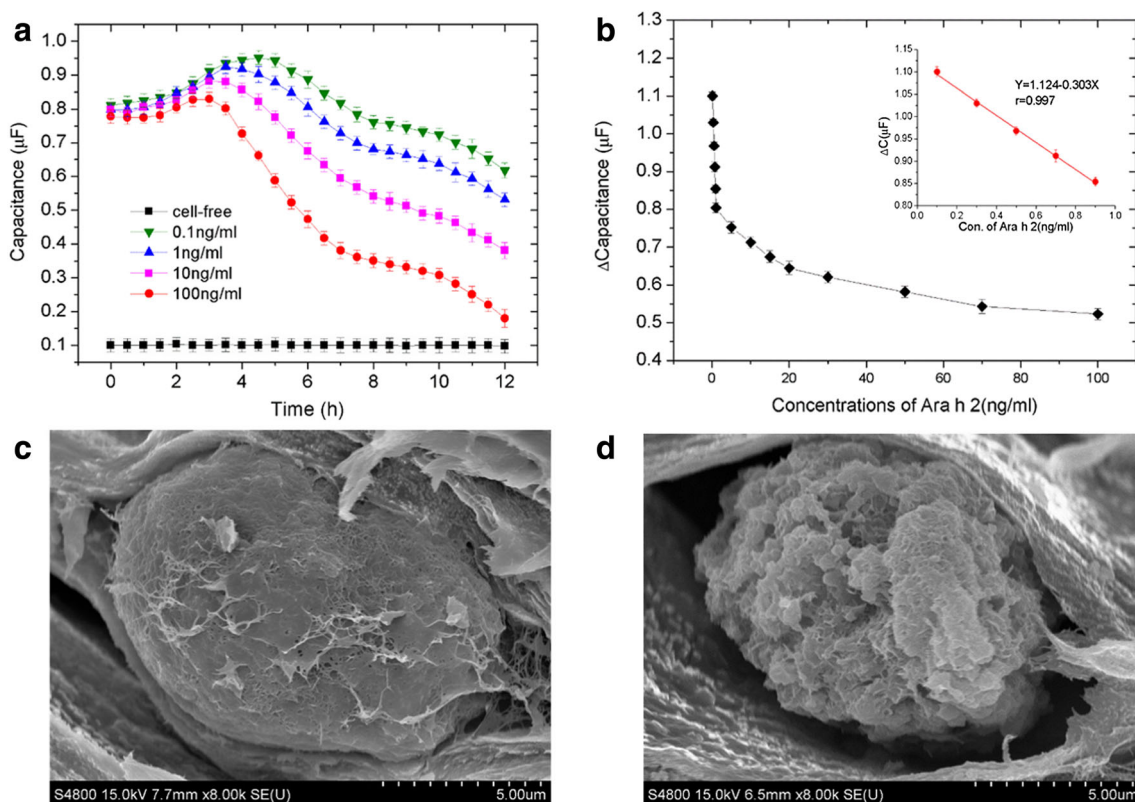


Fig. 4 Capacitance measurement results and morphology changes of RBL cells exposed to allergens. **a** RBL cells treated with different doses of Ara h 2: from 0.1 to 100 ng/mL. **b** Linear regression curve

between Ara h 2 concentration and ΔC values. The SEM observation for the morphology of **c** naive RBL cells and **d** RBL cells treated with Ara h 2

Table 1 The concentration and content of Ara h 2 in real samples

Sample	Raw peanut	Fired peanut
Capacitance value ($\mu\text{F} \pm \text{SD}$)	1.113 ± 0.02	1.114 ± 0.05
Concentration ($\text{ng/mL} \pm \text{SD}$)	0.034 ± 0.005	0.032 ± 0.004
Content ($\text{mg/g} \pm \text{SD}$)	4.552 ± 0.02	$4.221 \pm 0.03^{**}$

$^{**} p < 0.01$, $n = 5$

degranulation are shown in Supplementary Fig. S6 which confirmed that our cell-based sensor had an excellent performance.

Determination of Ara h 2 in Food Samples

The capacitance cell sensor's performance on rapid identification allergen targets from real food samples was assessed by taking raw peanuts and fried peanut extracts. Supplementary Fig. S3 shows that the purified Ara h 2 was applied to the gel and SDS-PAGE was performed. One major protein band was present at 17 kD, which was recognized by Coomassie blue staining (Supplementary Fig. S3 A and B, with arrow). This indicated the successful purification on the Ara h 2. The stimulation of the RBL cells with these two peanut extracts led to a dramatic decrease on cell's capacitance. The contents of Ara h 2 in the samples were calculated from the standard curve of Ara h 2 (taken from Fig. 4b) are shown in Table 1. The results acquired by the capacitance cell sensors were similar to reported methods, which verified that the developed cell sensor could effectively identify and determinate allergen in complex food samples.

Conclusion

A sensitive, disposed, and portable paper-based capacitance cell sensor was proposed by immobilizing the living mast cells in the paper chip for real-time monitoring peanut allergens. PGHAP hydrogels synthesized, characterized, and fabricated the paper fiber to encapsulate the RBL-2H3 cells. The sensor showed an admirable correlation with the number of cells, as the cell number increased, the impedance correspondingly increased. Therefore, dynamic physical changes of cell could be monitored in real-time by measuring the capacitance between the two screen-printed electrodes on the paper chip. In order to non-invasively monitor peanut allergen in real-time with the capacitance cell sensor, the exposure to Ara h 2 standards induced an obvious dose-dependent capacitance signal. The capacitance changes indicated the presence of target allergens, and the cell responses were monitored in real-time. Capacitance values were proportional to the Ara h 2 concentrations, which ranged from 0.1 to 10 ng/mL, with a

correlation coefficient of 0.997 and a detection limit of 0.028 ng/mL ($\text{RSD} < 5\%$). The detection signal intensity of the capacitance reduced as the Ara h 2 concentrations decreased. Moreover, accurate results can be obtained in less than 15 min for all tests, which showed stronger practicability and high efficiency than traditional cell model tests. These advantages make this cell sensor a potential point of care testing (POCT) tool for satisfying the needs of customers who lack professional analytical equipment. In addition, peanuts are not the only allergenic raw material widely used in food processing, which requires comprehensive and versatile assessment methods. Therefore, the proposed highly sensitive and convenient paper-based capacitance cell sensing method could not only determinate peanut allergen Ara h 2 but also estimate whether or not products contained other allergen protein. As long as the specified IgE or IgG antibody to stimulate mast cells changes, the quantitative determinate of different allergen proteins can be easily realized. It can be easily integrated into various portable devices for high-throughput screening and monitoring of food hazards.

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Compliance with Ethical Standards

Conflict of Interest Donglei Jiang declares that he has no conflict of interest. Hui Jiang declares that she has no conflict of interest. Lifeng Wang declares that he has no conflict of interest.

Ethical Approval All institutional and national guidelines for the care and use of laboratory animals were followed.

Informed Consent Not applicable.

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A novel paper based capacitance mast cell sensor for evaluating peanut allergen protein Ara h 2.

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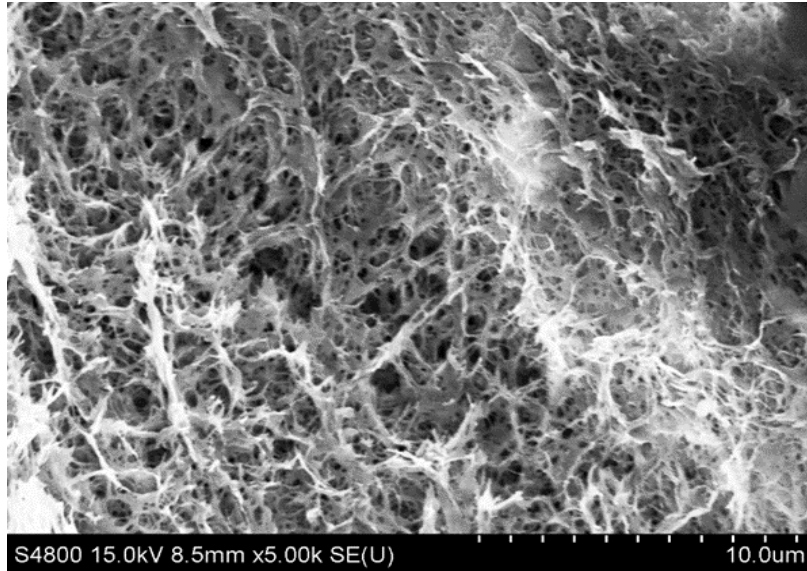


Fig. S1. SEM image of PGHAP gel hydrogel

The PGHAP gel had a dense and porous network structure as shown in Fig. S1. This structure enabled the mast cells to be well fixed in the fibers of the PGHAP gel and enabled 3D culture of the cells. The porous network structure not only kept the cell activity but also prevented the influence of the external environment on the cell and also facilitated the material exchange between the cell and the external environment. The above results proved that mast cells were successfully immobilized in the paper chip, which laid the foundation for the next step of cell sensing.

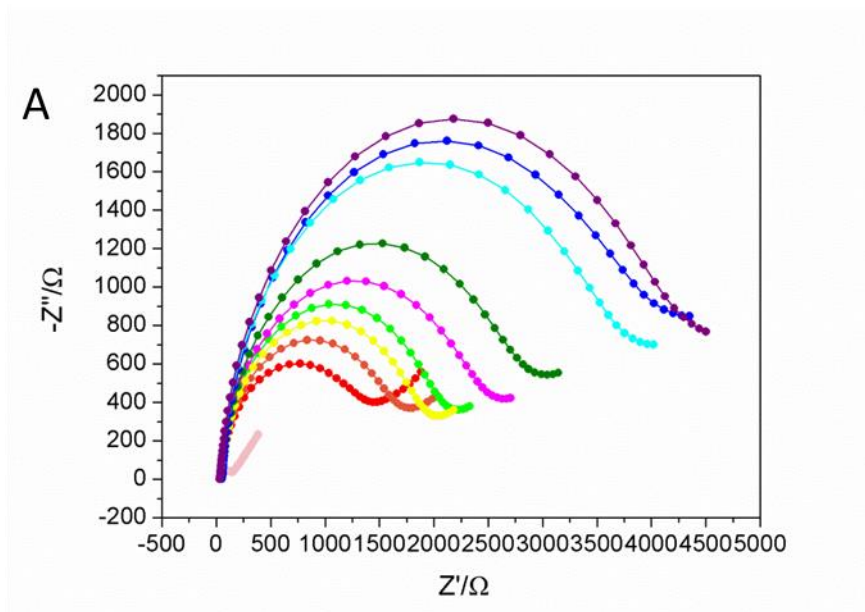


Fig. S2. (A) Nyquist curves of different concentrations of PGHAP gel. From 0 mg/mL to 9 mg/mL.

Then figure showed the Nyquist curves of PGHAP gel at different concentrations. As the concentration of PGHAP in the paper chip increased, the impedance began to decrease. However, when the concentration of PGHAP was over 5.5 mg/mL, the decrease degree of the capacitance value became slowly

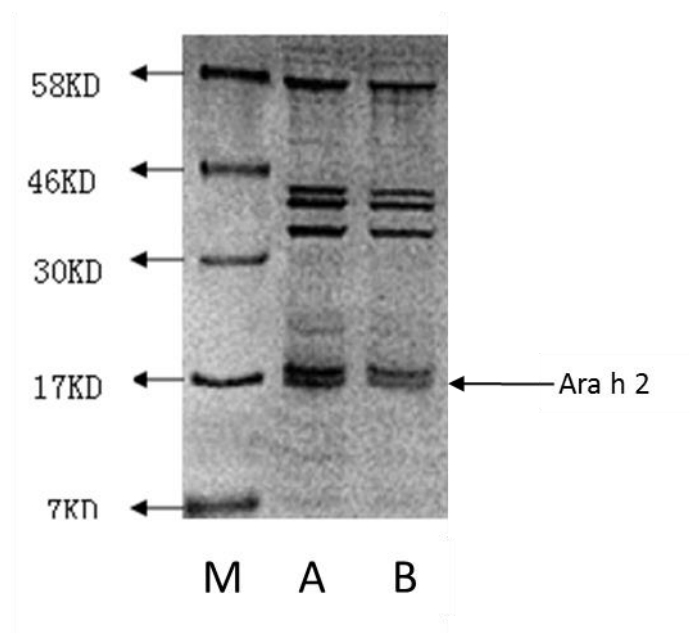


Fig. S3. SDS-PAGE analysis for Ara h 2 from raw and fired peanut purification. M: protein marker (58, 46, 30, 17, 7kDa). A: raw peanut, B: fired peanut.

In order to demonstrate the feasibility of our sensor that can identify the allergens from the real food samples quickly, raw and fired peanuts extra were used to test the performance of this sensor. Samples were prepared according to previously described procedures with minor modifications. Briefly, after homogenization in a mill at 10,000 rpm during 20 s, 1 g of sample was extracted with 10 mL of Tris-HNO₃ buffer (pH 8.2, 1% NaCl) at 60 °C during 30 min. Subsequently, samples were subjected to a first centrifugation step (5000 rpm) during 5 min. An aliquot (1 ml) of the supernatant was further centrifuged (4 °C) at 10,000 rpm for 3 min and the resulting supernatant was used directly to perform the electrochemical assays. Dilutions of the final extracts with 0.1 M Tris-HNO₃ pH 7.2 buffer were made when necessary. Then the extract solutions were subjected into anti-peanut Ara h 2 IgG pre-sensitized RBL-2H3 cells which were detected by electrochemical workstation for impedance analysis.

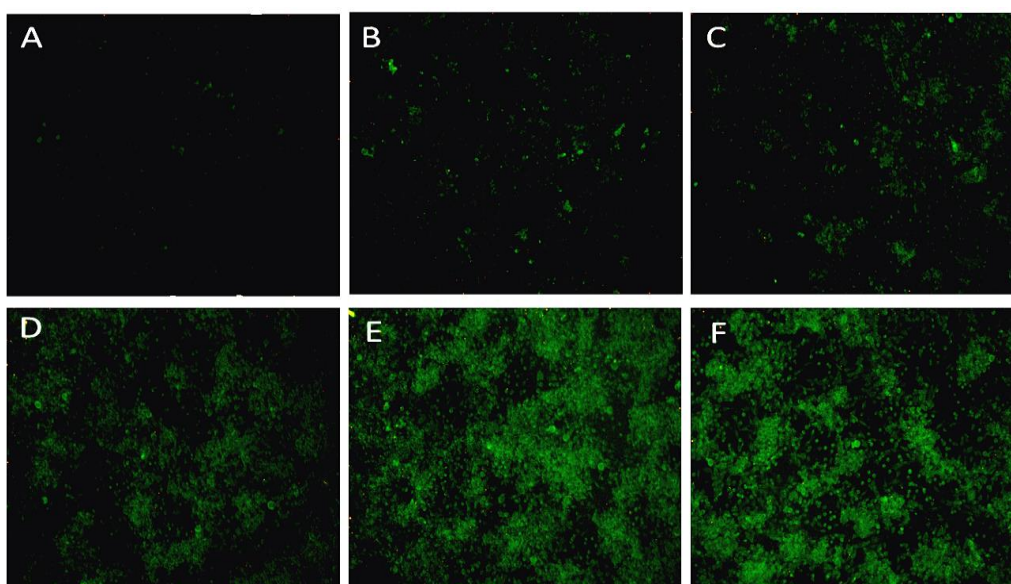


Fig. S4 Fluorescent detection of different concentrations of Ara h 2 sensitized RBL cells. (A) blank (B) 0 ng/mL (C) 0.1 ng/mL (D) 1 ng/mL (E) 10 ng/mL (F) 100 ng/mL

In the [Fig. S4](#), Group A was blank without RBL cells and Ara h2. [Group B](#) showed the fluorescent image of the mast cell stimulated without Ara h 2, and a very weak green fluorescence was observed under a blue excitation light through the fluorescent microscope. [Group B](#) was the fluorescent image of mast cells stimulated with 0.02 ng/mL Ara h 2. Obviously, some of the mast cells degranulated and showed slight green fluorescence when observed under blue laser, indicating that the degranulation reaction was started in a small number of cells. When 0.04 ng/mL of Ara h 2 was used to stimulate mast cells showed in [Group C](#), the degranulation degree increased compared with the cells in B group, which showed stronger fluorescence intensity than B group. When 0.06 ng/mL Ara h 2 was used to stimulate mast cells in [D group](#), compared with the previous three groups, stronger fluorescence was observed, indicating that large scale of degranulation was occurred in cells. [E group](#) use 0.08 ng/mL Ara h 2 to stimulate mast cells, and the fluorescence was significantly stronger than the D group. Finally, strongest fluorescence was observed in the [F group](#) which 0.10 ng/mL Ara h 2 was used to stimulate, imply that degranulation was happened in almost every mast cells. According to the above observations we came to the conclusion that with the increase of the concentration of Ara h 2, the degranulation degree of mast cell became more obvious, and the stronger cell fluorescence was observed, which well verified the electrochemical detection results in the manuscript.

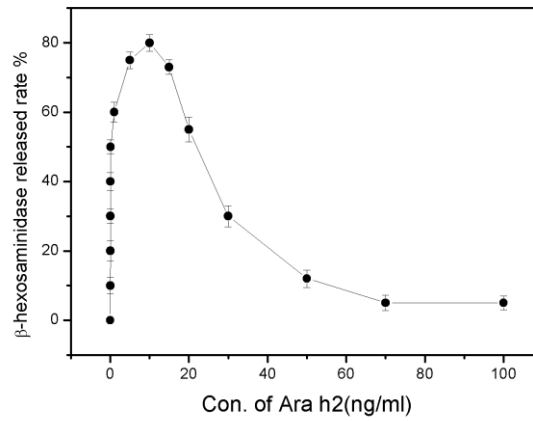


Fig. S5. β -hexosaminidase assay for the confirmation of capacitance measurement results.

As seen from [Fig.S5](#), β -hexosaminidase assays were performed to confirm the capacitance measurement results. This indicated that the β -hexosaminidase release rate increased gradually with the growth of allergen concentrations. These data agreed well with the dose-response curve obtained from impedance measurements.

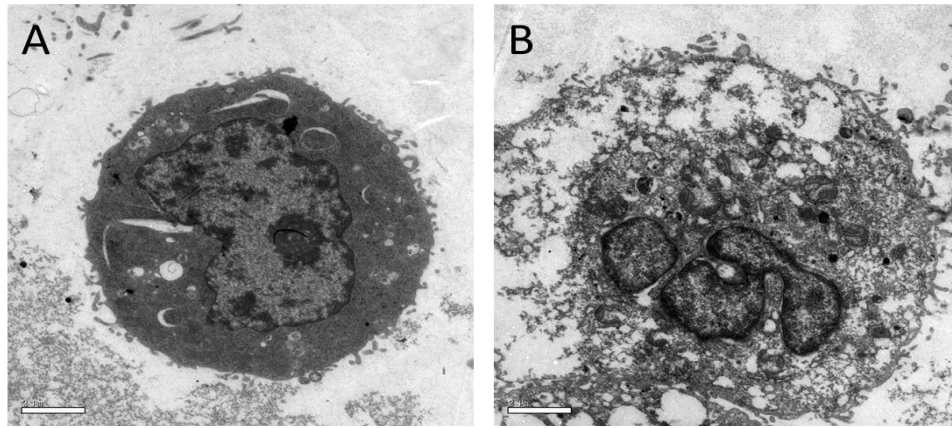


Fig.S6. TEM images of RBL mast cells and its cellular degranulation.

After incubation with 10 ng/mL Ara h 2, we found that Ara h 2 significantly increased degranulation in RBL cells. Compared with control group (Fig. S6A), the cells exhibited obvious morphological changes such as deformation, shrinkage, secretory granule release, sparse intracellular particles, and vacuolated cytoplasm, altogether indicating the occurrence of degranulation (Fig.S6B)

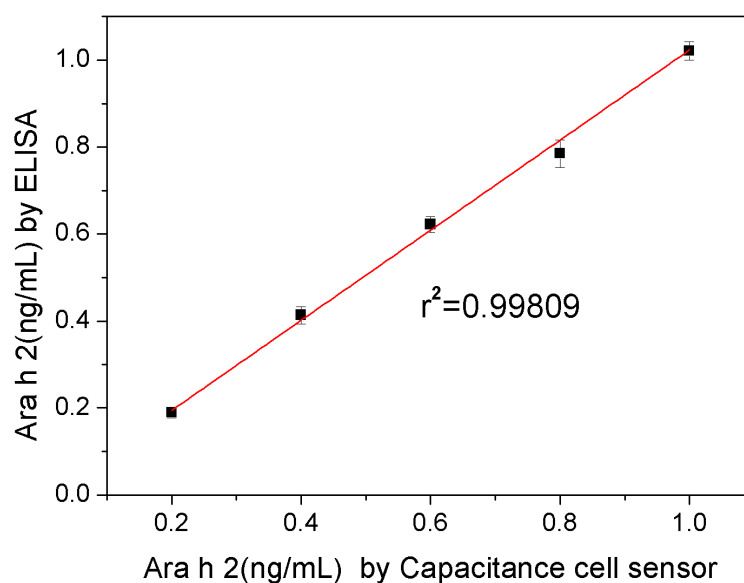


Fig.S7. The comparison between the capacitance measurement and the ELISA assay. Error bars estimated as triple of the standard deviation (n = 3)

An excellent correlation was found between the Ara h 2 concentrations measured with the ELISA kit and those obtained with the capacitance cell sensor (Fig. S7) with the confidence intervals (at a significance level of, $\alpha=0.05$) for the slope and intercept including the unit and the zero values, respectively. However, the analysis time of the cell sensor was only a quarter of that of the ELISA method (1 h *versus* 4 h), and the amount of samples and reagent consumption required in the whole analysis process was far less than that of the ELISA method (10 μL *versus* 100 μL). Hence, this will facilitate the significant reduction of detection cost and the development of portable rapid detection equipment. As a result, the cost of each analysis can be drastically reduced, and it is easy to prepare the automated portable analytical instruments. These advantages make the capacitance cell sensor an attractive tool to monitor routinely the food quality and to perform decentralized analysis in comparison with the ELISA method.