A MOLECULAR IMPRINTED POLYMER (MIP)-BASED NT-PROBNP SENSING ELECTRODE USING LG9 PEPTIDES FOR HEART FAILURE MONITORING

Liang-Kai Wang[1], Chih-Hung Lin[1], Yu-Ting Cheng[1], Hsiao-En Tsai[2] and Chih-Kuo Lee[3]*

[1] Institute of Electronics Engineering, National Yang Ming Chiao Tung University, Hsin-Chu, Taiwan

[2]Department of Internal Medicine, National Taiwan University Hospital Hsin-Chu Branch, Taiwan.

[3]Department of Surgery, National Taiwan University Hospital Hsin-Chu Branch, Taiwan.

ABSTRACT

The abstract presents the first molecular imprinted polymer (MIP)-based N-terminal pro-B-type natriuretic peptide (NT-proBNP) sensing electrode using LG9 peptides as the template molecule for clinical study. The MIP-based sensing electrode is an electrochemical polymerized o-phenylenediamine (o-PD) film, which can accomplish the detection in 25 min., exhibit a sensitivity of $8.24x10^{-1}$ $(ng/mL)^{-1}$ and the limit of detection (LoD) of 14.55 pg/ml in the concentration of NT-proBNP from 0.01 to 1.25 ng/ml. and be reused over than 6 times. As compared to other sensing techniques, the presented electrode exhibits excellent characteristics including high specificity and sensitivity, short testing time, and low cost suitable for ICU applications.

KEYWORDS

Molecular Imprinted Polymer, LG-9, serum test, NT-proBNP.

INTRODUCTION

Recent studies have found the importance of NT-proBNP combined with troponin and lactate assays for decision-making to guide immediate treatment and further diagnosis in patients who might have acute myocardial infarction or myocardial injury caused by other etiologies [1-3]. NT-proBNP test has been identified as an independent risk factor for pulmonologists not only to rule out cardiac dyspnea but also to predict mortality in patients with severe COVID-19, thereby improving individual risk stratification [3]. Although MIP-based sensing technique can exhibit superior characteristics such as high specificity and sensitivity for blood test, the main drawback of this technique is that recognition sites can be easily damaged during the elution process due to agile 3D protein structures and non-cross-linked imprinted film, thereby degrading device performance. Using small or fragmental peptide instead of a full one or protein as the template molecule for the complexity reduction of the electrode fabrication can well resolve the reliability issue [4]. In this work, we develop, characterize, and apply microfabricated MIP-based NT-proBNP sensing electrodes using LG-9 peptides, LQESPRPTG, as the template molecule for serum test. Its high specificity and sensitivity, low limit of detection (LoD) and cost-effective characteristics can improve the diagnostic, therapeutic and prognostic aspects of patients potentially with heart failure syndromes requiring intensive cardiac care.

EXPERIMENTAL

Fig. 1 shows the fabrication flow and measurement set up of the NT-proBNP sensing electrode where electrochemical polymerization of o-PD film is performed using conventional silicon process and linear sweep voltammetry (LSV) measurement is performed via a three-electrode configuration in the potassium ferricyanide solution (Fig. 1 (a) and (b)). Higher current response of the MIP film after the elution process as compared to that of non-imprinted film (NIP) film indicates the LG9 peptides have been successfully embedded in the o-PD film to form a MIP electrode (Fig. 1(c)). The major fabrication processes include 1) Ti (10nm)/Au (30nm) electrode deposition and patterning on a Si_3N_4(800nm) /SiO_2 (500nm) /Si substrate, 2) electrochemical polymerization of o-phenylenediamine film onto the Au electrode in an acetate buffer solution (0.5M, pH 5.2) containing 7.5 mM o-phenylenediamine and 1 µg/ml of LG-9 peptides using cyclic voltammetry from 0 to 1.1V, 3) LG-9 peptide removal in the solution of NaOH/Ethanol. Fig. 2 shows liquid chromatography–mass spectrometry (LC/MS) analyses of the LG9 peptide/PBS and the NT-proBNP solution digested with thermolysin @65°C for 15min. The existence of both signals, i.e., 984.500 and 492.756 representing LG9 peptide molecule with one and two positive charges respectively indicates the protease digestion is effective.

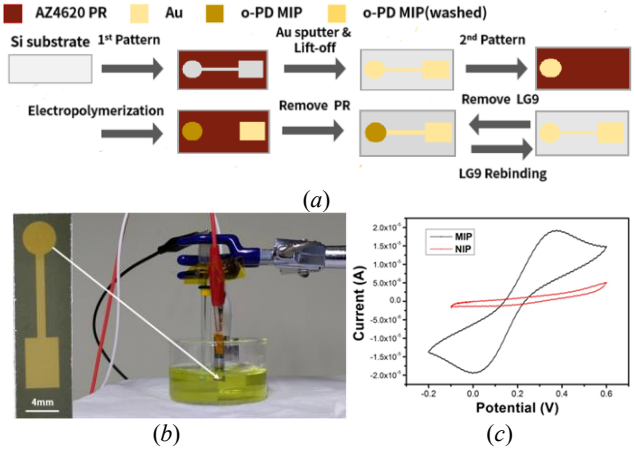

Figure 1: (a) The fabrication processes of the MIP-based Troponin T sensor, (b) Measurement set up: sensor immersion in the blood serum for 10 min (left) and LSV measurement using a three-electrode configuration in the mixture of the 1mM $[Fe(CN)_6]^{3-}$, 1mM $[Fe(CN)_6]^{4-}$ and 0.1M KCl solutions, and (c) I-V response of LSV measurement.

RESULTS AND DISCUSSION

Fig. 3 shows the sensor characterization and blood test results. The sensor electrode exhibits two sensitivity, i.e. $6.36x10^{-2}$ and $8.24x10^{-1}$ in the high (>3 ng/ml)/ low (<1 ng/ml) concentration region, respectively, as shown in Fig. 3(a). The derived LoD for the sensing electrode is based on the following equation [5]:

$$\text{LoD} = 3\sigma/s \quad (1)$$

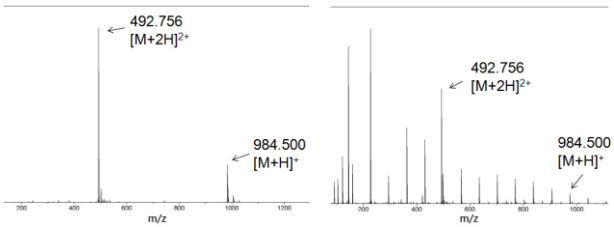

Figure 2. The profile of the LC/MS. (a) LG9 solution, (b) NT-proBNP solution digested with thermolysin for 15min.

978-1-940470-04-7/HH2022/$25©2022TRF
DOI 10.31438/trf.hh2022.108

Table 1. Comparison of NT-proBNP biosensing methods.

	D. Harpaz et al. [6]	University Hospital*	*M. Karimi et al* [7]	**This Work**
Sensor type	SPR	ECLIA*	Electrochemical (Cyclic Voltammetry)	**Electrochemical (LSV)**
Recognition element	Antigen-Antibody	Antibody with ECL	Antigen-Antibody	**MIP**
Testing time	15 min	60 min	70 min	**25 min (15 min+10 min) [5]**
Reusability	One time	One time	One time	**Multiple times**
Measurement Range	127.5 pg/ml	3.59 pg/ml	6 pg/ml	**14.55 pg/ml**
Storage temp./lifetime	4°C/short	4°C/short	4°C/short	**Room temp./Long**
Cost	High	High	Moderate	**Low**

*ElectroChemiLuminescence ImmunoAssay, ECLIA, *https://diagnostics.roche.com/global/en/products/instruments/cobas-e-411.html*

where σ is the standard deviation of the electrode response in the blank solution, and s is the slope of the curve of the sensor output vs. analyte concentration. The LoD was estimated to be ca. 14.55 pg/mL, indicating that the electrode was quite adequate for clinical requirements. Fig. 3(b) and (c) shows the same current response for each NT-proBNP concentration and lower current difference in response to the bovine serum albumin (BSA)/PBS solution indicating the sensor can exhibit excellent reusability and specificity respectively.

Fig. 3(d) shows the correlation of 4 sets of blood test results measured by the MIP sensor and ECLIA methods indicating 94.5% matching in terms of detected NT-proBNP concentration. The detail measurement of the NT-proBNP using the MIP sensor in the serum can be referred to our prior work [5]. The results validate not only the effectiveness of the MIP sensor in comparison with the formal examinations in a tertiary university-affiliated hospital but also the feasibility of the NT-proBNP test for doctors' therapeutic guidance and prognosis assessment. Table I lists the comparison of the present NT-proBNP sensing technologies, among which the MIP-based sensor can exhibit the advantages of high specificity and reusability, short testing times, and cost effective with long storage time [6,7].

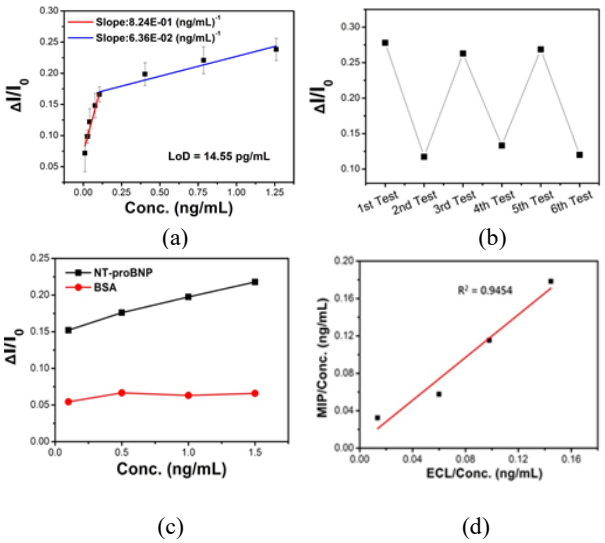

Figure 3: Sensing electrode characterization and blood test results: (a) sensitivity measurement of MIP sensor response, (b) reusability tests: the MIP sensor response immersed repeatedly in the NT-proBNP PBS solutions with different concentrations, i.e. 2 ng/mL and 0.05 *ng/mL, (c) specificity test: the sensor response in the NT-proBNP PBS and BSA/PBS solution, respectively, and (d) statistic correlation of 4 sets of blood test results measured by the MIP sensor and ECLIA methods indicating 94.5% matching in terms of detected NT-proBNP concentration.*

CONCLUSION

A MIP-based NT-proBNP sensing electrode has been successfully developed. The electrode exhibits superior characteristics such as rapid response, good reusability, and high specificity. The clinical blood test results have validated that it is feasible to apply the electrode in clinical applications for detecting heart failure status by monitoring the NT-proBNP concentration accurately.

ACKNOWLEDGEMENTS

This project is funded by the Higher Education Sprout Project by the MOE in Taiwan and in part by MOST 110-2321-B-009-004 and the NTUH Hsin-Chu Branch: 111-HCH001 and 110-HCH080.

REFERENCES

[1] S. Satyan, R. Light, and R. Agarwal, "Relationships of N-terminal pro-B-natriuretic Peptide and Cardiac Troponin T to Left Ventricular Mass and Function and Mortality in Asymptomatic Hemodialysis Patients", Am J Kidney Dis. 50, 1009, (2007).

[2] D. Westermann, J. T. Neumann, N. A. Soerensen, and S. Blankenberg, "High-sensitivity Assays for Troponin in Patients with Cardiac Disease", Nature Reviews Cardiology, 14, 472 (2017).

[3] L. Gao, D. Jiang, X. -S. Wen, X. -C. Cheng, M. Sun, B. He, L.-N. You, P. Lei, X.-W. Tan, S. Qin, G.-Q. Cai, and D.-Y. Zhang, "Prognostic Value of NT-proBNP in Patients with Severe COVID-19", Respir Res., 21, 83, (2020).

[4] A. M. Bossi, P. S. Sharma, L. Montana, G. Zoccatelli, O. Laub, O and R. Levi, "Fingerprint-imprinted Polymer: Rational Selection of Peptide Epitope Templates for the Determination of Proteins by Molecularly Imprinted Polymers", Anal. Chem., 84, 4036, (2012).

[5] Y. T. Lin, L. K. Wang, Y. T. Cheng, C. K. Lee, and H. E. Tsai, "Molecularly Imprinted Polymer/Anodic Aluminum Oxide Nanocomposite Sensing Electrode for Low-Concentration Troponin T Detection for Patient Monitoring Applications." ACS sensors, 6, 2429, (2021).

[6] D. Harpaz, B. Koh, R. C. Seet, I. Abdulhalim, and A. I. Tok, "Functionalized Silicon Dioxide Self-referenced Plasmonic Chip as Point-of-care Biosensor for Stroke Biomarkers NT-proBNP and S100β, Talanta, 212, 120792, (2020).

[7] Y. Zhuo, W. J. Yi, W. B. Lian, R. Yuan, Y. Q. Chai, A. Chen, and C. M. Hu, "Ultrasensitive Electrochemical Strategy for NT-proBNP detection with Gold Nanochains and Horseradish Peroxidase Complex Amplification", Biosens. and Bioelectron., 26, 2188, (2011).

CONTACT

*Yu-Ting Cheng, tel: +886-3-5712121, ytcheng@nycu.edu.tw