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A novel electrochemical mast cell-based paper biosensor for the rapid detection of milk allergen casein

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ABSTRACT

Developing low-cost, portable and simple analysis tools is of vital importance for food safety point-of-care testing. Therefore, herein, a new low-cost, simple to fabricate, disposable, electrochemical mast cell-based paper sensor is proposed and developed to sensitively determine the major milk allergen casein. Then, a graphene (GN)/carbon nanofiber (CN)/ Gelatin methacryloyl (GelMA) composite material with high conductivity and good biocompatibility was modified on the cell-based paper sensor to improve the electrical conductivity and provide a sensing recognition interface for the immobilization of rat basophilic leukemia (RBL-2H3) mast cells. The cyclic voltammetry and differential pulse voltammetry measurement of the mast cells in the paper sensor revealed an irreversible anodic peak, whose peak current is proportional to the number of cells in the range from 1×10^2 to 1×10^8 cells/mL. For the milk allergen detection tests, mast cells exposed to the casein caused a significant reduction in the current signal, displaying an inverse dose-dependent relationship. The developed cell sensor exhibited a range of linearity between 1×10^{-7} and 1×10^{-6} g/mL of casein with a detection limit of 3.2×10^{-8} g/mL and a great reproducibility and selectivity. The electrochemical responses obtained using the cell-based paper sensor were well consistent with the conventional detection assay, with good stability and reproducibility. Therefore, a simple and novel electrochemical method for food allergens detection was developed, demonstrating its potential application in the food safety determination and evaluation.

1. Introduction

Food allergies are a serious threat to public health, affecting around 1–2% of the adult population and up to 8% of children (Renz et al., 2018). The widespread use of cow's milk in the food industry has made milk allergies a major health concern, as reactions are often severe, prevalent, and persistent within those who are affected. Due to the strong heat stable property, casein occupies 80% and has become the most prevalent allergen in bovine milk (Cheng et al., 2017a; Xu et al., 2017). So far, there are no known cures or effective treatment to reduce this severe allergic reaction. Therefore, reliable methods for detection and quantification of milk allergens are required for improving the allergen early warning system and for ensuring food safety.

Cell-based biosensors, as a new strategy, have emerged as attractive alternatives to the traditional methods offering comparable sensitivities and selectivity, while allowing for on-site detection (Radhakrishnan, 2015; Sobhan et al., 2017; Vasilescu et al., 2016; Zhu et al., 2016). They have a distinct advantage in reflecting cellular physiological metabolism rather than just quantitative detection as well as providing insight into physiological effects of analytes. Therefore, the use of the natural ability of mast cells to recognize food allergens and elicit proinflammatory responses offers a stable and accurate strategy mimicking physiological conditions (Curtis et al., 2008; Jiang et al., 2015; Jin et al., 2011). Mast cells line express a large array of Fc receptors on their surface, including FcεRI, FcγRI, and FcγRIII, which could selectively bind murine IgE or IgG antibodies so that the subsequent cross-link with an allergen would trigger cellular degranulation (Graham et al., 2015). This triggers a sequence of intracellular events, including cellular degranulation and the release of chemical mediators such as histamine, serotonin, and β-hexosaminidase. Hence, RBL mast cells

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show excellent potential for biosensor applications due to their dramatic exocytotic response to antigen within minutes. They can detect target antigens and convert biological recognition into a signal that can be recorded and quantified quite easily.

Recently, paper has recently attracted increasing attention as a novel platform for various point-of-care applications due to their merits of low cost, simplicity, portability, disposability, and excellent biocompatibility (Morbioli et al., 2017; Oliveira et al., 2018). Through the modification of its physical and chemical properties, paper can better mimic the in vivo cell microenvironment and show great potential as a three-dimensional (3D) cell culture platform for developing a new generation of biosensors (Lee et al., 2016; Liang et al., 2016). This platform offers the possibility for the precise control of interactions between cells and sensitive monitoring of cell responses to exogenous hazards, which has attracted considerable attention for a simple and miniaturized cell based biosensor exploitation (Güder et al., 2016; Wang et al., 2017, 2016).

Based on the outstanding advantages of high sensitivity, rapid response, ease and convenience of instrument operation required, electrochemical analysis methods have increasingly becaaaaaaaome promising techniques in the paper-based analytical devices (Mettakoonpitak et al., 2016; Teengam et al., 2017a). To date, a series of new paper analytical devices have been developed for the sensitive detection of small molecules, pathogens, and DNA based on the electrochemical techniques (Bhardwaj et al., 2017; Cinti et al., 2017; Lu et al., 2012; Teengam et al., 2017b). These studies have demonstrated that the disposable paper analytical device combined with electrochemical technology would be a new efficient label-free technology, representing a powerful alternative for monitoring interfacial property changes.

In the fabrication of traditional electrochemical cell sensors, various conventional electrodes, such as gold and glassy carbon electrodes have been widely used (Ding et al., 2008; Ron and Rishpon, 2009). However, the electrodes can be easily contaminated during the electrochemical assay process and the need to be polished and regenerated for the subsequent detection (March et al., 2015; Promphet et al., 2015; Sajid et al., 2016). As a result, in practice, the complexity of the biological sample and time-consuming operations in the conventional electrochemical analysis greatly restrict the application of the cell-based biosensors. Accordingly, development of a low-cost and disposable electrode-carrying platform for the electrochemical cell sensors is of vital importance.

Currently, the prevalent screen-printed carbon is considered a good electrode material due to its low cost, wide potential range, chemical inertness and low noise (Jo et al., 2017; Nicholas et al., 2018). However, the mass printing of carbon electrodes on the paper matrix also brings some problems, such as limited sensitivity and large electrochemical resistance. The efforts to overcome these problems include the use of graphene, which has been extensively used in electronic devices in particular by considering biosensors with electrochemical transduction, due to their peculiar properties like fast electron transfer, high thermal conductivity, high mechanical flexibility, optical transparency, and good biocompatibility (Antiochia et al., 2017; Bollella et al., 2017; Mazzei et al., 2015). Therefore, graphene-modified carbon electrodes could exhibit superior performance in terms of their electrocatalytic activity and electrical conductivity (Chakrabarti et al., 2017; Niu et al., 2018). Moreover, graphene has also been used in combination with various types of functional materials to fabricate high-performance electrodes. Among them, carbon nanofibers, which are high-aspect ratio graphitic materials, have been widely used for electrochemical applications due to their unique mechanical and excellent electrical properties as well as high connectivity for transport of electrical currents (Li et al., 2017; Oularbi et al., 2017). The unique physical and chemical properties significantly improve the compatibility of carbon nanofibers with both chemical and physical modifications, which allows them to serve as an outstanding platform for cell immobilization.

In this work, a highly-efficient electrochemical mast cell sensor was developed on a novel folding paper-based device. The collapsible paper was successfully used to replace the traditional three-electrode system by simply folding the device, which was operated before the electrochemical assays were conducted. The sensor was based on carbon nanofiber/graphene-modified screen-printed electrodes (SPEs), which formed an effective cell immobilization layer and allowed the immobilized mast cells to have high stability and bioactivity. Casein was defined and characterized as the major allergen in cow's milk. Thus, the determination of Casein levels was essential to food safety applications. Thus the casein antibody-sensitized mast cells were immobilized on the paper fibers through the biological affinity of the GelMA hydrogel. The experimental results showed that this new mast cell biosensor not only exhibited an outstanding analytical performance with a favorable linear concentration range, good stability and excellent reproducibility, but also showed potential for high-throughput on-line detection analysis. In addition, the electrochemical results were verified by comparison to the traditional cell assay. Accordingly, the exploitation of such a simple, disposable, yet inexpensive cell sensor enabled the fast and simple electrochemical detection of milk allergen without involving complex operations.

2. Experimental

2.1. Materials and apparatus

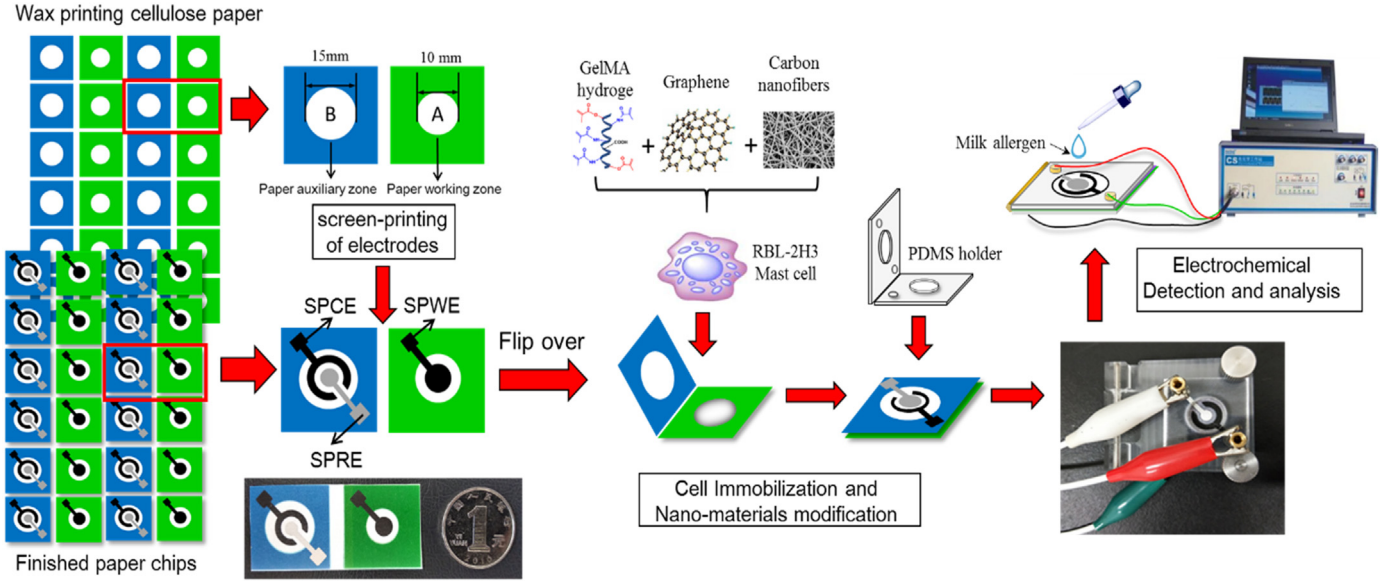
Casein standard was purchased from SenBeiJia Biological Technology Co. Ltd. (Nanjing, China). Casein antibody was purchased from Biocompare (South San Francisco, USA). Graphene and carbon nanofibers were obtained from Xian Feng Nanomaterials Technology Co. Ltd. (Nanjing, China). GelMA hydrogel was obtained from Tissue Ink Co. Ltd. (Zhejiang, China).

Rat basophilic leukemia (RBL) mast cells obtained from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China) were cultured in DMEM medium (Gibco, Gaithersburg, MD, USA) containing fetal calf serum (HyClone, Logan, UT), penicillin (1×10^{-4} g/mL), and streptomycin (1×10^{-4} g/mL) in a humidified 37 °C incubator with 5% CO_2. All cell assay kits were purchased from Beyotime Biotechnology Co. (Shanghai, China), all solutions were prepared with deionized water and all reagents were of analytical grade.

Origami electrochemical device was fabricated by a solid-wax printer (Fuji Xerox Phaser 8560DT). Transmission electron microscope (TEM) images were recorded on a Tecnai 12 TEM (Philips, Netherlands) operating at an accelerating voltage of 120 kV. Scanning electron microscope (SEM) images were obtained via S-4800 II thermal field emission SEM (Hitachi, Japan). Electrochemical measurements were carried out by a CHI 660E electrochemical workstation (CHI Instrument Company, Shanghai, China). The electrodes consisted of a screen-printed Ag/AgCl reference electrode and a carbon counter electrode on the paper auxiliary zone and a screen-printed carbon working electrode (5 mm in diameter) on the paper sample zones. All the electrochemical tests were performed in 0.1 mol/L phosphate buffer saline (PBS) of pH 7.4.

2.2. Design and fabrication of cell-based paper sensor

The paper-based device was fabricated on a piece of rectangular pure cellulose A 4 paper (21 cm × 29.7 cm), and the manufacture process (Scheme 1) consisted of wax-printing, baking, screen-printing and cutting. The detailed procedures were referred by previous report with some modification (Lu et al., 2012; Su et al., 2014), firstly, the shape for wax-printing of paper sheet was designed by Adobe illustrator CS6, and a wax printer was used for wax-printing in bulk (sheet A in Scheme 1). Each wax-patterned paper was comprised of a paper working piece (green square) and a paper auxiliary piece (purple square) with the same side length of 25 mm. On the paper working



Scheme 1. Schematic representation the fabrication and assay procedure of the electrochemical mast cell-based paper sensor. Paper sheets were firstly patterned in bulk using a wax printer. After baking, three electrodes were screen-printed on wax-patterned sheet. Reference electrode and counter electrode were printed on the B zone, while working electrode was printed on the A zone. The prepared sheet was cut to rectangular paper. After nano-materials modification and mast cell immobilization, the rectangular paper was folded and integrated with a device-holder for electrochemical assay.

piece, the wax-pattern contained a circular working zone (named zone-A with a diameter of 10 mm). Between two pieces, the unprinted line (1 mm in width) was defined as fold-line due to the difference of flexibility between the printed and unprinted area after baking. On the paper auxiliary piece, a circular auxiliary zone (named zone-B with a diameter of 15 mm) was designed which could properly and exactly cover all the paper working zones on paper cell tab after being folded along the predefined fold-line. The wax-patterned paper sheet was then baked in an oven at 130 °C for 150 s to melt the printed wax so that it penetrated through the paper to form the hydrophobic and insulating patterns. The unprinted area (paper working/auxiliary zone) still maintained good hydrophilicity, flexibility, and macroporous structure and will not affect the further modifications.

Then, the as-prepared wax-penetrated paper sheets were ready for screen-printing of electrode array after cooling to room temperature. In zone-A, carbon ink was used for screen-printing working electrode (SPWE, 8 mm in diameter); in zone-B, carbon ink and Ag/AgCl ink were used for screen-printing counter electrode (SPCE) and reference electrode (SPRE), respectively. Finally, by folding the rectangular paper, along the fold-line, the SPWE on zone-A will share the same SPCE and SPRE on zone-B once the paper electrochemical cell was filled with buffer solution. The three screen-printed electrodes and paper channels constituted a 3D electrochemical cell with a volume of 50 µL.

2.3. Electrochemical assay

As shown in Scheme 1, 10 µL of 3D-CN/GN/GelMA composite hydrogel was first dropped into the A zone. Then, 5 µL of 1% BSA (w/v) solution was dropped into the B zone to block the nonspecific binding sites. After a rinse with 0.1 mol/L PBS buffer (pH = 7.4), 3D-CN/GN/GelMA/SPWE was prepared and 10 µL of PBS containing 1×10^8 cells/mL RBL cells was dropped into the A zone, and incubated at 37 °C for 5 min. Then, the A zone was rinsed with 0.1 mol/L PBS buffer (pH = 7.4) to dispel the culture solution. The prepared cell sensor was blocked with 1% BSA for 30 min and rinsed thrice with 0.1 mol/L PBS buffer (pH = 7.4) for subsequent assay utilization. Afterwards, the sample zone was washed with 0.1 mol/L PBS buffer (pH = 7.4) to remove nonspecifically bonding. Next, the milk allergen casein was then added into the A zone to induce RBL cell degranulation with a more consistent and durable chemotactic response. the Cyclic Voltammetry (CV) and differential pulse voltammetric (DPV) measurements of the mast cell were monitored for casein content quantification, which were carried out with 10 µL of 0.1 mol/L PBS buffer (pH = 7.4) containing different concentrations of casein sample solution. The CV was scanned within a potential range from − 0.2 V to + 0.6 V with a scan rate of 100 mV/s. The DPV was recorded in a potential range between − 0.2 V and 0.6 V with a pulse amplitude of 0.05 V, a pulse width of 0.05 s and a pulse period of 0.2 s.

2.4. Conventional assay verification for electrochemical detection

The Intracellular Ca^{2+} levels were measured with a Fluo-3/AM, a visible wavelength calcium probe. Dye was added to the RBL cells for 1 h at 37 °C under 5% CO_2 in the dark, then the cells were washed with PBS (pH 7.4) and seeded into 96-well plates with different concentrations of casein for 3 h before measuring their fluorescence. Scanning electron microscope and Transmission electron microscope assay was used to cell morphological changes observation.

2.5. Statistical analysis

Data are expressed as mean ± S.D. The statistical analysis was carried out using SPSS 17.0 programs. The significance of difference was determined by the unpaired Student's *t*-test. $P < 0.05$ was considered the threshold for statistical significance between the control group and the experimental groups.

3. Results and discussion

3.1. Characterization of the cell-based paper senor

This novel paper-based cell sensor was fabricated on a pure cellulose paper. The hydrophilic electrochemical analysis area was formed by printing wax and SPEs on the paper surface. The scanning electron microscopy (SEM) images revealed that the carbon ink printed working electrode, half-ring like counter electrode (Fig. 1A) and Ag/AgCl ink printed reference electrode (Fig. 1B) were uniformly deposited on the hydrophilic area of the wax-patterned paper. Also, as showed in Fig. 1C,

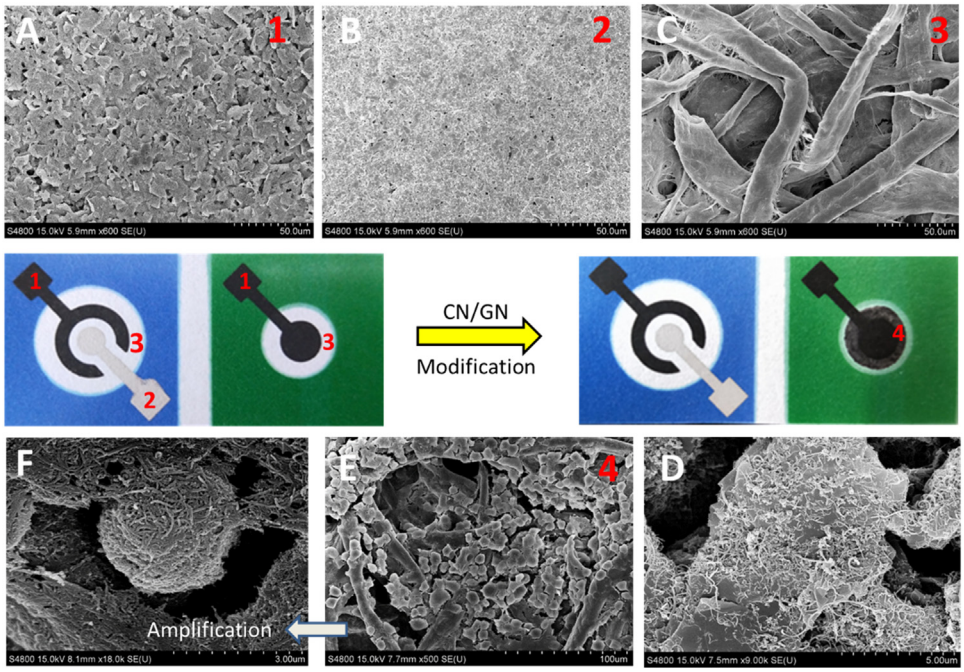


Fig. 1. SEM images of the prepared paper sensor. (A) carbon ink printed working electrode and counter electrode; (B) Ag/AgCl ink printed reference electrode; (C) pure cellulose paper; (D) Screen-printing working electrode modified with CN/GN nanoparticles (E) RBL mast cells immobilized on the CN/GN modified cellulose fiber; (F) Sign mast cell under 18k× magnification. Ps: the red words inset indicated specific location on the paper sensor.

the porous cellulose paper had a high ratio of surface area to weight with rough cellulose fibers, which maintained good hydrophilicity, flexibility and porous structure and could offer an excellent adsorption microenvironment for the CN/GN nanoparticles. After the CN/GN nanoparticles were successfully fixed by self-assembling on the cellulose fiber, a continuous and dense CN/GN conducting layer was formed on the cellulose fiber surfaces. The enlarged image of the CN/GN nanocomposite layer fixed on the cellulose fiber shown in Fig. 1D, revealed the uniform fixation of the CN/GN nanoparticles. This indicated that a good coverage of the CN/GN on the surfaces of the cellulose fibers was obtained and the paper working zone maintained excellent 3D structure of interwoven and incompact cellulose fiber networks after the modification, which would improve the area-to-volume ratio as well as increase the catalytically active sites to obtain a better electrochemical performance. The Raman characterization of CN/GN nanoparticles were showed in Supplementary material Fig. S1. In addition, the amount of CN/GN on the working zone was determined by the deposition time, which was optimized by measuring the DPV signals (Supplementary material Fig. S2).

After the RBL cells were introduced into the paper device, it could be seen that the RBL cells were firmly immobilized and uniformly distributed on the CN/GN coated cellulose fiber (Fig. 1E). Additionally, they showed long microvilli and filamentous pseudopods on the surface of their plasma membrane (Fig. 1F), which proved that the RBL cells preserved favorable physical conditions in the paper sensor and created a barrier against the electrochemical process, hindering the redox probe from accessing the electrode surface, thereby resulting in increased electron-transfer resistance. When the RBL cells were stimulated with the milk allergen casein, they triggered degranulation and the release of inflammatory mediators leading to cell apoptosis and cell death. As a result, the electron transfer rate of the working electrode was accelerated, thus enhancing the current activity.

3.2. Electrochemical characteristics of the cell -based paper sensor

The characteristics of the paper-based cell sensor was tested by CV and DPV in the presence of 1.0 mmol/L $Fe(CN)_6^{3-/4-}$. As shown in Fig. 2A, the voltammograms displayed a typical and reversible redox peak on the bare screen-printed working electrode (SPWE) surface (curve a). The current increased significantly when the paper working zone was modified with the CN/GN nanocomposite (curve b), due to its excellent electronic conductivity as well as the higher electroactive surface. Therefore, the CN/GN nanocomposite layer could not only enhanced the electrical connectivity of the paper working zone for RBL cells immobilization but also greatly improved the sensitivity of cell detection. Afterwards, a strong decrease in the peak current was observed when the paper working zone was flooded with RBL cells (curve c). This result may be caused by cell adhesion which increased the electrode resistance. Similar signal changes were shown in Fig. 2B. With bare paper sensor, the peak current was 3.1 μA (curve a), and then the current increased dramatically when the CN/GN nanocomposite was assembled on the bare paper working zone. A seven times increase of the I*p* value occurred at approximately from 3.1 μA (curve a) to 23.8 μA (curve b) as the CN/GE nanocomposite helped to accelerate the electrical conductivity. When the RBL cells were immobilized onto the CN/GN nanocomposite-coated cellulose fibers with the GelMA hydrogel, the I*p* value decreased to 18.8 μA (curve c). Besides, the incorporation of the CN/GN nanocomposite with the GelMA could offer a biocompatible, incompact, macroporous, and 3D microenvironment for subsequent cell immobilization. These results confirmed the successful preparation of the cell based paper sensor.

The DPV measurement was used to determine the relationship between the anodic peak current and the number of RBL cells. Although most electrochemical cell sensors measured cell concentration based on impedance signals, it is believed that amperometric methods that use the relationship between the voltammetric signals and the number of cells are more sensitive and selective. It was found that the peak current of RBL cells decreased gradually with increasing number of cells in the paper sensor, as showed in Fig. 2C. This indicated a higher number of immobilized cells in the paper sensor. The results presented in Fig. 2D showed that any decrease in peak current was proportional to the concentration of the cells with in a certain range. The peak current of RBL cells had a negative correlation with the number of cells in the range from 1.0×10^2 to 1.0×10^8 cells/mL, with a correlation coefficient of 0.993. When cell numbers exceeded 1×10^8, the impedance values were essentially unchanged. Therefore, cell number below 1×10^8 cells/mL was considered to be appropriate for following electrochemical analysis.

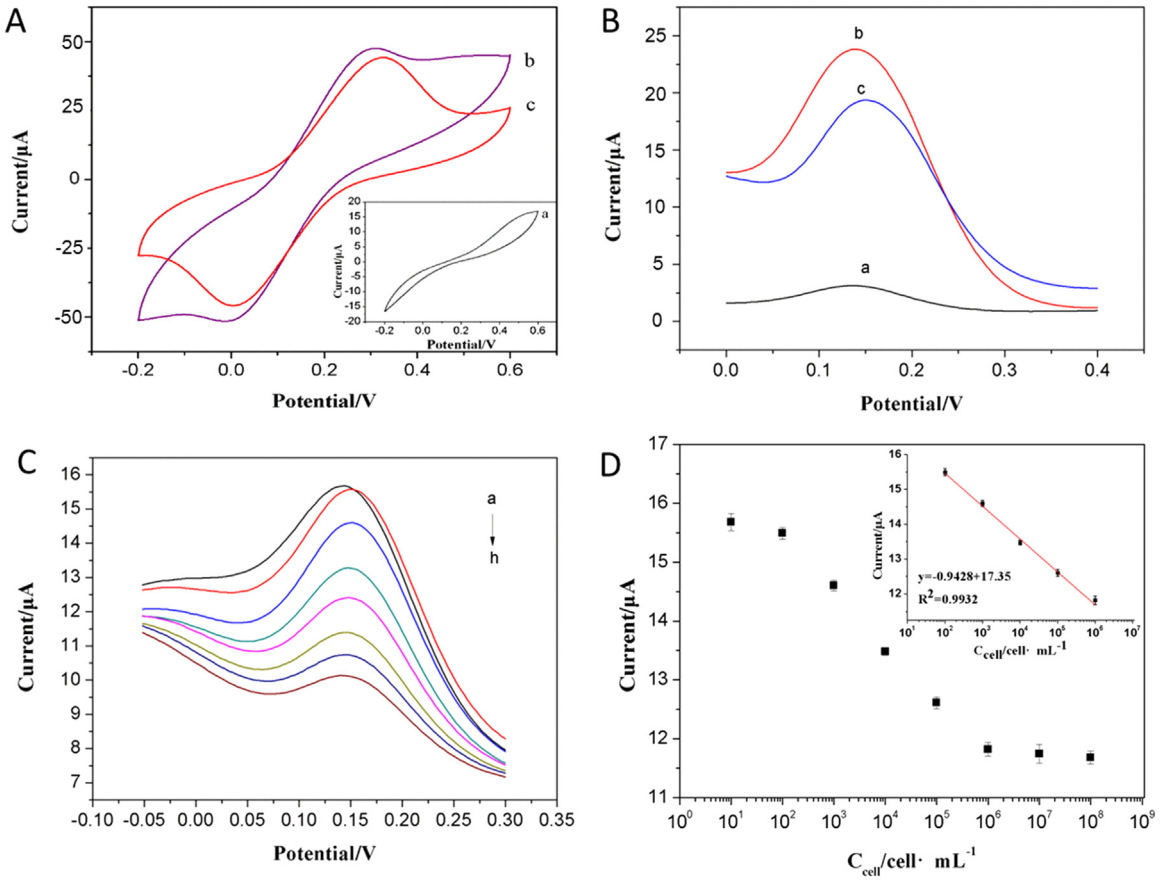


Fig. 2. Electrochemical characteristics of the cell based paper sensor. (A) Cyclic voltammograms and (B) Differential pulse voltammograms of (a) bare SPWE, (b) CN/GN/SPWE, (c) Cell/CN/GN/SPWE. (C) Differential pulse voltammograms of different cell numbers from a to h : 0 cells/mL, 1×10^2 cells/mL, 1×10^3 cells/mL, 1×10^4 cells/mL, 1×10^5 cells/mL, 1×10^6 cells/mL, 1×10^7 cells/mL and 1×10^8 cells/mL of RBL cells immobilized in the paper sensor. (D) Linear regression curve between cell concentration and Ip value.

3.3. Electrochemical detection of casein with the cell-based paper sensor

The increasing demand for reliable food safety monitoring is motivating scientists involved in development of highly sensitive and selective detection method to greatly strengthen the early prediction for food allergen. Due to the outstanding binding affinity and intelligent identification character of the RBL mast cells for special allergen protein, this paper-based cell sensor could be used for the detection of food allergen proteins. Thus, the analytical performance of this sensor for detection of milk allergen casein was examined in this work.

The DPV responses of the paper-based cell sensor corresponding to casein at different concentrations were displayed in Fig. 3. Under optimal experimental conditions (Fig. S2), the DPV responses decreased gradually over a casein concentration ranged from 1×10^{-7} to 1×10^{-5} g/mL. The linear regression equation was expressed as I_{DPV} (μA) = 12.02–1.25 C_{casein} (ng/mL) in the linear range from 1×10^{-7} to 1×10^{-6} g/mL with a correlation coefficient of R = 0.9954 (n = 5), where I_{DPV} was the DPV response and C_{casein} was the allergen casein concentration. The LOD for casein was calculated to be 3.2×10^{-8} g/mL according to the formula LOD = $3s/m$, where s represents the standard deviation of the blank sample (n = 5) and m represents the slope of the relative calibration curve for casein. The SEM and TEM images of RBL mast cells and their cellular degranulation were showed in Fig. 4. After incubation with 1×10^{-6} g/mL casein, we found that this allergen significantly increased degranulation in RBL cells. Compared with control group (A and C), 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 (B and D). In addition, the intracellular Ca^{2+} measurements performed to confirm the results of the electrochemical analysis that was showed in Supplementary material Fig. S3.

To further evaluate the performance of the developed sensor, the analytical performance of this cell sensor was compared with that of other detection methods listed in Table 1, which included the relevant LOD and the type of food matrices analyzed with each of the listed methods. The detection results obtained using the mast cell paper sensor were similar to those obtained with previously reported methods. However, the procedure with the newly developed sensor was much simpler with fewer operation steps, faster detection time, and lower cost, which confirmed that the proposed cell biosensor can be effectively applied to detect and predict real allergen samples. In addition, currently, there was no electrochemical cell sensor for determination of the milk allergen casein with a similar detection accuracy, especially, based on a disposable, portable paper platform.

3.4. Reproducibility and selectivity of the cell sensor

The reproducibility of this cell-based paper sensor was evaluated based on measurements of the same casein sample on 10 different paper chips (with RBL cells) prepared in different batches. The relative standard deviation (RSD) for the parallel detection of 1×10^{-7}, 1×10^{-6}, and 1×10^{-5} g/mL casein with 10 paper sensors, was 3.44%, 3.18%, and 3.51%, respectively. The long-time stability of the paper sensor (modified with CN/GN composite but without RBL cells immobilization) was studied on a 21-day period. After keeping it at room temperature for three weeks, the paper sensor was used to detect the same casein concentration (1×10^{-6} g/mL). The peak current varied in the

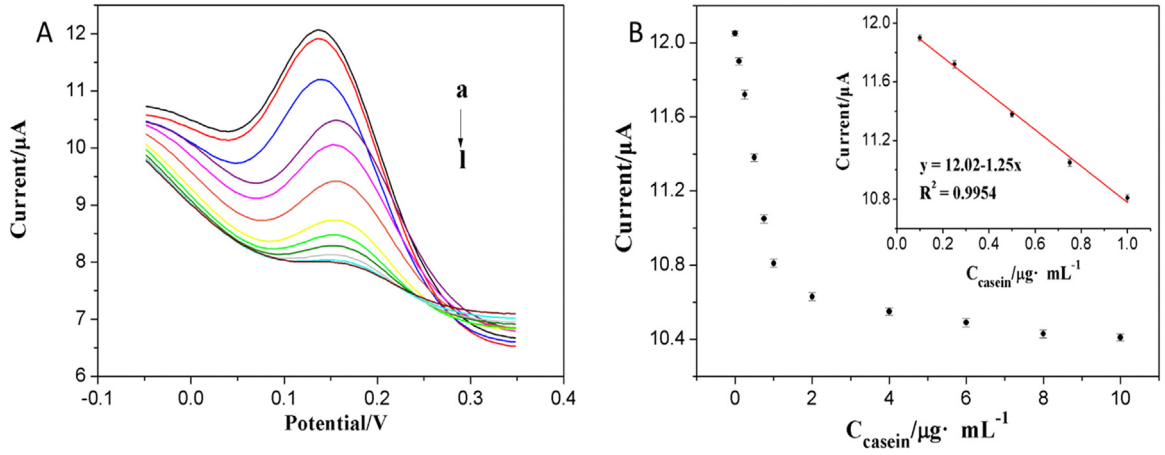


Fig. 3. Electrochemical detection of different concentrations of casein allergens. A: DPV responses of the paper cell sensor corresponding to casein at different concentrations, a to l: 0–1 × 10^{-5} g/mL casein; B: Current of cell based paper sensor corresponding to casein at different concentrations; inset: Calibration curve of casein obtained with the prosed assay. The DPV parameters were set to a pulse period of 0.2 s, 0.05 V pulse amplitude, 0.05 s pulse width and − 0.2 V initial potential to 0.6 V end potential.

range of 10.81 μA to 11.28 μA. As showed in Supplementary material Fig. S4, the peak current did not show an obvious change (lower than 5%), demonstrating that the paper sensor had a good stability. These results suggested that this paper-based chip has good reproducibility and precision as well as adequate stability during manufacturing, normal storage, or long-distance transport which suggest an excellent potential for the development of a portable detection instrument.

The specificity of the cell sensor plays an important role in analyzing biological samples in situ without separation. The effect of possible hindrances that might interfere with the determination of target analytes was investigated. The cell sensor was incubated in 1 × 10^{-6} g/mL casein containing some potential coexisting species (1 × 10^{-6} g/mL), including bovine serum albumin (BSA), egg albumin, soybean globulin and shrimp tropomyosin. It was found that the same concentration of these potential co-existing species had almost no influence on the detection of casein (Table S1). These results suggested that the cell sensor had good specificity for the determination of casein.

4. Conclusions

In this work, a novel low-cost, disposable, paper-based mast cell sensor is fabricated, which offers a simple platform for the electrochemical detection of the milk allergen casein. The RBL cells immobilized in such a paper sensor exhibited an irreversible voltammetric response related to the response of mast cells to the allergen and the peak current showed a positive relationship with the casein

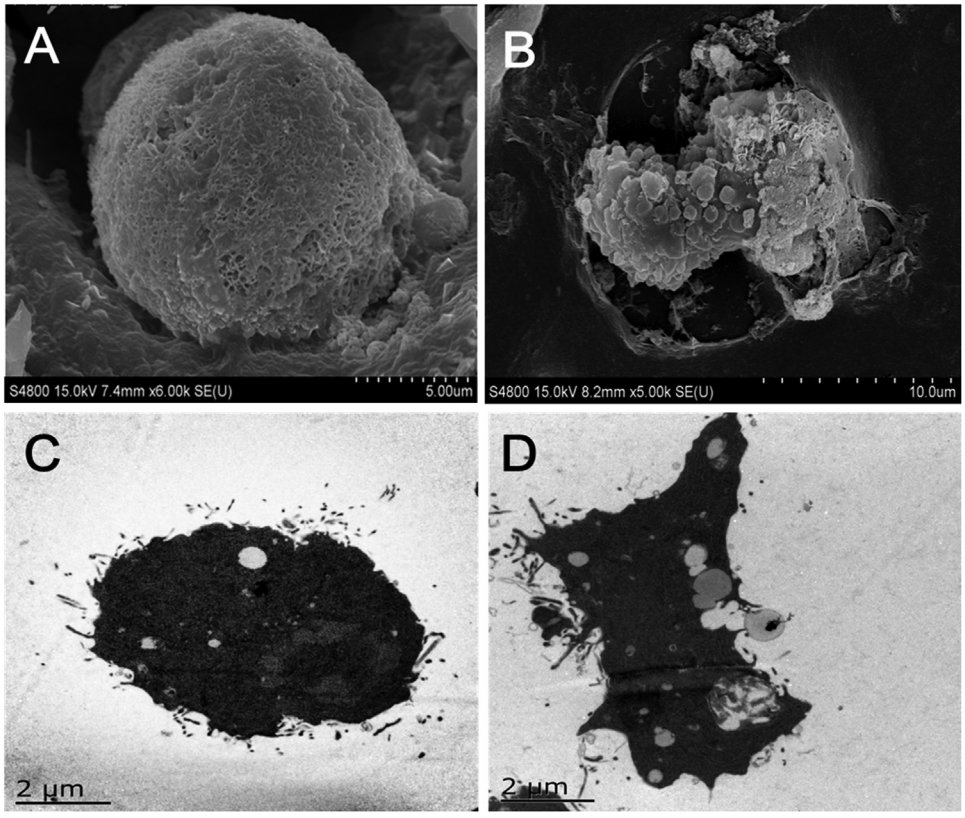


Fig. 4. The SEM and TEM images of RBL mast cells and its cellular degranulation. Scanning electron micrograph of RBL cells: (A) for control and (B) for RBL cells treated with 1 × 10^{-6} g/mL casein; Transmission electron micrograph of RBL cells: (C) for control and (D) for cells treated with 1 × 10^{-6} g/mL casein. SEM: S-4800, Signal Name = SE(U), Data Size = 1280 × 960, Accelerating Voltage = 15 kV, Magnification = 5 k~6 k, Working Distance = 7.4–8.2 mm; TEM: Tecnai 12, highest magnification = 650k times, Resolution = 0.24 nm, Resolution = 0.14 nm, highest acceleration voltage = 120 kV.

Table 1
Comparison of several reported methods for casein detection.

Methods	Detection target	Detection result	Detection time	Reference
Multiple reaction monitoring (MRM) mode in high performance liquid chromatography-mass spectrometry (HPLC-MS)	Milk allergen casein	The LOD = 5×10^{-4} g/mL in the range of 5×10^{-4} to 2.5×10^{-1} g/mL	1 h	(Feng et al., 2013)
Fluorescent microspheres lateral flow assay	Milk allergen casein	The LOD = 1×10^{-7} g/mL in the range of 1×10^{-7} to 1×10^{-5} g/mL.	2 h	(Cheng et al., 2017b)
A localized surface plasmon resonance (LSPR) immunosensor based on gold-capped nanoparticle substrate	Milk allergen casein	The LOD= 1×10^{-8} g/mL in the range of 1×10^{-7} to 1×10^{-5} g/mL	1 h	(Hiep et al., 2007)
Based on ultra performance liquid chromatography -quadrupole/electrostatic field orbitrap high resolution mass spectrometry(UPLC-Q/ Orbitrap MS)system	Milk allergen casein	The LOD= 2×10^{-7} to 5.5×10^{-6} g/kg in the range of 5×10^{-3} to 2.5×10^{-1} g/mL	1 h	(Zhan et al., 2017)
Sandwich-antibody enzyme linked immunosorbent assay	Milk allergen casein	The LOD= 7×10^{-9} g/mL in the range of 6×10^{-9} to 1.6×10^{-6} g/mL	2 h	(Wang et al., 2010)
Rapid immunoassay using disposable screen printed carbon electrode (SPCE) in connection with the differential pulse voltammetry (DPV).	Milk allergen casein	5 μL pure casein solutions	50 min	(Saito et al., 2012)
Electrochemical immunosensor based on gold nanoparticles and poly(L-Arginine)/multi-walled carbon nanotubes (P-L-Arg/MWCNTs) composite film	Milk allergen casein	The LOD= 5×10^{-8} g/mL in the range of 1×10^{-7} to 1×10^{-5} g/mL	1 h	(Cao et al., 2011)
Cell based paper sensor	Milk allergen casein	The LOD= 3.2×10^{-8} g/mL, in the range of 1×10^{-7} to 1×10^{-6} g/mL	20 min	This study

concentration. Additionally, the electrochemical quantitative detection of casein was investigated. The I*p* values were proportional to the casein concentrations, which ranged from 1×10^{-7} to 1×10^{-6} g/mL, with a correlation coefficient of 0.995 and a detection limit of 3.2×10^{-8} g/mL (RSD < 5%). The electrochemical results were consistent with those obtained by the conventional analysis assay, including SEM, TEM and Ca^{2+} concentration measurement, indicating that the proposed method can be used for the quantitative detection of food allergen. Typically, the simple, disposable, and low-cost paper-based cell sensor not only exhibited excellent analytical performance, but also remarkably avoided complicated operations, time consuming pretreatment and expensive electrode materials. Therefore, we anticipate that such versatile, convenient and new generation electrochemical cell based paper sensor will offer a valuable alternative protocol for the on-site monitoring and determination of various food allergens.

CRediT authorship contribution statement

Donglei Jiang: Conceptualization, Methodology, Software, Formal analysis, Writing - original draft, Writing - review & editing, Supervision, Project administration, Funding acquisition. **Panwei Ge:** Writing - original draft, Formal analysis, Data curation, Investigation. **Lifeng Wang:** Validation, Visualization, Supervision. **Hui Jiang:** Data curation, Investigation, Validation. **Ming Yang:** Data curation, Validation. **Limin Yuan:** Resources. **Qingfeng Ge:** Resources. **Weiming Fang:** Resources. **Xingrong Ju:** Validation, Resources.

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Declaration of interests

None.

Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at https://doi.org/10.1016/j.bios.2019.01.050.

References

Antiochia, R., Tortolini, C., Tasca, F., Gorton, L., Bollella, P., 2017. Graph. Bioelectron. 1–35.
Bhardwaj, J., Devarakonda, S., Kumar, S., Jang, J., 2017. Sens. Actuators B Chem. l 253, 115–123.
Bollella, P., Fusco, G., Tortolini, C., Sanzò, G., Favero, G., Gorton, L., Antiochia, R., 2017. Biosens. Bioelectron. 89, 152–166.
Cao, Q., Zhao, H., Yang, Y., He, Y., Ding, N., Wang, J., Wu, Z., Xiang, K., Wang, G., 2011. Biosens. Bioelectron. 26 (8), 3469.
Chakrabarti, B., Nir, D., Yufit, V., Aravind, P., Brandon, N., 2017. World Acad. Sci. Eng. Technol. 11 (9), 607–611.
Cheng, S., Yang, Y., Ni, X., Peng, J., Lai, W., 2017a. Food Agric. Immunol. 28 (6), 1017–1028.
Cheng, S., Yang, Y., Ni, X., Peng, J., Lai, W., 2017b. Food Agric. Immunol. 1, 1–12.
Cinti, S., Basso, M., Moscone, D., Arduini, F., 2017. Anal. Chim. Acta 960, 123–130.
Curtis, T., Naal, R.M.Z., Batt, C., Tabb, J., Holowka, D., 2008. Biosens. Bioelectron. 23 (7), 1024–1031.
Ding, L., Du, D., Zhang, X., Ju, H., 2008. Curr. Med. Chem. 15 (30), 3160–3170.
Feng, X., Zhang, J., Lü, M., Gao, M., Zhang, X., 2013. Chin. J. Chrom. 31 (6), 510.
Güder, F., Ainla, A., Redston, J., Mosadegh, B., Glavan, A., Martin, T., Whitesides, G.M.,

2016. Angew. Chem. Int. Ed. 55 (19), 5727–5732.
Graham, A.C., Temple, R.M., Obar, J.J., 2015. Front. Immunol. 6, 238.
Hiep, H.M., Endo, T., Kerman, K., Chikae, M., Kim, D.K., Yamamura, S., Takamura, Y., Tamiya, E., 2007. Sci. Technol. Adv. Mat. 8 (4), 331–338.
Jiang, D., Zhu, P., Jiang, H., Ji, J., Sun, X., Gu, W., Zhang, G., 2015. Biosens. Bioelectron. 70, 482–490.
Jin, C., Shelburne, C.P., Li, G., Potts, E.N., Riebe, K.J., Sempowski, G.D., Foster, W.M., Abraham, S.N., 2011. J. Clin. Invest. 121 (3), 941–955.
Jo, H., Her, J., Lee, H., Shim, Y.-B., Ban, C., 2017. Talanta 165, 442–448.
Lee, S.-M., Han, N., Lee, R., Choi, I.-H., Park, Y.-B., Shin, J.-S., Yoo, K.-H., 2016. Biosens. Bioelectron. 77, 56–61.
Li, L., Liu, D., Wang, K., Mao, H., You, T., 2017. Sens. Actuators B Chem. 252, 17–23.
Liang, L., Su, M., Li, L., Lan, F., Yang, G., Ge, S., Yu, J., Song, X., 2016. Sens. Actuators B Chem. 229, 347–354.
Lu, J., Ge, S., Ge, L., Yan, M., Yu, J., 2012. Electrochim. Acta 80, 334–341.
March, G., Nguyen, T.D., Piro, B., 2015. Biosensors 5 (2), 241–275.
Mazzei, F., Favero, G., Bollella, P., Tortolini, C., Mannina, L., Conti, M.E., Antiochia, R., 2015. Int. J. Environ. Heal. 7 (3), 267–291.
Mettakoonpitak, J., Boehle, K., Nantaphol, S., Teengam, P., Adkins, J.A., Srisa-Art, M., Henry, C.S., 2016. Electroanal 28 (7), 1420–1436.
Morbioli, G.G., Mazzu-Nascimento, T., Milan, L.A., Stockton, A.M., Carrilho, E., 2017. Anal. Chem. 89 (9), 4786–4792.
Nicholas, P., Pittson, R., Hart, J.P., 2018. Food Chem. 241, 122–126.
Niu, X., Chen, W., Wang, X., Men, Y., Wang, Q., Sun, W., Li, G., 2018. Microchim. Acta 185 (3), 167.
Oliveira, V.X., Dias, A.A., Carvalho, L.L., Cardoso, T.M., Colmati, F., Coltro, W.K., 2018. Anal. Sci. 34 (1), 91–95.
Oularbi, L., Turmine, M., El Rhazi, M., 2017. J. Solid State Electr. 21 (11), 3289–3300.
Promphet, N., Rattanarat, P., Rangkupan, R., Chailapakul, O., Rodthongkum, N., 2015. Sens. Actuators B Chem. 207, 526–534.
Radhakrishnan, R., 2015. Thesis (Ph.D.), Clarkson University, 2015.
Renz, H., Allen, K.J., Sicherer, S.H., Sampson, H.A., Lack, G., Beyer, K., Oettgen, H.C., 2018. Nat. Rev. Dis. Prim. 4, 17098.
Ron, E.Z., Rishpon, J., 2009. Whole Cell Sensing Systems I. Springer, pp. 77–84.
Saito, M., Kitsunai, M., Ahmed, M.U., Sugiyama, S., Tamiya, E., 2012. Electrochem 76 (8), 606–609.
Sajid, M., Nazal, M.K., Mansha, M., Alsharaa, A., Jillani, S.M.S., Basheer, C., 2016. TRAC Trend Anal. Chem. 76, 15–29.
Sobhan, A., Oh, J.H., Park, M.K., Kim, S.W., Park, C., Lee, J., 2017. Korean J. Chem. Eng. 23, 1–7.
Su, M., Ge, L., Ge, S., Li, N., Yu, J., Yan, M., Huang, J., 2014. Anal. Chim. Acta 847, 1–9.
Teengam, P., Siangproh, W., Tuantranont, A., Henry, C.S., Vilaivan, T., Chailapakul, O., 2017a. Anal. Chim. Acta 952, 32–40.
Teengam, P., Siangproh, W., Tuantranont, A., Vilaivan, T., Chailapakul, O., Henry, C.S., 2017b. Anal. Chem. 89 (10), 5428–5435.
Vasilescu, A., Nunes, G., Hayat, A., Latif, U., Marty, J.L., 2016. Sensors 16 (11), 1863.
Wang, B., Wu, Y., Chen, Y., Weng, B., Li, C., 2017. Sens. Actuators B Chem. 238, 802–808.
Wang, S., Yang, Z.L., Dong-Yue, M.A., Duan, S.X., Zhang, Y., Xi-Fen, L.I., Jiang, Y.M., Fang, L., 2010. Food Sci. Tech. 97 (8).
Wang, Y., Zhang, C., Chen, X., Yang, B., Yang, L., Jiang, C., Zhang, Z., 2016. Nanoscale 8 (11), 5977–5984.
Xu, N., Wang, Y., Pan, L., Wei, X., Wang, Y., 2017. Food Agr. Immunol. 28 (6), 1105–1115.
Zhan, L., Chen, Q., Gu, S., Deng, X., 2017. Chin. J. Chrom. 35 (4), 405–412.
Zhu, C., Dan, D., Lin, Y., 2016. Biosens. Bioelectron. 89 (1), 43–55.