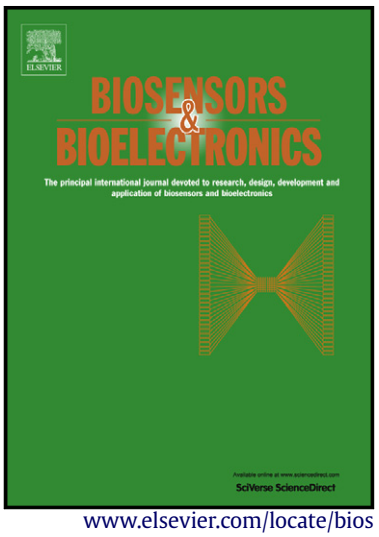

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PII: S0956-5663(13)00429-6
DOI: http://dx.doi.org/10.1016/j.bios.2013.06.032
Reference: BIOS6023

To appear in: *Biosensors and Bioelectronics*

Received date: 27 April 2013
Revised date: 14 June 2013
Accepted date: 14 June 2013

Cite this article as: Donglei Jiang, Jian Ji, lu an, Xiulan Sun, Yinzhi Zhang, Genyi Zhang, Lili Tang, Mast cell-based electrochemical biosensor for quantification of the major shrimp allergen Pen a 1 (tropomyosin), *Biosensors and Bioelectronics,* http://dx.doi.org/10.1016/j.bios.2013.06.032

Mast cell-based electrochemical biosensor for quantification of the major shrimp allergen Pen a 1 (*tropomyosin*)

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ABSTRACT

A novel cell-based electrochemical biosensor was developed to quantify major shrimp allergen Pen a 1(tropomyosin) and to assess its Immunoglobulin E (*Ig*E) -mediated hypersensitivity. Rat basophilic leukemia (RBL-2H3) mast cells, encapsulated in type I collagen, were immobilized on a self-assembled L-cysteine/gold nanoparticle (AuNPsCys) -modified gold electrode to monitor *Ig*E-mediated mast cell sensitization and activation. The exposure of dinitrophenol-bovine serum albumin (DNP-BSA), as a model antigen that stimulates mast cells, induced a robust and long-lasting electrochemical impedance signal in a dose-dependent manner which efficiently measured degranulation of anti-DNP *Ig*E-stimulated mast cells. Then this mast cell-based biosensor was applied into quantification for the shrimp allergen with anti-shrimp tropomyosin *Ig*E- sensitization. The electrochemical impedance spectroscopy (EIS) result showed that the impedance value (R_{et}) increased with the concentration of purified shrimp allergen Pen a 1(tropomyosin) in range of 0.5-0.25μg·mL^{-1} with the detection limit as 0.15μg·mL^{-1}, and the electrochemical result was confirmed by *β*-hexosaminidase assay and scanning electron microscopic morphological (SEM) analysis. Thus, a simple, label-free, and sensitive method for the determination of shrimp allergens was proposed and demonstrated here, implying a highly versatile biosensor for food allergen detection and prediction.

Key words: Electrochemical sensor, RBL-2H3 mast cells, shrimp tropomyosin, collagen

Introduction

Food allergy is regarded as a significant worldwide problem to public health. Among various kinds of food allergens, Shrimp is commonly identified as a cause of food hypersensitivity (Waring et al., 1985), and ingestion of shrimp may cause severe hypersensitivity such as urticaria, asthma, and anaphylactic shock(Davies et al.,1993; Ferrari and Eng, 2011). Thirteen different allergens were identified in brown shrimp *(Penaeus aztecus)*. The only major allergen (36 kd) that reacted to most subject's Immunoglobulin E (*Ig*E) was designated as Pen a 1 according to accepted International Union of Immunological Societies (IUIS) allergen nomenclature (Daul et al., 1991; Shanti et al., 1993). Amino acid sequence analysis of an internal Pen a 1 peptide identified this allergen as the muscle protein tropomyosin(Reese et al., 1995). Due to the severe responses of the immune system caused by shrimp tropomyosin, the detection for this protein in food has attracted increasing attention from the food industry and regulatory agencies over recent years.

Currently, multiple technical approaches have been designed to identify the presence of shrimp tropomyosin in food. However, most of the methods do not quantify the specific allergenic protein, but rather as an indicative marker for the presence of these proteins in food. Conventional skin test and RAST are considered as gold standard for the detection and characterization of shrimp tropomyosin (Daul et al., 1994 ; Fernandes et al., 2003), while, the allergic protein's level can not be quantified accurately as well as accompanied with risks. With the advent of monoclonal antibody (mAb), a series of mAb-based immunoassays have been developed to determine shrimp tropomyosin. These methods including enzyme-linked immuno sorbent assay (ELISA) (Zahradnik et al., 2011), quartz crystal microbalance (QCM)(Sun et al., 2010), immunoblotting (Yu et al., 2003). These methods are much more sensitive and specific than conventional methods, however, they require complicated sample pre-treatment procedures and are susceptible to contamination as well as high false-positive rate. Thus we need a rapid and sensitive tool by which the shrimp tropomyosin can be quantified accurately and the monitored signals can be read directly.

One new way to determine shrimp allergen tropomyosin is to measure their major allergen levels with cell biosensor which utilize living cells as sensing elements to detect target antigens through a specific biological recognition element and convert the recognition into a signal that can be recorded and quantified (Banerjee and Bhunia, 2010; Giaever and Keese, 1993). RBL Mast cells offer an excellent potential for serving in biosensor applications because they are robust and undergo a dramatic exocytotic response within minutes of antigen addition. A high affinity *Ig*E receptor (FcεRI) is abundant on the surface of these cells (Barsumian et al., 1981). Hence, the mast cells could be initiated by the encounter of an individual with an allergen leads to the production of *Ig*E, Subsequent come across with the allergen leads to cross-linking of the FcεRI -*Ig*E complex on the surface of mast cells triggering a sequence of intracellular events including cellular degranulation and releasing of chemical mediators such as histamine, serotonin, and *β*-hexosaminidase (Naal et al., 2004).

As a noninvasive and sensitive detection technique for studying living cells, Electrochemical impedance spectroscopy measurement is valid and holds promise for monitoring adhesion, viability, and environmental changes in cells(Hu et al., 2013). When cells attach to an electrode surface, a barrier for the electrochemical process is produced, thereby hindering the access of the redox probe to the electrode surface, resulting in an increase in the electron-transfer resistance(Hao et al., 2007). Thus sensitive and specific electrochemical signal generated by mast cells immobilized on electrode can be detected rapidly and accurately, especially when exposed to

allergen.

In these techniques anchoring cells on a proper surface invariably constitutes a key step. Thus in order to increase cell adhesion and keep their viability, type I collagen, which has excellent biocompatibility and high mechanical strength (Krewson et al., 1994), was used to encapsulate mast cells and provide a three-dimensional (3-D) capsule structure for cell proliferation. As mast cells are a kind of half-adherent cell (Metcalfe et al., 1997), they cannot firmly adhere on the surfaces of common electrodes, and thus, only by residing in collagen can they retain their viability and increase their adhesion. In this study, collagen around the cells played a role not only encapsulate cells but also block the unmodified surface of electrode thus avoid the nonspecific adsorption between protein and electrode surface. And collagen gels can be prepared with different pore sizes by varying the concentration for trapping different sized cells(Banerjee et al., 2008).

In recent years, Gold nanoparticles (AuNPs) are currently the subject of a wide field of research. They enhance the electrocatalytic response of the sensor owing to their similarity to a conducting wire or electron-conducting tunnel. In addition, they also provide more binding sites for the coupling of a wide range of molecules, including enzymes, antibodies, and cells (Minati et al., 2012). The use of L-cysteine (L-Cys) for the preparation of self-assembled monolayers on gold surfaces has been widely reported in many studies (Dong et al., 2010; Huang et al., 2010; Wei et al., 2010). L-Cys containing both sulfhydryl group and carboxyl group, is a promising compound to be used for bio-functionalization of gold nanoparticles(Milovanovic et al., 2010) and applied as a promoter for increasing the rate of electron transfer (Fu et al., 2011). Also these complexes can produce a bio-friendly polymer for the design of biological surfaces for sensing and for cells based devices(Fan et al., 2012). Consequently, the L-Cys played a role in providing a appropriate surface for cell immobilization and increasing the rate of electron transfer between cells and electrode.

Therefore, in this study, a novel mast cell-based impedance sensor was designed for quantifying major shrimp allergen Pen a 1(tropomyosin) and evaluating *Ig*E-mediated hypersensitivity. AuNPs were combined with L-Cys to form a novel architecture conjunction, which was further used to immobilize collagen-encapsulated RBL mast cells to fabricate a cell electrochemical sensor. The electrochemical behavior of dinitrophenol-bovine serum albumin (DNP-BSA) at the anti-DNP *Ig*E sensitized cell-modified electrode was then investigated as a model. Subsequently, different electrochemical responses caused by the stimulatory and allergic effects of shrimp tropomyosin on mast cells were recorded and the result showed excellent correlations between the allergen concentration and impedance signal. These effects can be used for the electrochemical identifying and quantifying the shrimp allergen Pen a 1(tropomyosin) level in food matrix, leading to a rapid, simple, and efficient biosensor for studying food allergens.

2. Experimental procedures

2.1. Materials and apparatus

Mouse monoclonal anti-dinitrophenyl *Ig*E antibody (Clone SPE-7) and dinitrophenyl-BSA, L-Cysteine, and chloroauric acid ($HAuCl_4 \cdot 4H_2O$) were obtained from Sigma-Aldrich Inc(St. Louis, MO, USA). Mouse monoclonal anti-shrimp tropomyosin *Ig*E antibody was purchased from Santa Cruz Biotechnology (Dallas, Texas, USA). Rat basophilic leukemia (RBL) cells were obtained from the Cell Bank of Chinese Academy of Sciences (Shanghai, China). Type I collagen (rat tail, collagen-I) was purchased from BD Biosciences (San Jose, CA, USA). RPMI 1640 medium and fetal bovine serum was 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 Auto Lab PGSTAT302N electrochemical workstation (Metrohm Autolab, Utrecht, Netherlands) using a conventional three-electrode system in a solution containing 2.5 mM of $Fe(CN)_6^{3-/4-}$ and 1.0 M KCl. The morphologies of L-Cysteine and gold nanocomposites and cells on the gold electrode were characterized using scanning electron microscopy (model Hitachi S–4800, Hitachi Co., Ltd., Tokyo, Japan). RBL cells were incubated in a CO_2 incubator (Thermo Scientific Forma Series II Water Jacket, Thermo Fisher Scientific Inc., Rockford, IL, USA).

2.2. Shrimp Allergen extracts and Purification

The preparation of shrimp extracts has been described as follows(Daul et al., 1994). Briefly, fresh brown shrimp (*Penaeus aztecus*) were boiled for 15 minutes in ion-depleted water, peeled, and deveined. The meat was homogenized with cold acetone and centrifuged by 10000 rpm for 10 min at 4℃, and the preparation of acetone powder from shrimp muscle was preserved at -18 ℃. Initial extraction was prepared by mixing acetone powder with 1 mol/L KCl, and 500 mg/L dithiothreitol in PBS 1:5 (W/V). After centrifugation at 12,000g for 15 min at 4℃, the supernatant was dialyzed (12–14 kD, Union Carbide Corporation) for 48 h at 4℃ against 10 mM PBS, pH 7.0, and then the resultant was lyophilized and stored at -80℃. Pen a 1 was separated and purified by a combination of ammonium sulfate and isoelectric precipitation as described by Sun(Sun et al. 2010): Pen a 1 extracts was precipitated with 30–50% ammonium sulfate saturation, the precipitation was dissolved in 0.01 M PBS, pH 7.0 and dialyzed. Further purification was obtained after precipitation with isoelectric at pH 4.6. Sediments were obtained in the complex soluble in 0.01 M PBS, pH 7.0. Protein dialysis solution was then purified using the AKTA system(GE Healthcare, Sweden), by Superdex 200 (16 × 250 mm) column chromatography to pH 7.0, containing 0.15 mol/L NaCl in 50 mmol/L phosphate buffer to wash off. The peaks were collected, SDS–PAGE identification(Bio-Rad ,California, U.S.A) and then freeze-dried(Reese et al., 1995).

2.3 Preparation of AuNPsCys-modified gold electrode

The Gold electrode (GE) was polished with 0.05μm alumina slurry on a polishing cloth, rinsed thoroughly with doubly-distilled water, and then sonicated in ethanol and doubly-distilled water for 10 min, sequentially. The modification of GE with AuNPs was achieved using electrodeposition in a de-aerated precursor solution of 0.5 M H_2SO_4 containing 1 mM of $HAuCl_4$, under a constant potential of -0.25 V, for an optimal time of 100 s (Finot et al., 1999) and then washed in doubly-distilled water. The obtained AuNP-modified GE was immersed in $1mg \cdot mL^{-1}$ L-Cys solution for 5 h and then washed in doubly-distilled water. Before the voltammetric measurements, the modified electrode was cycled between –0.6 and 0.6 V (scan rate, $100\ mV \cdot s^{-1}$)

several times until reproducible responses were acquired

2.4. Cells culture and immobilization

RBL-2H3 cells were cultured in a flask in RPMI 1640 medium supplemented with 10% fetal calf serum, penicillin (100 μg·mL^{-1}), and streptomycin (100 μg·mL^{-1}) at 37°C in a humidified atmosphere containing 5% CO_2. At the growth retardation stage after 3 d, cells reached to the logarithmic phase of growth after which they were used for experiments.

The general approach of encapsulating the RBL mast cells in collagen gel for biosensing is described as followed: Logarithmic phased grown RBL mast cells were collected, counted, washed, and about 1.5×10^7 viable cells/ml were resuspended in complete serum-free medium. 1ml cell-collagen mixture were prepared by mixing 200 μL collagen solution with 20 μL 10 ×phosphate-buffered saline and 12 μL 1N NaOH in precooled eppendorf tubes first. 768 μL cell suspension was then mixed with neutralized type I collagen (pH adjusted to 7 with 1 N NaOH in PBS) at a final density of 1.5 mg·mL^{-1}. Finally, 50 μL of cell -collagen matrix was dispensed onto the surface of the AuNPsCys-modified GE and incubated at 37°C for 30 mins to achieve cell immobilization for future electrochemical measurements. The morphology of the resulting structure was confirmed by SEM (supplementary Fig. S2).

In order to investigate *Ig*E-mediated hypersensitivity and to quantify shrimp Pen a 1. The cells were firstly sensitized by three to five-fold molar excess of anti-DNP *Ig*E (or anti-shrimp tropomyosin *Ig*E) over FcεRI for either 30 mins incubation at 37 ˚C before immobilized onto the electrode. Then stimulation of the RBL cells on electrode was initiated by addition of 50 μL of serially diluted DNP-BSA antigen (or purified shrimp tropomyosin antigen) in Tyrode's buffer, incubated at 37°C for 30 mins. The degranulation was stopped by placing the electrode at 4 °C for 5mins. Electrochemical impedance was measured after washing three times using PBS (pH 7.4); cells not exposed to DNP-BSA (or purified shrimp tropomyosin antigen) served as the control. The allergic effect of DNP-BSA and shrimp tropomyosin on cells was determined using the *β*-hexosaminidase assay.

2.5 β-Hexosaminidase assay for the measurements of cell of degranulation

β-Hexosaminidase assay was performed to validate the result of electrochemical studies by *Ig*E-mediated allergic response(Abassi et al., 2004). Cells growing in 96-well plates were washed and incubated in Tyrode buffer and stimulated with medium containing different concentration of allergens. After 1 h, the supernatant was removed and the cell monolayer was lysed in Tyrode buffer containing 0.5% TX-100 to obtain total cellular *β*-hexosaminidase activity. *β*-hexosaminidase activity was measured in both supernatant and lysates by adding 50 μl the substrate *p*-nitrphenyl -*N*-acetyl-*β*- glucopyranoside (1 mg/ml) to each well. After 1 h incubation at 37 °C, the reaction was stopped by the addition of 150 μl of 0.4 M glycine pH 10.7. The absorbance at 405 nm was read in ELISA reader (Biotek, Winooski, USA).The ratio of *β*-hexosaminidase release was calculated using the following formula: *β*-hexosaminidase release (%) = 100 × [(supernatant − blank supernatant)/(supernatant − blank supernatant) + (total cell lysate − blank total cell lysate)]. Cells incubated in medium without allergen served as blank

3. Results and discussion

3.1. Characterization of the AuNPsCys-modified Gold electrode

The process of electrode modification was described in Scheme.1. In general, AuNPs were deposited onto the surface of a clean bare GE quite uniformly, whose mean grain size was approximately 50±1.2 nm (seen in supplementary Fig. S1). This nanocomposite not only enlarged active surface area of the electrode but also increased the adsorptive capability to organic molecules. Then, L-Cys was adsorbed onto the AuNPs-modified GE through strong coordination interactions between thiol groups and AuNPs. After the *Ig*E-sensitizde cells were encapsulated in collagen, the cell -collagen mixture was dispensed onto the AuNPsCys-modified GE. The morphology of the GE surface after AuNPs modification and collagen-cell immobilization was characterized by SEM imaging (Sch.1, insert) .

Scheme.1

The RBL mast cells possess a high affinity *Ig*E receptor (FcεRI) on their surface which can selectively bind murine IgE antibodies and can be stimulated by crosslinking with protein allergen to initiate a sequence of biochemical events including cellular degranulation and releasing of inflammatory molecules(Dearman et al., 2005). According to this allergic mechanism, in order to identify and quantify the shrimp tropomyosin, mast cells have to be pre-sensitized by the anti-shrimp tropomyosin *Ig*E before the measurement started. Only in this way can they respond to the shrimp allergen specifically and trigger a sequence of *Ig*E-mediated hypersensitivity events. As shown in bottom of Sch.1, the allergic reaction process occurred spontaneously when the cells were pre-sensitized by the anti-shrimp tropomyosin monoclonal antibody *Ig*E. However, if the anti-shrimp *Ig*E pre-sensitized cells were exposed to other allergen proteins they will have no response, because the anti-shrimp tropomyosin *Ig*E on the surface of mast cell can not specifically recognize and crosslink with these protein allergen to initiate cellular degranulation.

3.2. Electrochemical characteristics of RBL mast cells on a modified electrode.

The characteristics of the RBL cells on modified GE were tested by Cyclic voltammetric (CV) and electrochemical impedance spectra (Zahradnik et al., 2011) in the presence of 2.5 mM $Fe(CN)_6^{3-/4-}$. As shown in Fig.1A, it can be seen that the voltammograms showed a typical reversible redox peak at the bare GE surface (curve a).The redox current increased when the electrode was modified with AuNPs (curve b), owing to its good conductivity and the increased effective area of the working electrode. The observed redox peak current decreased after modification of a L-Cys layer (curve c) and immobilization of cell- collagen(curve d), reflecting a blocking effect caused by the insulating properties of L-Cys and cell-collagen which indicated that the L-Cys layer and cell-collagen matrix were successfully assembled on the AuNPs/GE surface.

The electrochemical impedance spectroscopy (EIS) of the modified electrodes are shown in Fig.1B. With a bare GE, the redox process showed a low electron transfer resistance about 301 Ω (curve a). When AuNPs were assembled on the bare electrode, the semicircle decreased dramatically to 90 Ω(curve b) suggesting that this nanocomposite layer increased the interfacial electron transfer. Owing to the blocking effect caused by the insulating properties of L-Cys, the R_{et} was raised significantly (curve c) and continued to rise until 1.72 kΩ when cell-collagen were then attached to the AuNPsCys-modified GE (curve d).

The concentrations of attached RBL cells encapsulated in collagen were determined by the AuNPsCys/GE. With increasing cell concentration, the R_{et} increased, showing increasing diameter

of the semicircle on the Nyquist diagram (Fig.1C), which implied a higher number of immobilized cells on the electrode. The R_{et} values were proportional to the logarithmic value of RBL cell concentrations, ranging from 1.5×10^4 to 1.5×10^9 cells·mL^{-1} (Fig.1D), with a correlation coefficient of 0.998. The limit of detection was calculated to be about 6×10^3 cells·mL^{-1} based on the usual 3σ definition as 3 times the standard deviation of the blank sample. Therefore, an appropriate cell numbers for allergen analysis could be selected according to the result of this R_{et} measurement.

Figure 1.

3.3. Quantification of the shrimp allergen tropomyosin

*Ig*E-mediated RBL-2H3 mast cell activation in the presence of antigen leads to initiation of signaling cascade resulting in degranulation of secretory vesicles, which contain mediators of allergic reaction such as histamine, *β*-hexosaminidase. Since monitoring of cell-electrode impedance provides information about the parameters of cell morphology and metabolism, we sought to determine the cell-electrode impedance response of RBL-2H3 mast cells that were pre-sensitized with *Ig*E in the presence of antigen application.

In this study, DNP-BSA was applied as a model to indicate the R_{et} variation tendency of *Ig*E-mediated hypersensitivity. Electrochemical impedance signals from RBL-2H3 cells activated by different concentrations of DNP-BSA showed a dose-dependent relationship (Fig. 2A). The R_{et} value was proportional to DNP-BSA concentrations ranging from 1×10^{-3} to 10 ng·ml^{-1} with a correlation coefficient of 0.998, corresponding to the increased semicircle diameter in the Nyquist diagram. However, the R_{et} decreased with higher DNP-BSA concentrations, ranging from 100 to 1×10^4 ng·mL^{-1}. As DNP-BSA at low concentration stimulated cell degranulation, the R_{et} might be increased by the release from the cytoplasm to the intercellular spaces of various chemicals, such as histamine, tryptase, and *β*-hexosaminidase. While high DNP-BSA concentrations induced cell apoptosis and cytolysis(Rohlich et al., 1971), which led to cell detachment from the electrode and thus decreased R_{et}. A *β*-hexosaminidase assay was performed to confirm the results of the electrochemical analysis (Fig.2B). The *β*-hexosaminidase release rate increased gradually at concentrations < 100 ng·ml^{-1} DNP-BSA, while it decreased at concentrations >100 ng·ml^{-1} DNP-BSA. These data agreed well with the dose-response curve obtained from electrochemical measurements.

Fig.2

Quantification of shrimp allergen tropomyosin was achieved by RBL-2H3 cells exposed to different concentrations of purified shrimp Pen a 1 which was purified in AKTA system and analyzed by preparative SDS-PAGE. Result of one elution peak was obtained after shrimp allergen AKTA purification (Fig. 3A). The major allergen was collected and identified by SDS–PAGE analysis (Fig. 3B). It was clearly that the electrochemical impedance signals exhibited the same trend compared with the DNP-BSA activated cells. As shown in Fig.4A, the R_{et} was increased rapidly in range from 0.5μg·mL^{-1} to 4μg·mL^{-1} exhibiting a good linear relationship with the shrimp tropomyosin concentrations and the correlation coefficient was 0.998. The limits of detection (LOD) is calculated to be 0.15μg·mL^{-1} according to the formula: LOD=3s/m, where s represents the standard deviation of the blank sample (n=3) and m represents the slope of related calibration

curve for tropomyosin. In addition, it was also observed that as the concentration of shrimp tropomyosin increased the R_{et} decreased gradually ranging from 4 to 5μg·mL^{-1}.Perhaps because high doses of tropomyosin may induced cell apoptosis and cytolysis which led to cell injury or apoptosis and then decreased R_{et}. These measurements were performed three times to assess the reproducibility and precision of the freshly fabricated cell-sensor. The RSD of the detection results were all lower than 5%, showing acceptable performance. These imply that the sensitivity of our sensor could bear comparison with that of other biosensors for allergic reaction assessment reported in the literatures (Tab.1). The *β*-hexosaminidase assay confirmed the results of the electrochemical analysis (Fig.4B). Obvious changes in cell morphology were observed when cells were exposed to DNP-BSA and shrimp tropomyosin at the highest sensitization concentrations (insert of Fig.2B and Fig.4B), compared with the control group (supplement Fig.S1). It was clear that, cells exhibited obvious morphological changes, with cell deformation, cell shrinkage, secretory granule releasing, sparse intracellular particles, and vacuolated cytoplasm when they were exposed to different antigens which indicated the occurrence of degranulation.

Figure 3.

Figure 4.

Table 1

3.4. Investigation of specificity of the RBL cell sensor

To determine whether the recognition of shrimp tropomyosin was specific, shrimp allergen Pen a 1, soybean allergen *β*-conglycinin, peanut allergen Ara h 1 (perpared by our laboratory) were used alone to test RBL cells that pre-sensitized with anti-shrimp tropomyosin *Ig*E . To exclude non-specific interactions between cells and antigen, naive RBL cells without pre-sensitization were stimulated by the same samples in parallel. As shown in supplementary Fig.S5, soybean allergen *β*-conglycinin or peanut allergen Ara h 1hardly produced any obvious responses. Therefore contamination of soybean and peanut allergen did not influence the recognition of shrimp tropomyosin in the heterogeneous mixture food sample that contained different kinds of allergen.

4. Conclusions

In this study, a sensitive, simple, and label-free cell-based sensor was developed by immobilizing living mast cells on AuNPsCys-fabricated GE for shrimp tropomyosin quantification. AuNPsCys nanocomposites exhibited excellent electrochemical activity. The sensor exhibited good correlation to the logarithmic value of cell numbers ranging from 1.5×10^{4} to 1.5×10^{9} cells·mL^{-1}. The R_{et} value was proportional to model DNP-BSA concentrations ranging from 1×10^{-3} to 10 ng·mL^{-1} with a correlation coefficient of 0.998, corresponding to the increased semicircle diameter in the Nyquist diagram. Then the method was successfully applied into quantifying concentration of shrimp tropomyosin in range of 0.5 to 2.5μg·mL^{-1}, with the detection limit as 0.15μg·mL^{-1}(R.S.D. 5%). The detection signal intensity of R_{et} rose with the increases of the shrimp tropomyosin at low concentrations but decreased at higher concentrations, which was consistent with the results of *β*-hexosaminidase assay and SEM images of cell morphology. Thus the cell-based electrochemical sensor for quantification of major shrimp allergen Pen a 1 showed a high detection precision, a low detection limit, good detection precision, and was made using a

simple fabrication process. While more regeneration times and long-term storage of modified electrode need more experiments to verify. Furthermore, the assay could be readily adaptable into microfluidic devices to realize high-throughput analysis in future.

Acknowledgments

This work has been supported by National research program (No. 2011BAK10B03) , “973” National Basic Research Program of China (No. 2012CB720804), Program for New Century Excellent Talents in Jiangnan University , Commonweal project of the Ministry of Agriculture (No. 201203069–1), and Fundamental Research Funds for the Central Universities, (JUSRP21129.).

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Scheme.1. Schematic illustration of preparation of modified electrode (above) and principle of allergen reaction process t (below). Insert: for the SEM images cell-collagen 3D structure.

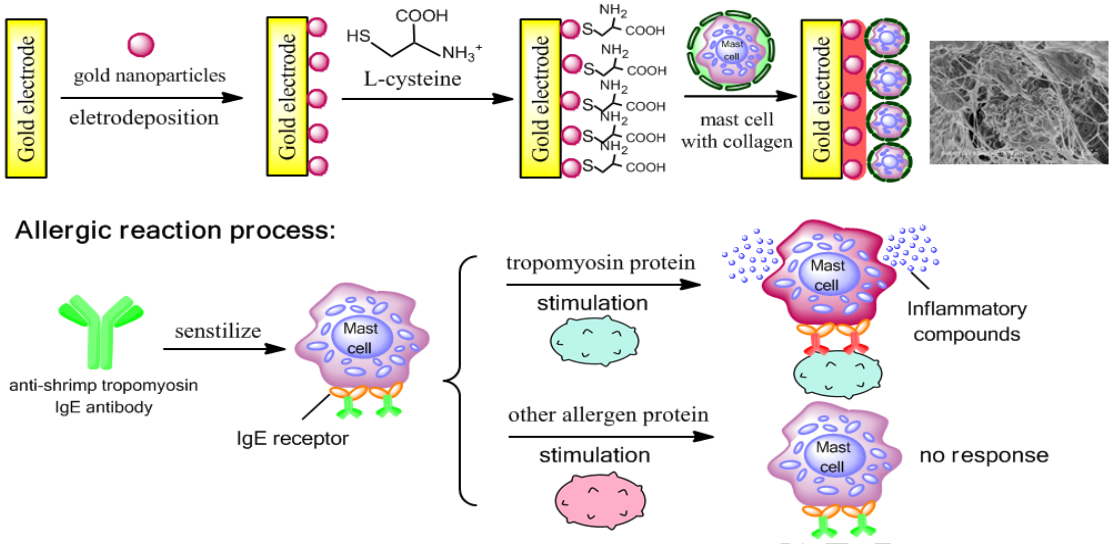
Figure 1. (A)Cyclic voltammograms of bare GE (a), AuNPs/GE (b), L-cys/AuNPs/GE (c), cells-collagen/ L-cys/ AuNPs/ GE (d), in $Fe(CN)_6^{3-/4-}$, Scan rate: 100mVs^{-1}. (B) Nyquist diagrams of electrochemical impedance spectra of bare GE (a), AuNPs / GE (b), L-cys/AuNPs / GE (c), cells-collagen/L-cys/AuNPs / GE (d).(C) Nyquist diagrams of (a) 1.5×10^4 cells mL^{-1}, (b) 1.5×10^5 cells mL^{-1}, (c) 1.5×10^6 cells mL^{-1},(d) 1.5×10^7 cells mL^{-1}, (e) 1.5×10^8 cells mL^{-1}, (f) 1.5×10^9 cells mL^{-1} RBL cells immobilized on AuNPsCys/gold electrode. (D) Equivalent circuit model, to which all of the experimental impedance curves were fitted.

Figure 2. Effects of *Ig*E-mediated hypersensitivity on RBL-2H3 cells: (A) electrochemical impedance spectroscopy for RBL-2H3 cells treated with different doses of DNP-BSA,(a→h) arrow indicates: control= 0 ng·mL^{-1} (R_{et}=3.5kΩ was not shown), 1×10^{-3}ng·mL^{-1}, 1×10^{-2}ng·mL^{-1}, 1×10^{-1}ng·mL^{-1}, 1ng·mL^{-1}, 10ng·mL^{-1}, 100ng·mL^{-1}, 1×10^{3}ng·mL^{-1}, 1×10^{4}ng·mL^{-1}.(B) *β*-hexosaminidase assay for confirmation of cell activated by DNP-BSA. Data represent mean±SE of three different experiments under similar condition.

Figure 3.(A)AKTA protein purification system was used to purify the shrimp protein, there was a distinct shrimp protein elution peaks after eluting with 50 mM PBS with 0.15M NaCl(pH=7.0).(B) Purified Pen a 1 was applied to the gel and SDS-PAGE performed. A major protein band was present at 36kD which were identified by Coomassie Blue staining. The results demonstrate substantial purification on Pen a 1.

Figure 4. Quantification of major shrimp allergen by RBL-2H3 cells: (A)electrochemical impedance spectroscopy for RBL-2H3 cells treated with different doses of shrimp tropomyosin, (a→k) arrow indicates: control=0μg·mL^{-1}, (a) 0.5μg·mL^{-1}, (b) 1μg·mL^{-1}, (c)1.5μg·mL^{-1}, (d)2μg·mL^{-1}, (e)2.5μg·mL^{-1}, (f)3μg·mL^{-1}, (g)3.5μg·mL^{-1}, (k)4μg·mL^{-1}, (j)4.5μg·mL^{-1}, (i)5μg·mL^{-1}, (h)5.5μg·mL^{-1}. (B) *β*-hexosaminidase assay for confirmation of cell activated by shrimp tropomyosin. Data represent mean±SE of three different experiments under similar condition.

Table 1. Sensitivity performances of several reported related biosensors for allergen detection.



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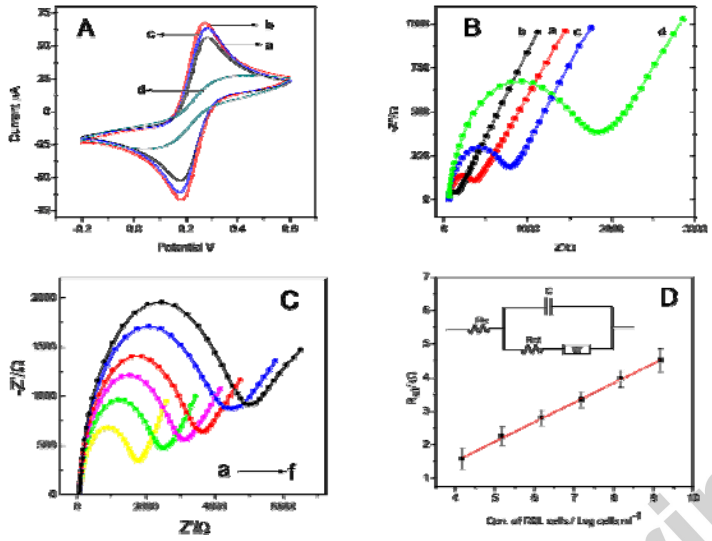

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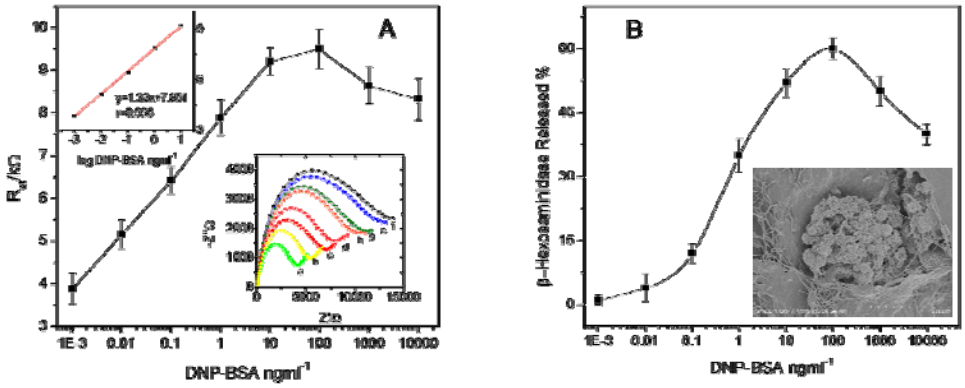

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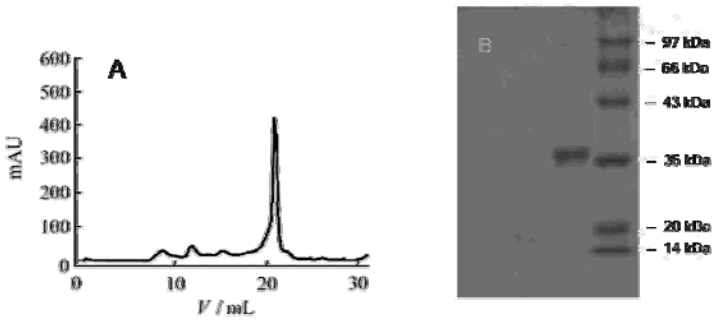

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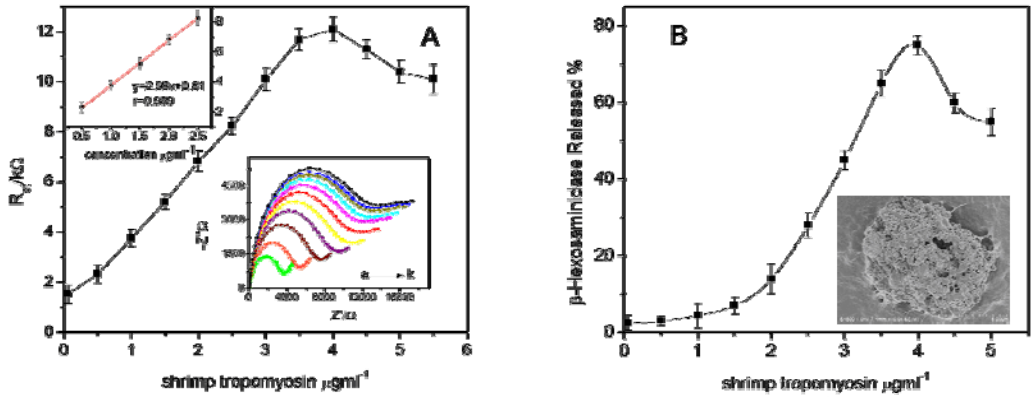

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Method and materials	research object or detection target	Linear range	Detection limit	Reference
quartz crystal microbalance-based Immunosensor	Shrimp tropomyosin Allergen	1to 300 μg/mL	0.333μg/mL	Sun et al Eur Food Res Technol 231 (2010):563–570
electrochemical DNA sensor	peanut allergen Ara h1	10^{-15} to 10^{-10} mol/L	3.2×10^{-13} mol/L	Sun et al. J. Agric. Food Chem. 60 (2012):10979−10984
optical immunochip biosensor	egg white allergen ovalbumin and ovomucoid	1 mg/mL to 1 ng/mL	1 ng/mL	Maier et al. Anal. Chem. 80 (2008) : 2694-703
Molecularly imprinted polymers(MIP) Quartz crystal microbalance (QCM).	rubber latex allergens (Hev b1)	10 to 1500 μg L^{-1}	1μg L^{-1}	Sontimuang et al. Analytica Chimica Acta 687 (2011) :184–192
electrochemical DNA-array for PCR amplified detection	hazelnutmajor allergens (Cor a 1.04, Cor a 1.03)	0.01 to 2μmol L^{-1}	Cor a 1.03 and Cor a 1.04 were 0.3 and 0.1 nmol L^{-1}	Bettazzi et al. analytica chimica acta 614 (2008) :93–102
Electrochemical Immunosensors	Peanut Allergen Ara h2	5 pg mL^{-1} to 1 μgmL^{-1}	0.5 ng mL^{-1}	Liu et al. Anal. Chem. 82 (2010) : 5865–5871
surface plasmon resonance (SPR) biosensor	recombinant group 1 house dustmite allergen (rDer f1)	0.2114 to 1.235 RU	rDer f1 in HBS-EP buffer and FBS was 15.4 and 32.1 ng/ml	Huang et al. Sensors and Actuators B 131 (2008) :417–423
ZnO quantum dots (QDs) and a CHOM bioconjugate	allergy chicken ovomucoid (CHOM)	1 ng/mL to 140 ng/mL	1 ng/mL	Yang et al. Journal of Electroanalytical Chemistry 660 (2011) :97–100
electrochemical DNA sensor	peanut allergen Ara h 1	10^{-15} to 10^{-10} mol/L	3.2×10^{-13} mol/L	Sun et al. J. Agric. Food Chem. 60 (2012):10979−10984
Surface plasmon resonance (SPR) immunoassays	peanut allergen	0.7–45 μg/ml	0.7 μg/ml	Mohammed et al. Analytica Chimica Acta 444 (2001): 97–102
Surface Plasmon Resonance	milk allergen β-lactoglobulin	1 pg mL^{-1} to 100 ng mL^{-1}	0.85 pg mL^{-1}	Eissa et al. Biosensors and Bioelectronics 38 (2012) :308–313
This Method	Shrimp tropomyosin Pen a 1	0.5-0.25 μg·mL^{-1}	0.15μg·mL^{-1}	

Highlights

- RBL-2H3 mast cell-based sensor for evaluating allergic reaction was developed
- Cells were encapsulated in type I collagen to form 3D structure on the fabricated electrode
- Electrochemical signal of cell showed an excellent linear relationship with the DNP-BSA and shrimp allergen Pen a 1, providing potential applications in allergen detection