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Daily Amperometric Monitoring of Immunoglobulin E in a Mouse Whole Blood: Model of Ovalbumin Induced Asthma

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ABSTRACT : There is an increasing interest in monitoring of specific biomarker for determining progression of a disease or efficacy of a treatment. Conventional method for quantification of specific biomarkers as enzyme linked immunosorbent assay (ELISA) has high material costs, long incubation periods, requires large volume of samples and involves special instruments, which necessitates clinical samples to be sent to a lab. This paper reports on the development of an electrochemical biosensor to measure total immunoglobulin E (IgE), a marker of asthma disease that varies with age, gender, and disease in concentrations from 0.3-1000 ng/mL with consuming 20 μL volume of whole blood sample. The sensor provides rapid, accurate, easy, point-of-care measurement of IgE, also, sequential monitoring of total IgE with ovalbumin (OVA) induced mice is another application of sensor. Taken together, these results provide an alternative way for detection of biomarkers in whole blood with low volumes and long-term *ex-vivo* assessments for understanding the progression of a disease.

Keywords : Electrochemical Amperometry (EA), Screen Printed Electrode (SPE), Ovalbumin (OVA), Immunoglobulin E (IgE), Allergic asthma

1. Introduction

Pulmonary disease and asthma, allergic reactions are heterogeneous disease with many symptoms and characterized by airway obstruction and an inflammatory process.[1-4] Ovalbumin (OVA) and polyhexamethylene guanidine phosphate (PHMG-p) is the one of agents for these pulmonary diseases. OVA is the main protein found in egg white, and it stimulate immune system as antigenic stimulus for pulmonary disease model.[5,6] The other agents, PHMG is biocide family of polymeric guanidine, which has been frequently used as an antiseptic and a disinfectant. However, it is restricted as a microbicidal additive because it causes a fatal lung disease when inhaled. The PHMG induces apoptosis and result in fibrotic lesion by inflammatory response and oxidative stress.[4,7,8]

Total immunoglobulin E (IgE) detection is important for prognosis and diagnosis of these pulmonary and allergic disease because IgE has a central effect on the pathogenesis of pulmonary disease.[9-11] The main function of IgE is providing immunity against parasites but recently, it linked with allergic reactions, playing an essential role in type I hypersensitivity and Th2 inflammatory responses.[12,13] To be specific, after allergen exposure, antigen-presenting cells (APC) sensitize naïve T-cells and direct their development towards Type 2 helper T cell. After they produced IL-4 and IL-13, it is increasing triggering the relevant B-cells to produce IgE that is specific to allergen.[14,15] How-

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ever, monitoring of total IgE shows some challenges related to their low concentration ranges in biological samples, which makes it difficult obtaining correct and reliable analytical results.[12] Moreover, conventional analytical system such as enzyme linked immunosorbent assay (ELISA) requires large volume of samples, expensive and physically large instruments to analyze samples. Therefore, a robust method is for a rapid, accurate, easy to use and inexpensive analysis of IgE quantification.

Electrochemical (EC) biosensor is one of the advanced analytical tools for total IgE because it has high sensitivity, and ultra low limit-of-detection (LOD) at low cost has numerous advantages compared to other analytical method.[9] Moreover, EC biosensor needs small volume of samples, allowing for total IgE quantification and continual monitoring of total IgE in a non invasive manner while consuming only small volumes of biological fluids.[16,17] As a result, quantification is possbile without killing animals in *ex vivo* experiments by using this tool.

Here, we developed a one of important biomarker for diagnosis of allergic response, total IgE EC sensor using a multichannel screen-printed electrode (SPE) with only comsuming 20 μL of per sample basd on amperometric analysis. Such screen printed electrode permit simple, rapid, cheap and fast electrochemical anslyses of microliter volumes.[18] Previously, we already reported that various biomarker quantification in biological fluid with high sensitivity, selectivity is possible based on electrochemical measurement.[19,20] Their performances were further assessed in applying *ex vivo* experiment with PHMG-p, Ova induced mice. During 40 days of experiment, we continuously measure IgE level in vail blood sample without killing mouse models, so we can know the critical point of agent effects for pulmonary diseases. Although, the basic concept of electrochemical immunosensors for measuring total IgE is not new, to the best of our knowledge, this concept has not applied to asthma model without killing mouse. In addition, the proposed electrochemical sensor might seems to apply to the toxicology field. The devleoped biosensor is versatile and can be used to detect various biomarkers in biological samples.

2. Experimental

2.1 Materials

Potassium ferricyanide ($K_3Fe(CN)_6$), ethanolamine hydrochloride, 11-mercaptoundecanoic acid (11-MUA), N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide (EDC), N-hydroxysuccinimide (NHS), bovine serum albumin (BSA), 3,3,5,5-tetramethylbenzdine (TMB), hydroquinone (HQ), sodium citrate dihydrate, phosphate buffer saline at pH 7.4 (PBS), 2-(N-Morpholino) ethanesulfonic acid (MES), mouse albumin lyophilized powder, fetal bovine serum (FBS), Streptavidin labled with poly horseradish peroxidase (poly-HRP-Strept) were purchased from Sigma-Aldrich (St. Louis, USA). Protein G (PG) was purchased from Thermoscientific (Cambridge, USA) and C57BL/6 mouse serum was purchased from Innovative reserach (Novi, USA). Mouse IgE capture antibody (anti-IgE), horseradish peroxidase (HRP) conjugated mouse IgE detection antibody (HRP-anti IgE), IgE standard proteins were used in the mouse IgE ELISA set from Bethyl laboratories (Texas, USA).

2.2 Apparatus and electrode

Screen printed electrode (SPE) array formed by eight electrochemical cells was purchased from Dropsens Co (DRP-8X220AT, Austurius, Spain). The working electrode (WE)'s and counter electrode (CE)'s regions were covered by gold and reference electrode (RE)'s region was covered by silver. Chronoamperometry (CA) and cyclic voltammetry (CV) measurements were carried out with a multi-channel potentiostat obtained from CH instruments (Texas, USA, Model no: CH 1030C) and the wizmac Co. Ltd. (Daejeon, South Korea, Model no: WIZECM-1200 Premium). Electrochemical impedance spectroscopy (EIS) was carried out with a pocketstat from Ivium Technologies B.V. (AJ Eindhoven, Netherlands). All EC measurements were carried out at room temperature in a Faraday cage for shielding.

2.3 Preparation of immunolayer on SPE

The electrodes were first treated with a self-assembled monolayer (SAM) to allow covalent bonding with proteins. To create the SAM, SPE was

treated with acetone, cleaned with ethanol and DI water, and dried in a stream of N_2 (99% purity). Electrodes were then incubated in 10 mM 11-MUA dissolved in anhydrous ethanol for 1 hour at room temperature. Next, equal parts 400 mM EDC and 100 mM NHS in pH 5.5 MES buffer were used to activate the carboxyl groups on the SAM. Next, 10 μg/mL protein G (PG) was immobilized on the SAMs by covalent bonding for 1 hour at room temperature (RT) in order to increase antibody (Ab) density and ensure Ab orientation. The unreacted activated carboxyl groups were blocked by 1 M ethanolamine for 10 min at RT. Finally, a solution of 10 μg/mL capture Ab (anti-IgE) with 20 μL volume was immobilized by PG-antibody affinity, followed by 1% BSA blocking (10 mg/mL for 30 min), which deactivates non-antibody area on the SPE preventing non-specific binding. After that, 20 μL volume of IgE as antigen (Ag) in different solution (in PBS, 10% diluted FBS, 10% diluted mouse blood by PBS) was dropped on the SPE and incubated for 30 min at RT. FBS and blood was diluted by PBS. Next, 20 μL of 1.37 μg/mL biotinylated Ab (HRP-anti-IgE) was added and incubated 60 min in dark. After washing using 0.05% PBS with tween 20 (3 times repeated), SPE was dried by N_2 and performed EC measurement.

2.4 Electrochemical Analysis of IgE

EC assay to quantify IgE based on amperometric measurement was performed with a SPE, including a working electrode (WE: Au), a counter electrode (CE: Au) and a reference electrode (RE: Ag). All three electrodes were connected to a potentiostat that measured current. To calibrate EC measurements, concentrations of IgE from 100 pg/mL to 1 μg/mL were spiked in 10% fetal bovine serum (diluted in 1:9 with PBS). Testing in fetal bovine serum (FBS) determined the effects of nonspecific binding.[20] After measuring the sensor's performance in FBS, 1:10 diluted whole mouse blood by PBS were tested "real sample" analysis indicates the potential for direct translation of this technique to the lab. The determination of IgE was accomplished by dropping 30 μL of a TMB solution onto the surfaces of the SPE immunosensor horizontally positioned and applying a detection potential of 0.10 V and allowed standing for 20 s. The steady state current corresponding to the electrochemical reduction of TMB was used as the analytical readout.

2.4.1 Collection and Analysis of a certified Blood sample

Six-week-old female BALB/c mice were purchased from Orient Bio Inc. (Seongnam, Korea). The mice were housed in an environmentally controlled animal room which was maintained at a temperature of 22±3 °C, a relative humidity of 50±20%, and an air ventilation rate of 10-20 changes/h with a 12 h light/dark cycle. Sterile pelleted food for experimental animals (PM Nutrition International, Richmond, USA) and UV-irradiated (Steritron SX-1; Daeyoung, Inc., Korea) and filtered (1 μm) tap water were provided. The mice were acclimatized for 6 days. All experiments were approved by the Institutional Animal Care and Use Committee of Korea Institute of Toxicology (KIT) and conducted according to the guidelines of Association for Assessment and Accreditation of Laboratory Animal Care International.

For OVA-induced asthma model, mice were intraperitoneally sensitized on days 1 and 8 with 20 μg of ovalbumin (OVA, Sigma-Aldrich, St Louis, Mo) emulsified in 1 mg of aluminum hydroxide (Thermo Fisher Scientific, Rockford, IL) in a total volume of 200 μL. On days 17, 18, and 19 after initial sensitization with OVA, the mice were inhaled to aerosolized 5% (volume/ volume) OVA for 30 minutes in whole-body exposure chamber.

For whole blood collection, we diluted a 5 mM EDTA solution with PBS before beginning collection. A mouse was gently placed in a restrainer and its tail was washed and cleaned using a wipe with 70% alcohol. After grasping the distal end of the tail vein line, we inserted a 1 mL needle, bevel side up, parallel to the skin. Once the needle was placed correctly in the vein, it was slowly removed. Blood was collected from the vein until at least 10 μL was obtained. A 10 μL aliquot of blood was directly mixed with 90 μL of an EDTA solution in a tube and stored at -80 °C.

2.5 Methods in statistics

All assays were run 5 times for checking repro-

ducibility and mean value/ standard deviation were calculated at each concentration to generate the calibration curve. Each replicate was measured with a new SPE. Electrolyte (TMB) was newly made at each time measurement to maintain fresh conditions. Nonlinear curve fitting was performed with origin 8.0.

3. Results and Discussion

3.1. TMB Amperometric Analysis

The schematic of the IgE analysis by using EC sensor is shown in Fig. 1(a). Herein, total IgE quantification was performed by EC enzyme linked immunosorbent assay (EC ELISA) based on enzyme-antibody conjugation. The enzyme reaction between HRP and TMB is known to be two-step oxidation reaction and each oxidized TMB product has a different color: TMB (transparent) → ox-TMB1 (λ_{max} = 650 nm, blue) → ox-TMB2 (λ_{max} = 450 nm, yellow) as shown in Fig. 1. (b). Especially, the first oxidation product of TMB (ox-TMB1) is easily converted to the second oxidation product of TMB (ox-TMB2) under acidic condition. Conventional ELISA is the optical density analysis because the product of TMB changed color when enzyme-antibody conjugate is bound to the substrate. The enzymatic property also can be measured by amperometric analysis of the reduction current generated by oxidation of TMB at an appropriate WE as called EC ELISA. The oxidation and reduction of TMB substrate by cyclic voltammetry (CV) is shown in Fig. 1(c). In CV, the TMB molecule in electric double layer of the working electrode of SPE is oxidized (TMB → ox-TMB1 → ox-TMB2) by the potential ramp at the positive direction, and then the oxidized TMB on the working electrode is reduced by the potential ramp at the negative direction. In this paper, we choose a working potential of 100 mV versus Ag was selected for the measurement of HRP enzymatic activity. These condi-

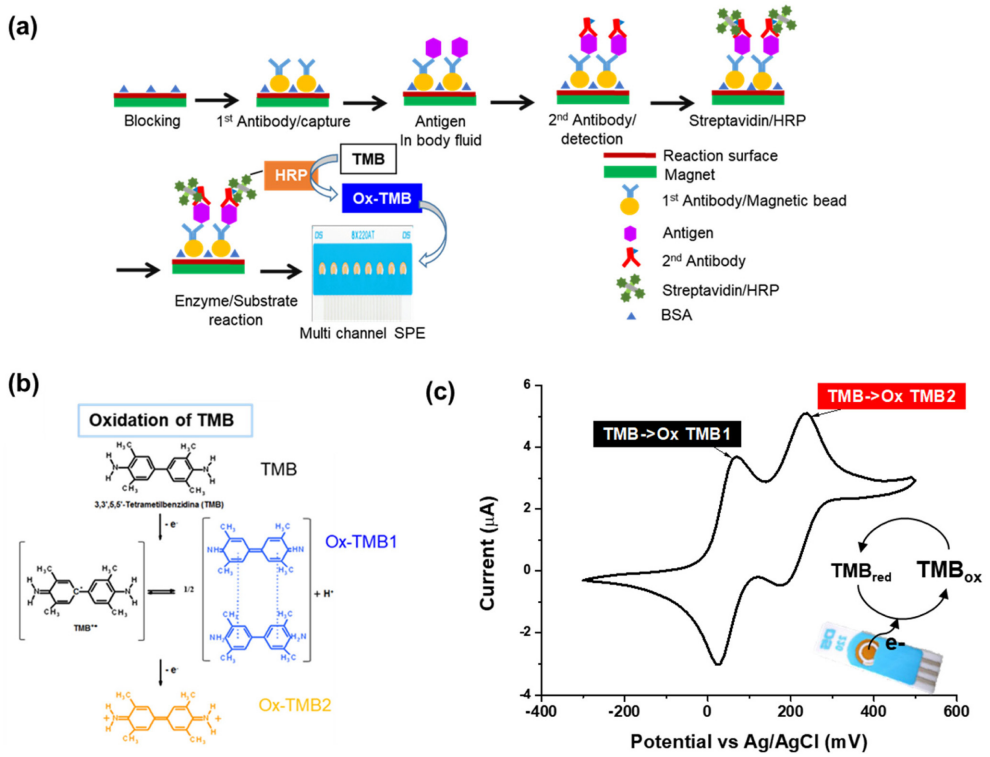


Fig. 1. (a) Schematic illustration of IgE immunosensor based on screen printed electrode. (b) Oxidation sequence of TMB. (c) Electrochemical performance of TMB using cyclic voltammetry. Electrode immersed in TMB solution and the scanning range was from -300 to 500 mV at the scanning rate of 50 mV/s.

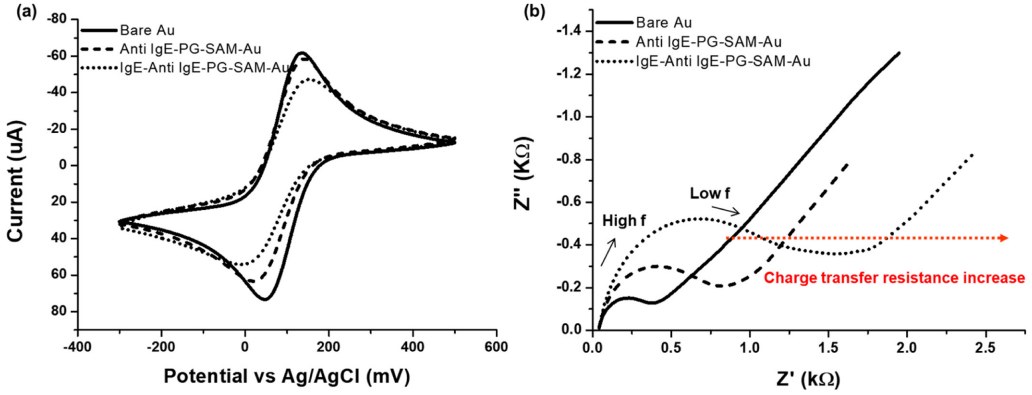


Fig. 2. (a) cyclic voltammogram (CV) of bare Au electrode (line), Anti IgE-PG-SAM-Au electrode (dash), IgE (1 μg/mL)-anti IgE-PG-SAM-Au electrode (dot). Potential applied -300 to 500 mV with scan rate 50 mV/s. 5 mM ferricyanide in PBS (pH 7.4) was used as electrolyte. (b) Nyquist diagrams of bare Au electrode (line), Anti IgE-PG-SAM-Au electrode (dash), IgE (1 μg/mL)-anti IgE-PG-SAM-Au electrode (dot). Analysis has been done in 5 mM ferricyanide in PBS (pH 7.4), in a frequency range 10 kHz and 100 mHz, with disturbance of 0 V.

tions are the optimum because in the EC ELISA, the second oxidation product of TMB was reduced for the quantification of the enzyme reaction by the application of a single reduction potential to the working electrode instead of the potential ramp of CV analysis for quantification of ox-TMB2.

First, Cyclic voltammogram (CV) and electrical impedance spectroscopy (EIS) analysis were performed to characterize the developed EC immunosensor performance. The EC response signals of the bare Au electrode (line), Anti IgE-PG-SAM-Au electrode (dash), and response change after adding IgE 1 μg/mL (dot) are shown in Fig. 2(a). As shown in the figure, current and the integrated area of CV is decreased continuously with modifying the electrode surface. This is contributed from the electrical property of SAM and biomolecule. Due to the insulating nature of SAMs which is bond to the Au electrode via thiol-Au bond, electron transport from the electrolytes to the working electrode is interfered. Also, the CV response is further decreased after immobilizing the PG via amide bonding. Anti-IgE bonding to PG and IgE binding to anti-IgE caused hinder in electron transport, so CV response is decreased continuously. As same reason, we expected that binding of IgE cause increased immunolayer, which creates a dense and thicker electrical isolation layer.[21] EIS result supports these mechanisms. As shown in Fig. 2. (b), the charge transfer resistance (R_{ct}) is continuously increased after biomolecule adsorption (SAM-PG-Ab-Ag). This is caused by the immunolayer's electrical property.

After characterization, we optimize the variables in the preparation of the EC immunosensor for the determination of IgE in real sample. All experiments were performed individually for determination of each variable as a) the concentration of HRP secondary antibody; b) incubation time for secondary antibody; c) capture antibody concentration; d)

Table 1. Optimization of the experimental variables affecting the performance of IgE immunosensor.

Variable	Tested	Selected
HRP-Secondary antibody, μg/Ml	13, 1.3, 0.13, 0.013	1.3
incubation time for HRP-Ab, min	30-90	60
Capture antibody, μg/mL	100, 10, 1, 0.1	10
TMB reaction time, min	3, 5, 10, 15	5

TMB reaction time and SWV signal was used to find variables. TMB concentration and the detection potential value were same than those employed in previous paper.[22] Detail on these optimization studies are provide in Table 1 and supporting information.

3.2. Daily IgE measurement in blood

In this work, an immunoassay platform was prepared by immobilizing anti-IgE antibodies to multichannel screen-printed electrode, and IgE in biological sample was bind to anti-IgE antibodies, then HRP conjugated secondary antibodies treated for IgE quantification. TMB was used for the quantification of the amount of bound IgE, and then the amperometric analysis was carried out. As we applied oxidative potential (100 mV), oxidation current was measured and we found after 15 sec, the current was stable. Finally, the steady current corresponding to the electrochemical oxidation of TMB was used as the analytical current. As shown in Fig. 3(a), the amperometric measurements were initially carried out using IgE solutions over the concentration range of 0.3-1 μg/mL. The amperometric response reached a plateau within 20 sec. Absolute current values increased as a function of increasing oxidized-TMB concentration. Fig. 3(b) is the semi-log calibration curve using the current values obtained at the 20 second of the amperometric measurements. EC sensor has a limit of detection (LOD) of 30 ng/mL and a linear range from 30 ng/mL to 1 μg/mL. Above 3 μg/mL, the signal is saturated and we found that sensing range of EC sensor for IgE measurmeent is 30 ng/mL to 3 μg/mL.

Finally, we performed the continuous monitoring of IgE, which occurs by repeated exposure of allergen. Allergic response initiates in the first allergen sensitization, which is characterized by production of specific IgE driven to the allergen by B cells, and produced IgE binds to high-affinity receptor FcεR1 on the surface of mast cells and basophils, resulting in releases of various mediators including histamine and leukotriene. In challenge phase with same allergen, mast cells and basophils in the airways cause an immediate hypersensitivity through FcεR1. Through this series of processes, IgE-mediated asthma is pathophysiologically characterized by airway inflammation, epithelial hypertrophy, goblet cell hyperplasia and airway hyperresponsiveness (AHR). We performed sequential monitoring of total IgE in whole blood of OVA-induced allergic asthma model. PHMG was injected to the mouse day 7, 10, 14, 15, 16 and OVA was injected day 14, 15, 16. OVA is very sensitive and critical to the mouse so OVA was injected to the mouse last 3 days. Also, last 3 days (day 14, day 15, day 16), we reduced PHMG concentration as one third because continuous injection of high concentration PHMG cause killing mouse (Fig. 4(a)). Our results showed that IgE slightly increased after the first OVA challenge and rapidly increased in secondary OVA sensitization as well as during OVA challenge. Native control is the group which repeated exposure of air (no PHMG, Fig. 4(b)). In the case of PHMG 0.1

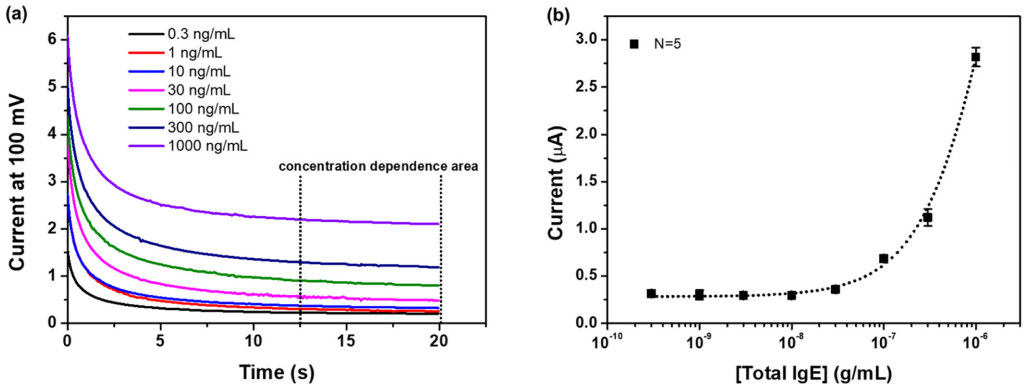
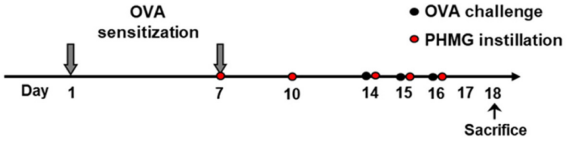


Fig. 3. (a) Amperometry measurement of the EC sensor at 100 mV for different IgE concentrations. (b) EC calibration curve for IgE obtained from IgE solution in mouse blood (n=5).

(a)



Group	Asthma Induction[a)]	PHMG (mg/kg)[b)]	PHMG (mg/kg)[c)]	No. of Animals	Result Figure
Naïve control	X	0	0	6	(b)
T2	X	0.1	0.033	6	(c)
T4	X	0.2	0.067	6	(d)
Asthma control	O	0	0	6	(e)

[a)] X: Without OVA, O: With OVA
[b)] Day 7, Day 10 PHMG concentration
[c)] Day 14, Day 15, Day 16 PHMG concentration

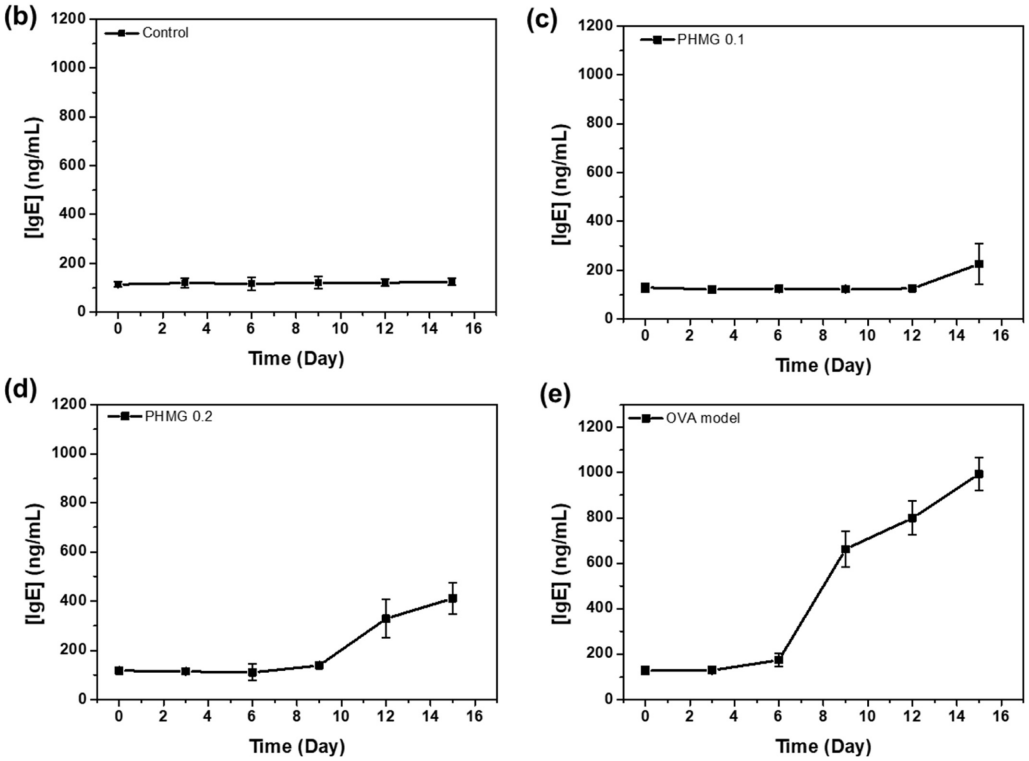


Fig. 4. (a) Specific experimental schedule of sensitization of BALB/c mice to ovalbumin (OVA) and PHMG. (b) Daily monitoring of IgE in native mouse model. (c) Daily monitoring of IgE in PHMG 0.1 mg/kg mouse model. (d) Daily monitoring of IgE in PHMG 0.2 mg/kg mouse model. (e) Daily monitoring of IgE in OVA induced mouse.

mg/kg group (Fig. 4(c)), IgE level is quickly increased after 12 days. However, IgE level quickly increased after 8 days (PHMG 0.2 mg/kg group, Fig. 4(d)) and 6 days (OVA group, Fig. 4(e)). These results proved that IgE level is increased cause of allergic stimulation. Therefore, whole blood IgE measurement using our electrochemical biosensor is a useful tool for sequential monitoring of the onset and progression of the allergic response in the asthma model.

As shown the Table 2, although previous paper has demonstrated biomarker detection levels as low

Table 2. Comparison of different methods for IgE detection

Immunosensor	technique	Linear range	LOD	Sample	Ref
MWCNT-aptmaer	DPV	0.5 ~ 30 nM	37 pM	Spiked serum	1
Carbon SPE-chitosan	CV	1~400 ng/ml	0.5 pg/ml	Spiked serum	2
Fe3O4/r-GO/GCE/aptamer	EIS	-	5 nM	Spiked serum	3
Au/GCE/Zwitterionic peptide/aptamer	DPV	0.1~10 pg/ml	42 fg/ml	Spiked serum	4
Well plate	FACS	0.5-4 μg/mL	0.5 μg/ml	Whole blood	
				Whole blood	
SPE-Ab-Ag	CA			Whole blood	This work

as pg/mL level using aptamer or carbon nanotube structure, this ultra-high sensitivity is not a critical factor for IgE disposable sensor operation. The technique mentioned in here enables IgE as asthma biomarker detection from whole blood or any other physiological fluid without the challenges associated with tailoring sensor operation for the medium of interest or engineering nanosensors that can withstand complex fluid media. Furthermore, the need for ultra-sensitivity in electronic detection may not be essential with such an integrated platform because of IgE cutoff level is rather high in OVA-induced mice.

4. Conclusions

We demonstrated that the modified screen-printed electrode enables to detect IgE at low concentrations in undiluted blood serum. The sensors have many advantages over conventional methods of detection: it does not require fluorescence; analysis time is short; it does not require costly equipment, such as a plate reader, it has a high S/N ratio; and it requires very little sample (30 μL). Also, allergic responses affected to IgE level by continuous monitoring of mouse group which treated PHMG and ovalbumin. This demonstrates the potential of EC sensors to be used in detection of other biomarkers in blood, serum. EC sensors will become a powerful tool for point-of-care biomarker assessment.

Supporting Information

Supporting Information is available at https://doi.org/10.5229/JKES.2022.25.1.13

Notes

The authors declare no competing financial interest

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References

1. Lötvall J, Akdis CA, Bacharier LB, Bjermer L, Casale TB, Custovic A, Lemanske RF Jr, Wardlaw AJ, Wenzel SE, and Greenberger PA, *J Allergy Clin Immunol*., **127(2)**, 355-360 (2011).
2. Wendell SG, Baffi C, and Holguin F, *J Allergy Clin Immunol.,* **133(5),** 1255-64 (2014).
3. Aggarwal T, Wadhwa R, Thapliyal N, Sharma K, Rani V, and Maurya PK, *J Cell Physiol*., **234(3),** 2067-2082 (2019).
4. Kim HY, Kim MS, Kim SH, Joen D, and Lee K, *Molecules*., **23(8),** 1974 (2018).
5. Hines EA, Szakaly RJ, Leng N, Webster AT, Verheyden JM, Lashua AJ, Kendziorski C, Rosenthal LA, Gern JE, Sorkness RL, Sun X, and Lemanske RF Jr, *PLoS One.,* **9(12),** e112997 (2014).
6. Bai F, Fang L, Hu H, Yang Y, Feng X, and Sun D, *Biosci Biotechnol Biochem.*, **83(3),** 531-537 (2019).
7. Song J, Kim W, Kim YB, Kim B, and Lee K, *Toxicol Appl Pharmacol*., **345,** 94-102 (2018).
8. Park EJ, Park SJ, Kim S, Lee K, and Chang J, *Toxicol Lett.*, **286,** 108-119 (2018).
9. Salimi A, Khezrian S, Hallaj R, and Vaziry A, *Anal Biochem*., **466,** 89-97 (2014).
10. Carrington R, Jordan S, Pitchford SC, and Page CP, *Pulm Pharmacol Ther*., **51,** 73-78 (2018).
11. Samitas K, Delimpoura V, Zervas E, and Gaga M, *Eur Respir Rev*., **24(138),** 594-601 (2015).
12. Prado IC, Souza ALA, Provance DW Jr, Cassella RJ, and De-Simone SG, *Anal Biochem*., **538,** 13-19 (2017).

13. Djukanovic R, Hanania N, Busse W, and Price D, *Respir Med*., **112,** 128-129 (2016).
14. Patel TR and Sur S, *Curr Opin Allergy Clin Immunol*., **17(1),** 42-49 (2017).
15. Gould HJ and Sutton BJ, *Nat Rev Immunol*., **8(3),** 205-217 (2008).
16. Nwankire CE, Venkatanarayanan A, Glennon T, Keyes TE, Forster RJ, and Ducrée J, *Biosens Bioelectron*., **68,** 382-389 (2015).
17. Magliulo M, Mallardi A, Gristina R, Ridi F, Sabbatini L, Cioffi N, Palazzo G, and Torsi L, *Anal Chem*., **85(8),** 3849-3857 (2013).
18. Cinti S, Valdés-Ramírez G, Gao W, Li J, Palleschi G, and Wang J, *Chem Commun (Camb)*., **51(41),** 8668-8671 (2015).
19. Lee JK, Bubar CT, Moon HG, Kim J, Busnaina A, Lee HY, and Shefelbine SJ, *ACS Sens*., **3(12),** 2709-2715 (2018).
20. Zhao S, Yang W, and Lai RY, *Biosens Bioelectron*., **26(5),** 2442-7 (2011).
21. Lee JK, Cho SH, Lee JH, Ryu HY, Park JG, Lim SH, Oh BD, Lee CW, Huang W, Busnaina A, Lee HY, *J. Biotechnol.*, **168(4)**, 584-588 (2013).
22. Riahi R, Shaegh SA, Ghaderi M, Zhang YS, Shin SR, Aleman J, Massa S, Kim D, Dokmeci MR, and Khademhosseini A, *Sci Rep*., **6,** 24598 (2016).