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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.

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