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A novel mast cell co-culture microfluidic chip for the electrochemical evaluation of food allergen

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ABSTRACT

In this study a novel cell-to-cell electrochemical microfluidic chip was developed for qualitative and quantitative analysis of food allergen. Microfluidic cell culture, food allergen-induced cell morphological changes, and cell metabolism measurements were performed simultaneously using the aforementioned device. RBL-2H3 mast cells and ANA-1 macrophages have been used within a cell co-culture model to observe their allergic response when they are introduced to the antigen stimulus. Two cell cultivation microfluidic channels are located in the microfluidic chip, which is fabricated with four groups of gold electrodes, with an additional "capillary". In order to detect the allergic response, the cells were stimulated with dinitrophenylated bovine serum albumin (DNP-BSA) without anti-DNP IgE incubation. When exocytosis occurs, the cell-secreted inflammatory cytokines were measured by enzyme-linked immuno sorbent assay (ELISA) and cell impedance changes were detected using cell-based electrochemical assay. Results indicate that the real-time cell allergic response are accurately monitored by this electrochemical microfluidic chip, which provides a general example of rapidly prototyped low-cost biosensor technology for applications in both food allergen detection and investigation.

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1. Introduction

Food allergies have become a more serious and prevalent issue within global health over recent years (Montserrat et al., 2015). According to a recent survey, the estimated prevalence of food allergies in the population of industrialized countries is almost 2% of adults, and up to 8% of children (Gomaa and Boye, 2015). This is especially concerning because allergic reactions are potentially fatal, with other harmful symptoms possibly occurring (Baumert, 2014). An allergic reaction due to food is an adverse immune response to certain types of food, defined as an allergen-specific IgE-mediated type I response (Matsuo et al., 2015). These responses are caused by the release of chemical mediators, such as histamine and leukotrienes from activated mast cells and basophils. Cross-linking of IgE receptors with an allergen is required for these cells to activate (Baumert, 2014). Since even a very minimal hidden allergens in final food products can induce severe immunological reactions (Byun et al., 2002), and there is no way to avoid allergens completely (Nwaru et al., 2014), successful and widely-available allergen management tools are required to detect their presence in foods and to ensure food safety (Yokooji et al., 2014). An effective method for evaluation of allergens in foodstuffs is a key component of allergy treatment, as it aids prevention of exposure to allergens.

Many methods are used to identify the presence of allergens in food including surface plasmon resonance (SPR) (Alves et al., 2015), real-time polymerase chain reaction (RT-PCR) (Eischeid, 2016), and enzyme-linked immunosorbent assay (ELISA) (Montserrat et al., 2015; Palle-Reisch et al., 2015). These methods possess a strong sensitivity with even higher accuracy; however, complicated pre-treatment procedures as well as a high false-positive rate remain a costly deficiency. The rat basophilic leukemia (RBL-2H3) cell line possesses abundant FcεR receptor on its surface, which can selectively bind murine IgE antibodies so that subsequent identify specified allergen through the cross-link of the FcεRI-IgE complex on the surface of mast cells (Ribatti, 2015). A series of intracellular contents will be activated through this method, which leads to cellular degranulation and the release of chemical mediators such as histamine, serotonin,

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and β-hexosaminidase (Yu et al., 2015). Hence, RBL-2H3 mast cells can locate target antigens and convert biological recognition into a signal that can be recorded and quantified visually (Jiang et al., 2013, 2015).

In recent years, microfluidic chip technology has gained the interest of researchers while creating new opportunities in the biological and food safety detection research (Xu et al., 2013). The integrated microfluidic device allowed the performance of high-throughput bioassay and was widely applied in cell culture, cell metabolism, and pollution analysis (Materne et al., 2015). Microfluidic chips coupled with photology, fluorescence, and electrochemistry technologies were by far the most widely used technique for analytic identification (Fan et al., 2015). These biochips can be fabricated with materials that are non-toxic with flexible properties, such as Polydimethylsiloxane (PDMS) (Bricks et al., 2014).

Electrochemical impedance spectroscopy (EIS) measurements have been consistently proven valid as a noninvasive and sensitive detection technique for studying living cells, showing promising application in to monitoring adhesion, viability, and environmental changes within cells (Abiri et al., 2015). Recent studies on biosensors have demonstrated that EIS measurements produce significantly more sensitive responses than cyclic voltammograms (CV) in the as far as fabrication of a label-free cell-based biosensors (Gu et al., 2015). Thus, a sensitive and specific electrochemical signal generated by cells immobilized on an electrode can be detected rapidly and accurately, especially when exposed to allergens.

In this paper, a new cell co-culture microfluidic chip for food allergen identification and evaluation is proposed. The function of this proposed cell chip is mimicked by the co-culture of the ANA-1macrophage as an antigen-presenting unit and RBL-2H3 mast cell as effective apparatus in microfluidic biochips. These cells are cultivated on the PDMS channels, where electrochemical-plating electrodes fabricate the bottom. Thus, they are able to form a sensitive immune sensing system with properties similar to those of the human intestine that allows the quick detection and study of the mechanisms of the allergic reaction. Simultaneously, electrochemical impedance technology, along with inflammatory cytokines ELISA detection, is utilized in order to provide a comprehensive evaluation for the study of food allergens. Therefore, we present a novel method of designing a cell co-culture microfluidic sensor to evaluate the allergy in foodstuff, which laid a foundation for the development of portable detection equipment.

2. Materials and methods

2.1. Materials and apparatus

Dinitrophenyl-BSA of mice was obtained from Sigma-Aldrich Inc (St. Louis, MO, USA). Rat basophilic leukemia (RBL) cells and ANA-1 macrophage were obtained from the Cell Bank of Chinese Academy of Sciences (Shanghai, China). IL-6 ELISA kit, TNF-α ELISA kit, INF-γ ELISA kit were purchased from R&D Systems (Minneapolis, CA, USA). RPMI 1640 medium and fetal bovine serum were obtained from Gibco Laboratories (Gaithersburg, MD, USA). All solutions were prepared with deionized water and all reagents were analytical grade.

All electrochemical experiments were performed with an AutoLab PGSTAT302N electrochemical workstation (Metrohm Autolab, Utrecht, Netherlands). Cells were incubated in a CO_2 incubator (Thermo Scientific Forma Series II Water Jacket, Thermo Fisher Scientific Inc., Rockford, IL, USA).

2.2. Fabrication and design of the microfluidic device

The method was referred to in Mao's report (Mao et al., 2012) with some modifications. A microfluidic chip, coupled with gold electrodes, was developed in order to investigate Cell-to-Cell Communication affected by the food allergen DNP-BSA. The chip was divided into two parallel channels, up and down; four groups of working electrodes and counter electrodes were electroplated in each of the channels. A delicate “capillary” connected these two channels. When the liquid flow in the two channels exhibited the same direction and same speed, no liquid will be able to pass the capillary because of the shared pressure on both capillary ends. This is referred to as the “parallel model”; when the injection of the liquid in one channel was stopped, the liquid of the other channel will flow through the capillary due to the pressure difference, which realize the connection of these two channels. The entire chip was designed by our lab independently, and was processed by Wenhao chip technology co., LTD. (Suzhou, China).

The making craft of the microfluidic chip is composed of two parts. One part is the building of PDMS flow channels layer through soft lithography and replica molding techniques. As shown in Fig. 1D, the PDMS layer was 44,670.83 μm length, 31,200 μm wide, 4 mm thick, and contained up and down channels which were 1000 μm wide, and 31,673.16 μm length. Each end of the channels contained a diameter of 2500 μm socket mouth, where cell suspension, as well as sensitization drugs, could be injected into the channels. There was a 100 μm wide, 10,000 μm length capillary in the middle of these two channels. For easy insertion of the miniature Ag/AgCl reference electrode, each end of the capillary was equipped with a diameter of 2000 μm port. The design of capillary referred to the surface tension of the solution in the end of the narrow channel, and the solid-liquid interface principle of curvature radius (Mao et al., 2012). The capillary is easily integrated into the chip; no other auxiliary equipment should be needed to see the connection between these two channels. The size of chip was calculated using the following formula:

$$\mu = \frac{\gamma h w}{4\pi r l T}$$

Among them, μ represents linear velocity in main channel, γ is the surface tension (γ = 0.07 N/m, 37 °C), h and w are the height and width of main channel, l is the distance between end of the capillary and the end of channel, r is the equivalent diameter of the channel, and T is the coefficient of the viscosity solution.

The other part concerns the bonding of the PDMS flow channel layer and the electric plating electrode glass wafer. As shown in Fig. 1E, the four groups of gold work electrodes and the counter electrodes were electroplated in glass wafer surface plating, each work electrode was 1000 μm in diameter, surrounded by the 1000 μm wide counter electrode ring, and they were all guided by 500 μm wide line link to the edge of the gold finger (4000 μm wide). The surface of PDMS substrate was activated by the plasma, and then sealed to the slide irreversibly to obtain a complete cell co-culture microfluidic chip (Fig. 1F). Finally, the chip was dried under vacuum at 60 °C for 2 h to enhance the bonding effect.

2.3. Cell co-culture on microfluidic chip

The ANA-1 cells were maintained in Dulbecco's modified Eagle's minimal essential medium (DMEM) with a high glucose supplement containing 10% fetal bovine serum (FBS), nonessential amino acids, 100 units/mL penicillin, and 100 units/mL streptomycin in a humidified atmosphere of 95% air and 5% CO_2 at 37 °C. The RBL-2H3 mast cells were maintained in RPMI 1640 with high glucose supplemented with 10% FBS, 100 units/mL penicillin, and

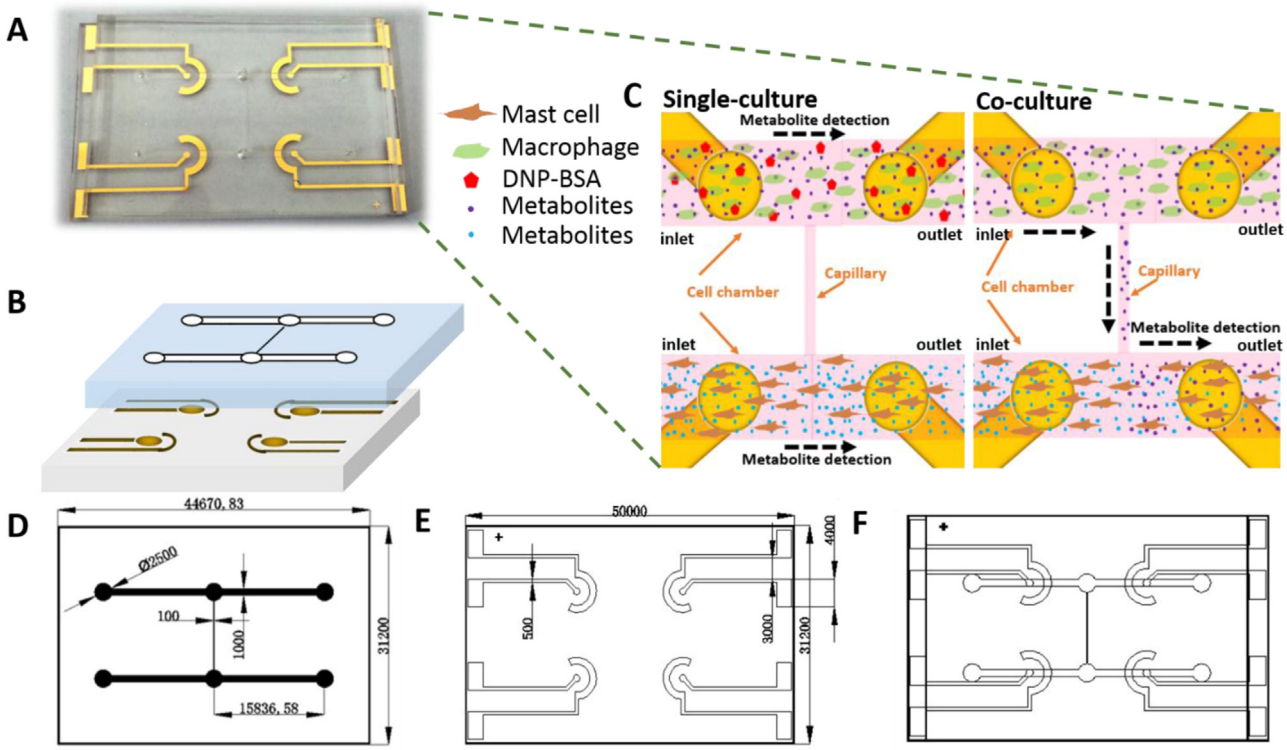

Fig. 1. Photograph of a two-layer cell co-culture microfluidic chip (A). The chip was composed with two layers (B). The first part is the build of PDMS flow channels layer through soft lithography and replica molding techniques. The second part is the bonding of PDMS flow channel layer and the electric plating electrode glass wafer. A magnified schematic of a bioreactor on the microfluidic device (C). Structure of PDMS (D) and electrodes (E) in microfluidic chip (μm). Assembly diagram of the microfluidic chip (F).

100 units/mL streptomycin in a humidified atmosphere of 95% air and 5% CO_2 at 37 °C. The ANA-1 macrophages and RBL-2H3 mast cells were maintained in Petri dishes for 2–3 days prior to commencing the microfluidic experiments. All the experiments were carried out when the cells were in the exponential growth phase.

When microfluidic chip assembly was finished, the cell-culture channels were filled with 0.1% poly L-lysine solution at room temperature for 1 h and then washed by PBS. The modified channels were sterilized under UV light for 5 min before cells were loaded, and then rinsed with 75% ethanol and sterile PBS. Cells were then detached from the Petri dishes with 0.25% trypsin, suspended in corresponding cell-culture medium, counted with a hemocytometer and injected into the cell-culture channel at a density of 1×10^6 cells/mL. The top surface of the microfluidic chip was covered with cell-culture medium to decrease liquid evaporation. Then, the seeded microfluidic device was placed into a humidified atmosphere of 95% air and 5% CO_2 at 37 °C for more than 6 h, and cells attached to the bottom of the channels. After the cells adhered, syringes filled with the corresponding cell-culture medium were connected to the cell-culture channels with polytetrafluoroethylene (PTFE) tubes, as shown in Fig. 2. Cells could be serially cultured in the microchannel by the injection pump.

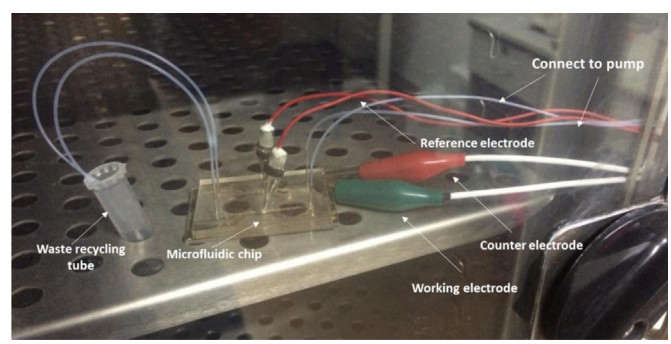

Fig. 2. Schematic of continuous culture for cells in microfluidic chip.

2.4. Detection with microfluidic system

100 ng/L DNP-BSA of allergen model initial solution was prepared by the RPMI 1640, and was then diluted to 10^{-3} ng/L–10 ng/L, respectively. After the two cells were incubated in the two main channels with a cell density of 10^6 cells/mL, respectively, an injector filled with solution containing different concentrations of DNP-BSA connected to the inlets of the ANA-1 macrophage cell-culture channels. The allergen solutions flowed into the channel to stimulate the macrophage cells in single-culture model with a flow rate of 10 μL/min for 1 h (Fig. 1C left). Then, the allergen solutions were stopped and changed to complete medium for mast cells activation in co-culture model (Fig. 1C right). The metabolites containing signalling molecules either from single-culture or co-culture were then introduced into micro recycling tube for three kinds of inflammatory cytokines IL-6, TNF-α, INF-γ production ELISA detection.

AutoLab PGSTAT302N electrochemical workstation was used for the real-time monitoring and analysis of impedance signals for single-culture or co-culture to detect the cell electrochemical signal changes in the microfluidic chip, as well as to verify the inflammation factor detection results of ELISA assay. Impedance-Time curves were recorded under the optimized scanning frequency of 10 kHz, voltage amplitude of 50 mV (Fig. S1). Signal acquisition was completed within a 2 s time interval.

2.5. Statistical analysis

The impedance data from the analyzer is transferred to a computer, analyzed and processed by the integrated software. All values are presented as mean ± s.e.m.. One-way ANOVA (LSD) or independent-samples *t*-test was used for statistical analysis using software (SPSS Statistics 21.0 for Windows, IBM Corp., NY, USA).

3. Results and discussion

3.1. Fabrication of microfluidic system

As shown in Fig. 1, to observe the single-culture or co-culture of ANA-1 macrophages and RBL-2H3 mast cells in the microfluidic chip, we designed two straight channels on the chip, sized 1000 μm in wide and 31,673.16 μm in length, where both ends of the channels existed two outlet holes with a diameter of 2500 μm. Between these two channels there was a 100 μm wide and 10,000 μm length capillary that connect these two main channels to monitor the control of the liquid flow in parallel or in series. Within the capillary ends there were two open holes (2000 μm in diameter), which were advantageous for the micro reference electrode insertion. In addition, four groups of gold electrodes have been electroplated on the surface of glass chip, with the insertion of the counter electrodes; three-electrode system will be constituted to realize the detection of cell electrochemical impedance values. All the channels in chip were 100 μm in height.

As shown in Fig. 3A, when the red and blue dye solutions were injected simultaneously, at the same speed and the same direction into channels by the digital injection pump, these two colours liquid would flow in their channel respectively and would not have contact with each other due to the same gas liquid pressure at the ends of the capillary. When the injection of blue liquid was stopped and red liquid continued, the red liquid will be pushed through the capillary and flow into the blue liquid's channel (Fig. 3B), and red liquid began to occupy half of the below channel gradually due to the liquid pressure at the end of capillary is higher than the gas pressure (Fig. 3C).

3.2. Cell co-culture on microchip

The injection amount of cells should be controlled since the channel of microfluidic chip were all micron grade size. Dense cell density will lead to the blocking of injection port, resulting in the death of the channel cells. On the contrary, insufficient concentration of cells will prevent the substance exchange between cells and environment, thus affecting the cell signal links and growth (Palková et al., 2004). According to the cell numbers in 96-well plate and the actual area of main chip (31.67 mm^2), the required number of cell in channel was calculated about 3000 cells. The distribution of different concentrations of ANA-1 macrophages and RBL-2H3 mast cells suspension (10^4 cells/mL–10^7 cells/mL) has been observed after injection (Fig. 4A and B). When the injection cell density was 10^6 cells/mL, cell numbers in the channel numbered approximately 2862 cells, which was close to the theoretical calculation value of 3000 cells. Furthermore, after 24 h incubation, the cells grew sufficiently on the gold electrodes with a uniform distribution (Fig. 4C and D). Therefore, an appropriate cell numbers of 10^6 cell/mL have been selected for cell injection.

3.3. ELISA detection for inflammatory factor of cell co-culture system

In the case of allergic inflammation, macrophages and mast cells will release its synthesized cellular content to perform its physiological function, including histamine and leukotriene, trypsin, and a series of inflammatory factors, such as IL-6, TNF-α and INF-γ. They are likely the key link between the inducing period and effective stage during an allergic reaction. Thus, in order to verify the feasibility of this cell co-culture system for the recognition of food allergen, the release quantity of inflammatory factor (IL-6, TNF-α or INF-γ) in cell co-culture supernatant was measured by the ELISA method. Different concentrations of allergen standard (DNP-BSA), such as 10^{-3} ng/mL, 10^{-1} ng/mL, or 10 ng/mL, were employed to stimulate the macrophages and mast cells co-culture or single culture system, and 1 μg/mL lipopolysaccharide (LPS) as the positive control. Cells supernatant under different cultivation way were collected respectively, as determined by ELISA.

As shown in Fig. 5A, C and E, IL-6, TNF-α and INF-γ production of cells co-culture system increased with different concentrations of DNP-BSA when compared with blank group (without DNP-BSA). By comparison, IL-6, TNF-α and INF-γ (macrophage treated with 1×10^{-1} ng/mL DNP-BSA) were obviously higher than that of the subjects treated with 1×10^{-3} ng/mL and 10 ng/mL treatment groups, and there were significant statistical differences within the data (One-way ANOVA, $^{*}p<0.05$, $^{**}p<0.01$, n=3). While, there was no significant statistical difference between the 1×10^{-3} ng/mL DNP-BSA treated groups and the 10 ng/mL DNP-BSA treated groups (One-way ANOVA, $^{ns}p>0.05$, n=3). Moreover, when compared with 0 h groups, IL-6, TNF-α and INF-γ release quantity of 12 h groups increased significantly, which was treated with different concentrations of DNP-BSA (*t*-test, $^{**}p<0.01$, n=3). Interestingly, there were no significant statistical difference in the treatment group between 12 h and 24 h, 12 h and 48 h (*t*-test, $^{ns}p>0.05$, n=3).

The IL-6, TNF-α, and INF-γ release quantities of macrophages and mast cell co-culture system treated with different concentration of DNP-BSA and then incubated for 12 h were obviously higher than that of macrophage or mast cells single-culture. As shown in Fig. 5B, D and F, there were significantly statistical differences among these groups (*t*-test, $^{**}p<0.01$, n=3). Hence, all these experiment results depicted an obvious synergistic effect existed in the macrophages and mast cells co-culture system for secretion of IL-6, TNF-α and INF-γ.

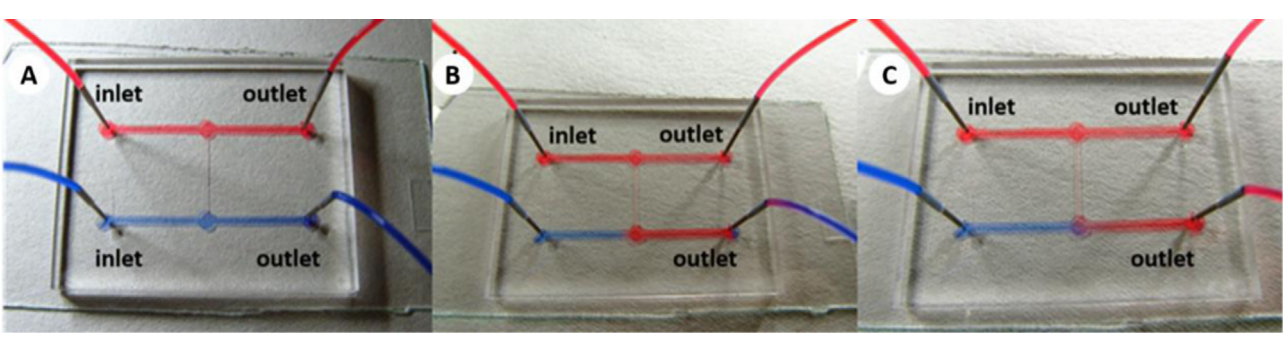

Fig. 3. Capillaries in microfluidic chip test.

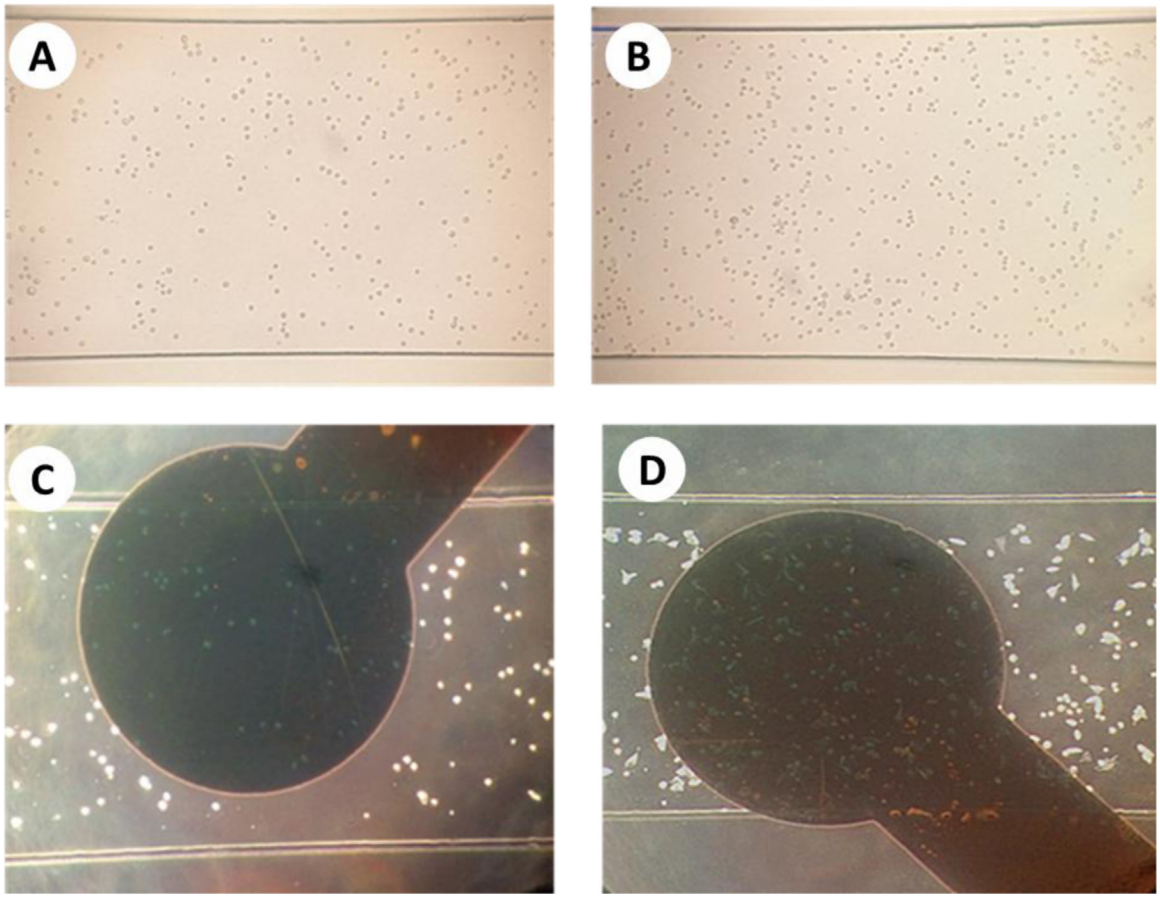

Fig. 4. Observation of cells in microfluidic chip. The distribution of ANA-1 macrophages and RBL-2H3 mast cells in the channels (A and B). The distribution of ANA-1 macrophages and RBL-2H3 mast cells on gold electrodes (C and D).

3.4. Electrochemical impedance assay for the evaluation of allergen

Cell proliferation is a divisive, differential, migratory, and dynamic process (Thomson et al., 1998). The growth of cells in the process is affected by a variety of external factors. Conventional cell detection method are based on the terminal result, which cannot reflect the entire process of metabolism of cell, thus real-time monitoring of cell physiological activities is unable to achieve (Nwankire et al., 2015). Nevertheless, the emergence of electrochemical sensing technology will result in vast improvement, only a small amount of cells are required for detection and high flux analysis (Si et al., 2015). Electrochemical Cell Impedance Sensing (ECIS) is a type of measurement that detects the resistance change between cell layer and the electrode caused by cell division, migration, and contact with others. In this study, microfluidic chip electroplated with gold electrode was used for real-time monitoring of the cell impedance spectrum in the co-culture system.

To utilize the real-time monitoring of impedance signal of cells in co-culture system, Normalized Cell Index (NCI) was applied as the evaluation Index, which will improve the consistency of cell sensor detection results.

The cell growth condition on chip electrode was monitored through the ECIS system. First, the NCI value of ANA-1 macrophages was monitored in real time through ECIS during their single-culture stage, with 20 h being the normalization point. As shown in Fig. 6A, after 1 or 2 h rest, cells started to grow and cover the surface of the electrode gradually, and bare area of electrode surface began to decrease, making the surface of the electrode impedance values continually increasing rapidly. This indicates that the macrophage were in a rapid growth period. From 20 to 40 h, the NCI values grew steadily, and the electrode surface was continuously covered by the newborn cells which presented a steady growth state. After 48 h cultivation, cells entered a stable period, the NCI values were maintained with slightly fluctuations. Although the electrode surface had been covered with cells, the aging cells will be washed away and replaced by the new cells due to the continuous culture function of chip, so the NCI value remained smooth and steady. In addition, the black curve and the red curve in the graph represented the NCI measured by the two gold electrodes in the same channel; the measurement results exhibited the same trend, which demonstrated that the microfluidic chip had a good reproducibility.

While in Fig. 6B, the NCI value of RBL-2H3 mast cells were also monitored by ECIS during their single-culture stage, 20 h was selected as the normalized point. The measurement results were similar to that of macrophage cell proliferation. The difference was that mast cells grow significantly faster at the same time compared with macrophages, which lead to the rapid increase of cell NCI value.

Fig. 6C was the growth curve of macrophages and mast cells in co-culture. Among them, curve *a* and *b* represented cell NCI value of macrophages on the two electrodes in the same channel, respectively. Curve *c* was the NCI value of mast cells after they contacted with macrophages metabolites. The NCI value declined over a period time for the stop in nutrient delivery of mast cells at 30 h. After that it would quickly recover and sustain growth, proving that macrophages could promote the proliferation of the mast cell, in agreement with Tamang's report (Lama Tamang et al., 2012) as well as the ELISA detection results. Curve *d* demonstrated that the mast cells gradually entered into a decline phase and

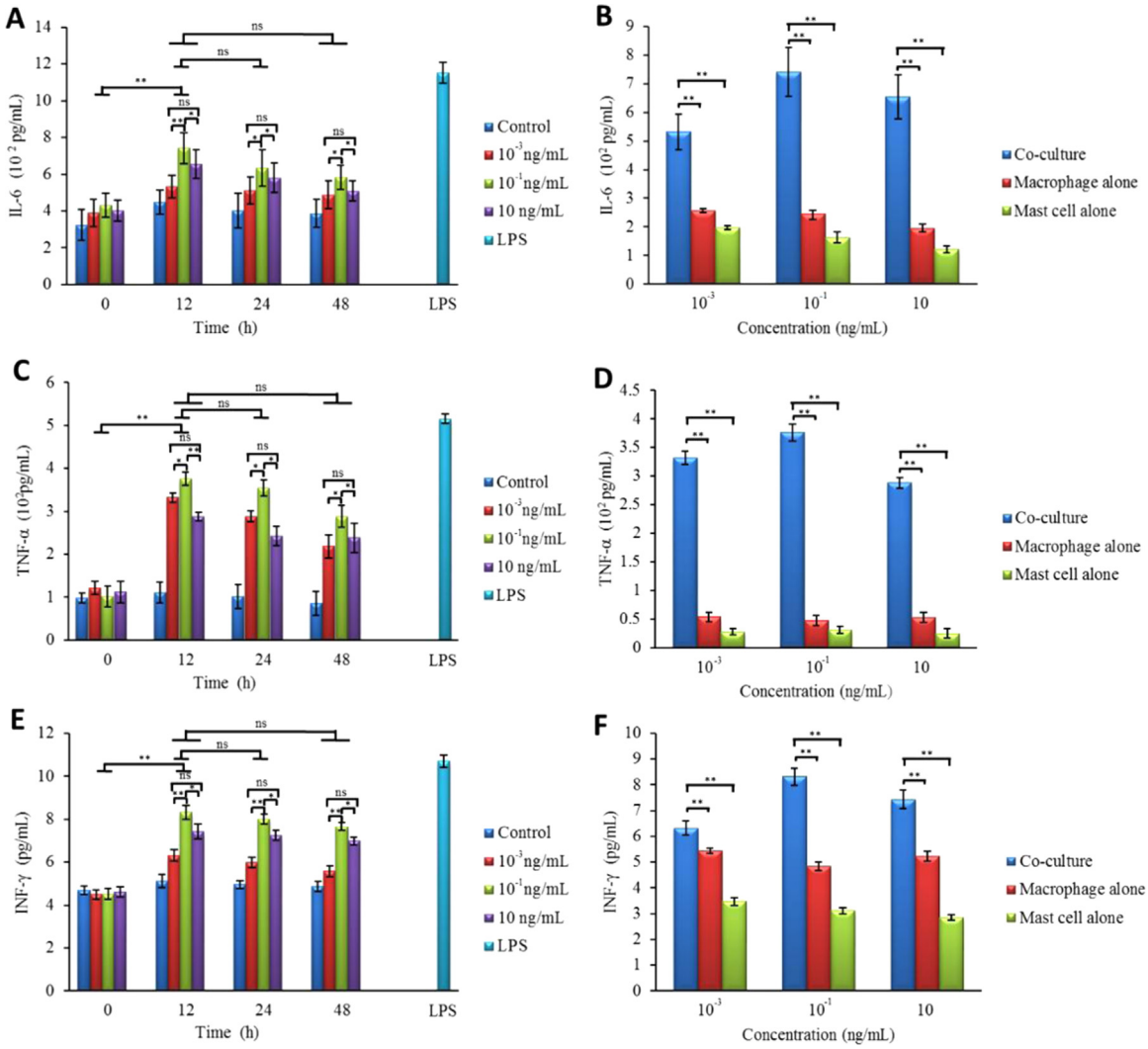

Fig. 5. IL-6, TNF-α and INF-γ secretion level of cell co-culture system affected by DNP-BSA in different time (A, C and E). IL-6, TNF-α and INF-γ secretion level of cell co-culture system and individual culture system affected by DNP-BSA in 12 h (B, D and F). All data shown is mean ± s.e.M. and *p*-value obtained by One-way ANOVA (LSD) or independent-samples *t*-test. (^{ns} $p > 0.05$, $^{*}p < 0.05$, $^{**}p < 0.01$, n = 3).

turned to cell apoptosis and dead without culture nutrition supply, which lead to the dramatic fall of the NCI value.

Next, culture medium containing different concentrations of DNP-BSA (10^{-3} ng/mL, 10^{-2} ng/mL, 10^{-1} ng/mL, 1 ng/mL and 10 ng/mL) were injected into the macrophages and mast cells co-culture system, and the electrochemical impedance signal were detected simultaneously. The NCI values at 20 h were used as the starting point. As shown in Fig. 6D, when treated with concentrations of DNP-BSA, the growth of mast cells were suppressed, resulting in decreasing degrees of the NCI value. The decline speed and amplitude of NCI were related to the concentrations of DNP-BSA and incubation time. Among them, the NCI value-declining rate of 10^{-1} ng/mL group fell most obviously when compared with that of other groups. Although a large drop occurred in the NCI value of high concentrations group (1 ng/mL and 10 ng/mL), the decline rate was less than the 10^{-1} ng/mL group. This proved that without the help of DNP-IgE, the cell co-culture system could only recognize antigen and rendered the allergic signal to mast cells in a certain extent. Hence, the proposed method had a certain saturation value that was suitable for the qualitative identification of allergen antigen in a certain concentration range. Low allergen levels could not cause the sensitivity of cell co-culture system, thus the NCI value of 10^{-3} ng/mL and 10^{-2} ng/mL group remained nearly unchanged. In addition, the medium without DNP-BSA was used as the negative control group whose NCI value kept stable; while 10 ng/mL DNP-BSA solution was employed as the positive control group.

4. Conclusion

In this study, a novel cell co-culture microfluidic chip was developed, which observed the ANA-1 macrophages and RBL-2H3 mast cells co-culture or single-culture. According to the principle of surface tension, two culture channels in the microfluidic chip could be connected through the creative capillary, which will then observe the free control of parallel-series connection for mast cells and macrophages. ELISA measurement results proved that proper levels of DNP-BSA could be the catalyst in the increase of IL-6, TNF-α and INF-γ secretion in cell co-culture system significantly, in which cells treated with 10^{-1} ng/mL DNP-BSA for 12 h performed most obviously. It was demonstrated that DNP-BSA could promote the expression of IL-6, TNF-α and INF-γ in macrophages and mast cells co-culture system, also showing that there was a synergistic

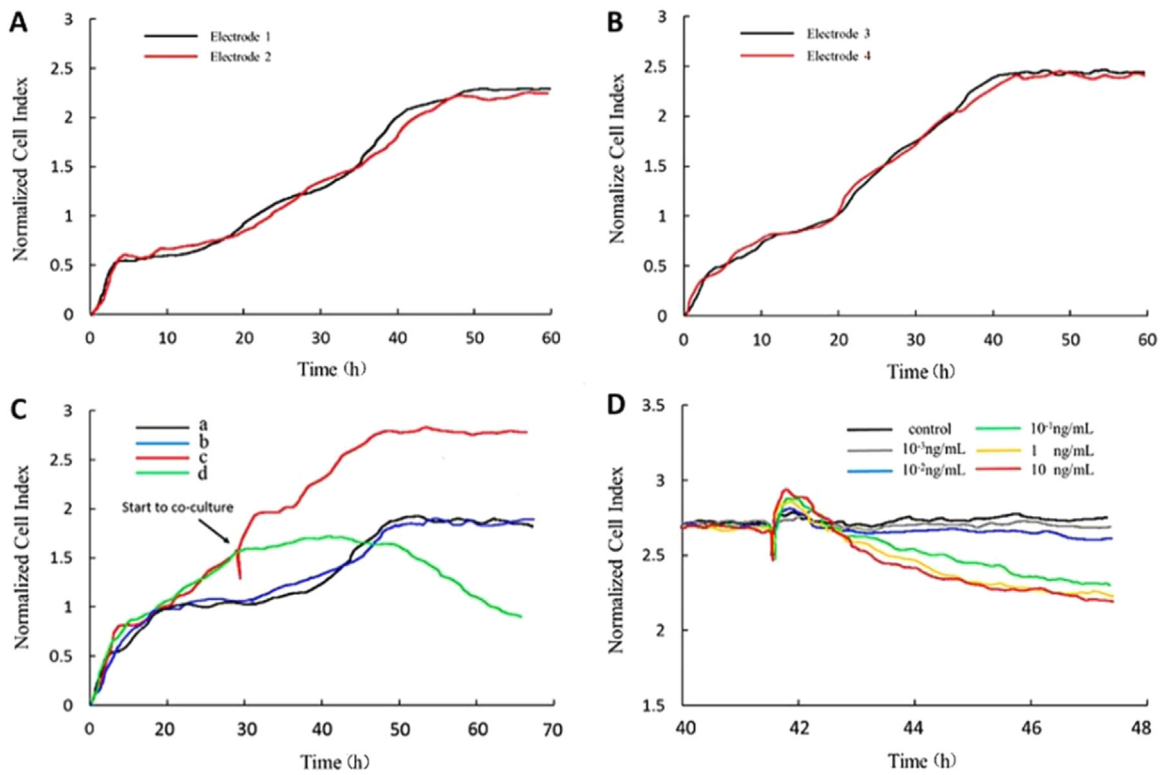

Fig. 6. Growth curve of ANA-1 macrophages in microfluidic chip (electrode 1 and 2: electrodes in the upperstream) (A). Growth curve of RBL-2H3 mast cells in microfluidic chip (electrode 3 and 4: electrodes in the downstream) (B). Growth curve of ANA-1 macrophages (*a* and *b*) and mast cells (*c* and *d*) in co-culture (C). Growth curve of RBL-2H3 mast cells in co-culture system affected by DNP-BSA (D). (For interpretation of the references to color in this figure, the reader is referred to the web version of this article).

effect on the production on macrophages and mast cells. Finally, cultivation process of macrophages and mast cells co-culture system were monitored in real time through the EIS system, where the NCI value was used to reflect the variation trend of the cell impedance value. Results show that the growth of macrophages and mast cells would achieve a stable state at 40 h, and under the condition of co-culture, macrophages could effectively promote the proliferation effect of mast cell in a certain extent. When different concentrations of DNP-BSA were injected into the cell co-culture system, the NCI value of mast cells decreased obviously with the increase of DNP-BSA. Among them, 10^{-1} ng/mL DNP-BSA has shown the most significant effect. A dose of DNP-BSA that is too low or high cannot cause allergic reactions of mast cells in co-culture system, these conclusions were agreed with the ELISA detection results for the secretion of inflammatory factor in cell co-culture system, which improved the development of the cell co-culture microfluidic chip system and provided a new practical method for the detection and evaluation of food hazards.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.bios.2016.04.028.

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Supplementary information

A novel mast cell co-culture microfluidic chip for the electrochemical evaluation of food allergen

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1. The assembly process of the PDMS layer

Polydimethylsiloxane, which has good biocompatibility and permeability, was employed as the main material. In brief, an integrated microfluidic device was fabricated using PDMS by soft lithography and replica molding techniques. First, an SU-8 2075 negative photoresist was spin-coated on a cleaned silicon wafer, and then the wafer was soft-baked in the oven. After being cooled to room temperature, the wafer with SU-8 was exposed to UV light (7 mW) for 30 s and developed by developing solution. Finally, the microchip mold was hard-baked for 5 min at 65 °C. A 10:1weight mixture of PDMS prepolymer and curing agent (Sylgard184, Dow corning) was then poured onto the silicon mold and baked in the oven at 85 °C for 2 h. Then, the PDMS was peeled off from the wafer mold, and connection holes were punched before the PDMS replica was irreversibly sealed with glass slides by oxygen plasma treatment.

2. The definition of Cell Index (CI) and Normalized Cell Index (NCI)

To utilize the real-time monitoring of impedance signal of cells in co-culture system, the CI was used as measure parameter for the characterization of cell impedance changes under specific frequency points, the formula is as follows:

$$CI(t) = \frac{Z_{cell}(t) - Z_0}{Z}$$

Among them, the *CI (t)* represents the cell index in *t*, $Z_{cell}(t)$ represents the impedance value of cells in *t* time, Z_0 represents the initial impedance value, and *Z* indicates electrode impedance values before cell inoculation, usually $Z_0 = Z$.

The CI (which used as the evaluation index) was beneficial to equally compare and evaluate the impedance values of the cells cultured on different surface, and avoid background noise values of the chip. In addition, the CI value can intuitively reflect the real state of cell growth, and the value of the CI decrease indicates the reducing of the cell numbers on the electrode surface. On the contrary, the CI value increases, proving that the growth of cells quantity on the electrodes surface and spread to the cell adhesion area (Tran et al. 2013).

There will be differences between the measurement results of original CI curve due to the differentiation of cell growth on the electrodes, which was unsuitable for the evaluation index of the cell sensor. Therefore, to reflect the cell impedance changes clearly and intuitively, NCI was applied as the evaluation Index, which will improve the consistency of cell sensor detection results. The relationship between normalized cell index and cell index was as follows:

$$NCI(t) = \frac{CI(t)}{CI(t_n)}$$

Among them, the *NCI (t)* represents the normalized cell index in *t* time, *CI (t)* represents cell index in *t* time, $CI\ (t_n)$ indicates cell index in normalized point t_n.

In conclusion, the normalization method can more intuitively reflect the change of cell impedance value compared with CI method. Therefore, the cell impedance value were shown as NCI in all following result of the experiments.

Figures Caption:

Fig. S1 Impedimetric spectroscopy of the microfluidic system and the microfluidic system without cells, i.e., control.

Fig. S2 Specific detection of allergens by co-culture systems. Quantification of the cell responses to stimulation with 10 ng/ml DNP-BSA, 10 ng/ml BSA, 10 ng/ml C48/80. Response of RBL-2H3 mast cells to these stimuli were recorded in parallel.

Fig. S3 The repeatability test of microchip and relative detection response.

Table S1 Comparison of several reported methods for cell co-culture investigation.

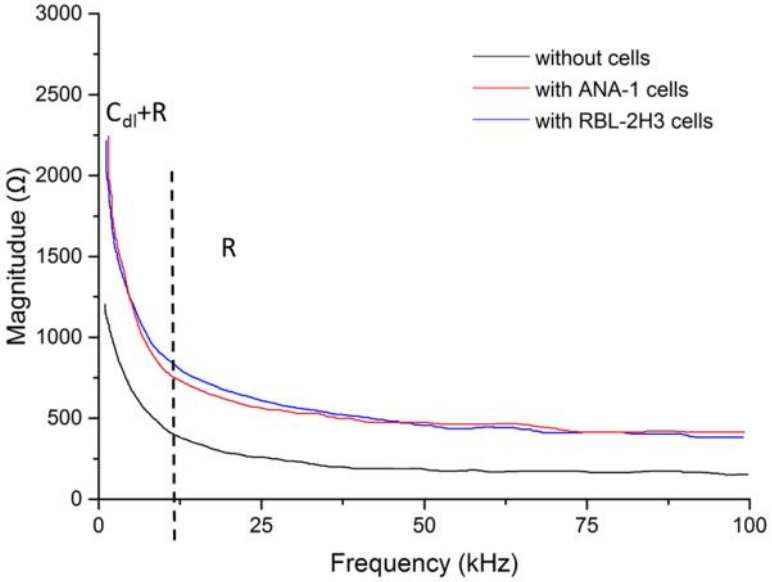

Fig. S1 Impedimetric spectroscopy of the microfluidic system and the microfluidic system without cells, i.e., control.

Cells suspension at a cell density of 1×10^6 cells/ml was loaded into the culture chamber and impedance measurement was performed to investigate the electrical characteristics of the microfluidic system. The impedimetric spectroscopy of the microfluidic system is shown in Fig. S1 and compared with the microfluidic system without cells, i.e., control. Research has shown that too high or too low frequency is not suitable as the signal for the cell impedance test. Intermediate frequencies are usually employed as the best detection frequency which generally ranged from 10 kHz to 40 kHz (Wang et al. 2008). Hence, as shown in Fig. S1, at the frequency below 10 kHz, the impedance magnitude decreased rapidly compared with the control. The total impedance below 10 kHz represented the combination effect of capacitance and resistance. At the frequency above 10 kHz, the total impedance was mainly dominated by the resistance of cells and medium. Therefore, it is generally suggested that the

measurement frequency below 10 kHz can better describe the presence of cells on the electrodes.

In the high-speed data acquisition system with the same condition of the sampling rate, the lower frequency of the sinusoidal signal was, the more points of the discrete signal could be collected in a sinusoidal cycle which would improve the accuracy for the calculation of sinusoidal periodic amplitude. Therefore, the scanning frequency of 10 kHz, voltage amplitude of 50 mV were selected in this study.

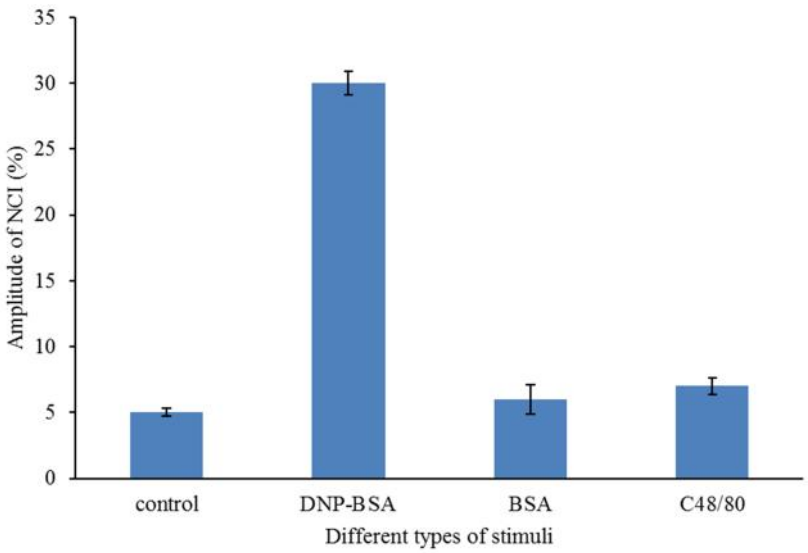

Fig. S2 Specific detection of allergens by co-culture systems. Quantification of the cell responses to stimulation with 10 ng/ml DNP-BSA, 10 ng/ml BSA, 10 ng/ml C48/80. Response of RBL-2H3 mast cells to these stimuli were recorded in parallel.

As shown in Fig. S2, BSA or C48/80 hardly produced any obvious response. Therefore, contamination of non-allergenic substance will not influence the recognition of allergens in food samples.

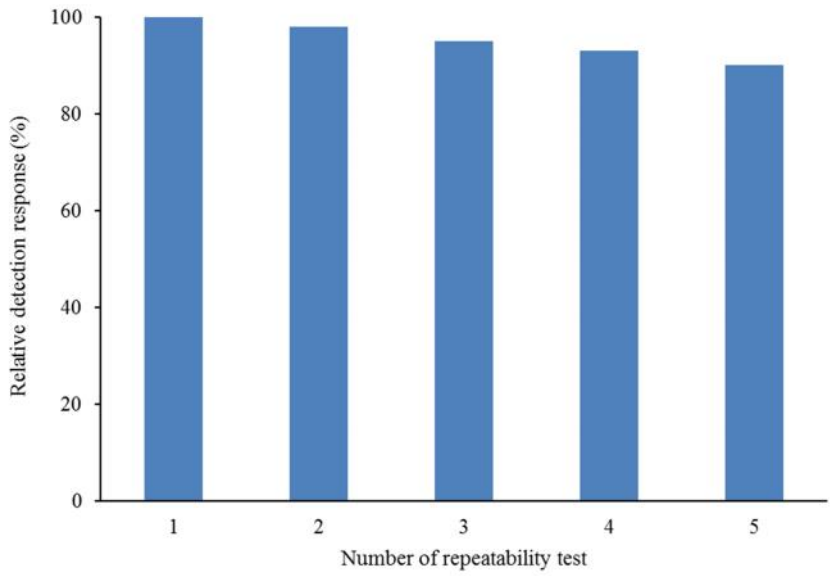

Fig. S3 The repeatability test of microchip and relative detection response.

The device is reusable after one experiment. When one test was finished, the cell-culture channels of the microfluidic chip were washed with 75% (v/v) ethanol in water at a flow rate of 10 μL/min for 3 min to remove cells, and rinsed with sterile PBS. Then, the channels were sterilized under UV light for 5 min before cells were loaded to determine next test. The device could be reused for 5 times. As shown in Fig. S3, after five rounds of regeneration, it also retained its 90% original detection response, which well illustrated the reusable characteristic of the set up. Also, a slow degradation rate of the set up was proved.

Table S1 Comparison of several reported methods for cell co-culture investigation.

Materials	Research object	Result	Reference
human meniscus cells，bone marrow mesenchymal stromal cells	the effect of interleukin-1β treatment	IL-1β induced MMP-1 expression in mono-cultures of MCs, MMP-13 expression in mono-cultured pellets of BMSCs and in co-cultured pellets.	(Chowdhury et al. 2013)
Sertoli cells, endothelial cells (ECs)	proliferation and immunogenicity	Sertoli cells promoted the proliferation of co-cultured ECs, accelerated post-transplant angiogenesis, while reduced EC immunogenicity and stimulus to lymphocytes.	(Fan et al. 2011)
amniotic membrane (AM), adipose-derived stem cell (ADSC)	bone regeneration	This study provides the basis for using ADSCs with an AM co-culture system as stem cell therapy and scaffold transplantation in clinical periodontology.	(Wu et al. 2015)
neurons, astrocytes	phenotypic behavioural traits of cells	Using ptychography as an assay to study the phenotypic behaviour of microglial cells in primary neuronal co-cultures through the addition of cyclosporine A.	(Suman et al. 2016)
hepatocytes, Kupffer cells	hepatotoxicity	HKCC model system may lead to enhanced or mitigate hepatocellular injury.	(Rose et al. 2016)
human adipose-derived stem cells, human osteoblasts	study for the differential osteogenic potential	Co-culture promoted better cell adhesion, higher cell proliferation, higher production of ALP.	(Rozila et al. 2016)
endothelial cells (ECs), smooth muscle cells (SMCs)	serelaxin signalling	Treatment of ECs with serelaxin caused marked cGMP accumulation in SMCs.	(Sarwar et al. 2016)
hepatocytes, stellate cells	monitoring liver cell signaling during injury	Our microsystem makes it possible to monitor paracrine crosstalk between two cell types communicating via the same signaling molecule (e.g. TGF-β).	(Zhou et al. 2015)
RBL-2H3 mast cells, Ana-1 macrophages	evaluation for food allergens	A real-time cell allergic response can be well monitored.	This study

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