A non-enzymatic micro-needle patch sensor for free-cholesterol continuous monitoring

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***Abstract*— A patch type non-enzymatic free-cholesterol sensor was newly developed for continuous monitoring by using stainless steel based micro-needle patch and nanoporous platinum (NPt) sensing electrodes. The formed micro-needle patch was coated with parylene/gold/parylene film and selectively dry-etched to form the gold electrodes at the tips of micro-needles. The bare gold tips were finally electroplated with nanoporous platinum. The sensor was then characterized and analyzed by using cyclic voltammetry and chronoamperometry measurement techniques. The fabricated sensor exhibited high sensitivity of 305nA/mM•cm^2 and correlation coefficient of 0.964 in 0.1M PBS (pH 7.4). In the recovery test, recovery rate was more than 89%.**

Keywords—micro-needle patch; non-enzyme; free-cholesterol; nanoporous platinum

I. INTRODUCTION

67% of cholesterol is esterified with fatty acids and 33% of it is in state of sterol in human blood serum, which contains the cholesterol in the range of up to 5.17mM (or 200mg/dl) for normal people. In particular, the higher concentration of cholesterol can cause several kinds of cardiovascular disease such as hypertension, ischaemic heart diseases, coronary-artery disease, arteriosclerosis, and cerebral infarction because blood vessels can get be blocked by cholesterol with weakened capability of vessels. In order to prevent these diseases, various researches of cholesterol assays based on electrochemical analysis have been conducted. Many conventional cholesterol sensors almost employed immobilized enzyme, which were cholesterol esterase and cholesterol oxidase [1-4]. However, these enzyme based sensors have many disadvantages of weakness for temperature, humidity, and pH. On the other hand, non-enzymatic sensors have several merits such as stability, reproducibility, and simplicity [5]. Therefore, the non-enzymatic sensor is highly valuable for continuous monitoring of cholesterol. Recently, a non-enzymatic cholesterol sensor based on tubular silver nanoparticles on glassy carbon electrode was reported [6].

Micro-needle was proposed in 1976 by Gestel and Place for the first time. Because interstitial or implantable type sensors have problem in bio-fouling caused by adsorption of protein, micro-needle which is aimed to insert into epidermis enables to measure bio-signal through interstitial fluid (ISF) [7]. Micro-needle has also benefits for continuous monitoring in aspect of use for patients because they feel less pain due to small diameter of needle and convenient for patch type product. Many researches related to micro-needle were studied, using nickel, SU-8 epoxy, and micro-machined silicon [8-10]. However, these micro-needles almost have been studied in the field of drug delivery.

In this research, non-enzymatic free-cholesterol sensor was developed on a patch type micro-needle array based on stainless steel (SUS) substrate. Non-ionic surfactant triton X-100 was used to build nanoporous structure of Pt by electroplating. Cyclic voltammetry and chronoamperometry were performed to analyze various electrochemical characteristics through redox reaction. The non-enzymatic free-cholesterol sensor makes it possible to monitor the free-cholesterol continuously.

II. EXPERIMENTAL

A. Reagents

Non-ionic surfactant, *t*-octylphenoxypolyethoxyethanol (Triton X-100, molecular biology), sodium chloride (≥99.5%, molecular biology) and chloroplatinic acid hydrate (HCPA, ≥99.9%) were prepared for electroplating nanoporous Pt. Cholesterol (≥99%, sigma grade), triton X-100 and 2-propanol were purchased from Sigma Aldrich. In order to improve solubility of the cholesterol in 0.1M phosphate buffered saline (PBS, pH 7.4) solution containing cholesterol because the cholesterol do not dissolve well in water-based solution [11]. Diluted 1M sulfuric acid (H_2SO_4) from concentrated H_2SO_4 (95-98%, ACS) was used for cleaning and measuring roughness of fabricated nanoporous Pt on micro-needle patch. All chemicals were purchased from Sigma Aldrich in this research.

B. Apparatus

The fabricated free-cholesterol sensor was measured and characterized through electrochemical method. CHI 660D (CH Instrument, USA) including three electrodes system of working electrode, counter electrode, and reference electrode was employed for all electrochemical analysis. The electroplated nanoporous Pt electrode formed in micro-needle patch, DC sputtered Pt, and Ag/AgCl with 3M NaCl were used as

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working, counter, and reference electrodes, respectively. Cyclic voltammetry was performed in 1M H_2SO_4 at room temperature and the potential range of -0.4V to 1.0V to analyze morphology of nanoporous Pt structure. Chronoamperometry was carried out in 1M phosphate buffer saline (pH 7.4) at room temperature and applied potential of 0.4V to characterize electrochemical performances.

C. *Fabrication*

Fig. 1 shows a fabrication sequence of non-enzymatic free-cholesterol sensor by using a micro-needle patch and nanoporous Pt electrode. In order to fabricate the micro-needle, SUS 306L which is medical grade for invasion into epidermis was prepared and it is pretreated for cleaning using 10% NaOH. And then, dry film was attached on both top and bottom sides of SUS substrate and patterned by using photolithography. Subsequently, the SUS substrate was wet-etched as the patterned film and the micro-needles were made stand physically. Chemical vapor deposition (CVD) and sputtering were carried out to deposit parylene and Ti/Au, which are for isolation and seed layer, respectively. So as to expose the tips of micro-needles, shadow mask was used. Finally, on the tips of micro-needles, NPt was electroplated by using Triton X-100, which is non-ionic surfactant [12]. Fig. 2 shows optical micro-

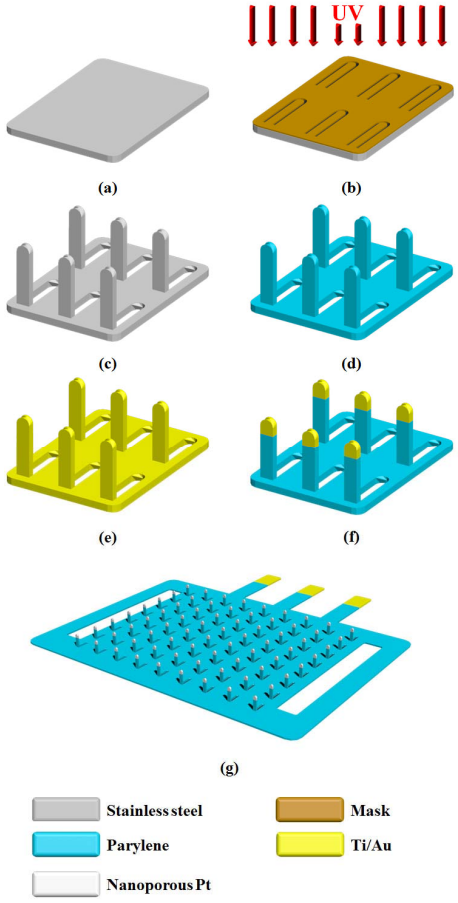

Fig. 1. Fabrication sequences of proposed non-enzymatic free-cholesterol sensor on micro-needle patch: (a) cleaning substrate, (b) photolithography for patterning of SUS, (c) forming micro-needles, (d) parylene/Ti/Au deposition, (e) parylene deposition, (f) opening tips of micro-needles, and (g) electroplating NPt.

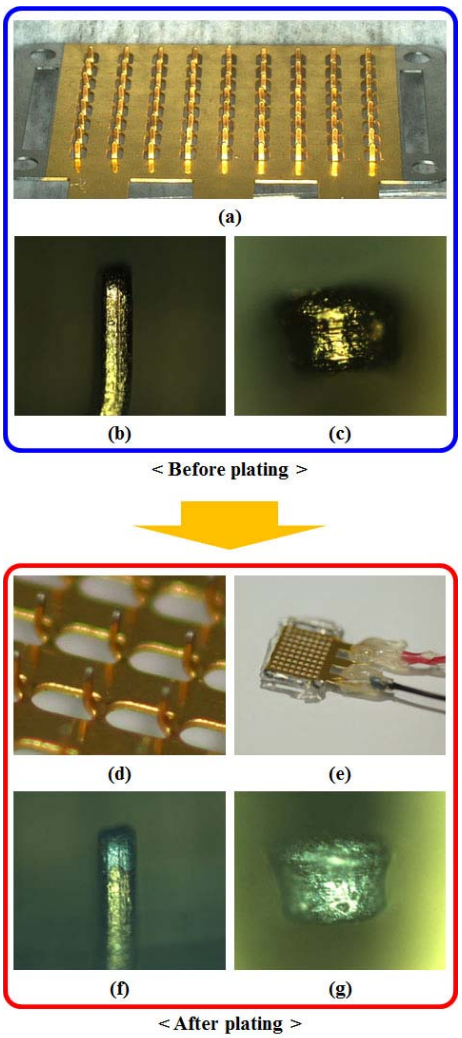

Fig. 2. Optical microscopic images before and after electroplating of micro-needle: (a) whole image before electroplating, (b, c) side and top view of a micro-needle before elecroplating, (d, e) whol image of after electroplating, (f, g) side and top view of a micro-needle after electroplating

scopic images of non-enzymatic micro-needle patch sensor for free-cholesterol detection, before and after electroplating.

III. RESULTS AND DISCUSSION

Fig. 3 shows cyclic voltammogram of the fabricated non-enzymatic cholesterol sensor in 1M sulfuric acid solution. The cyclic voltammogram was measured at the scan rate of 200mV/sec through the potential range from -0.4V to 1.0V versus Ag/AgCl reference electrode. As shown in Fig. 3, the redox reaction on NPt surface is much more vigorous than that on Au and the roughness factor can be calculated by a monolayer of adsorbed hydrogen at double reduction peak. The calculated roughness factor of NPt on the micro-needle patch was 195 as the charge value in the region of hydrogen

adsorption was divided by conversion factor of 210μC/cm^2 [13].

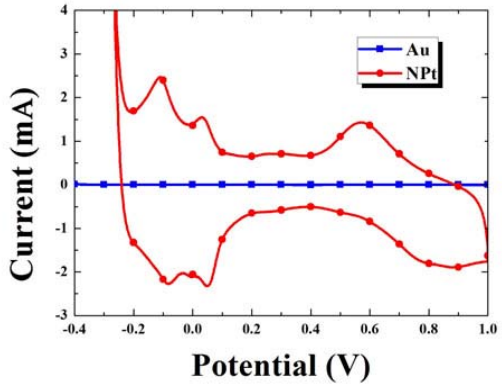

Fig. 3. Comparison of cyclic voltammetry of bare gold and Npt formed on micro-needle patch in 1M sulfuric acid at scan rate of 200mV/s in potential range of -0.4V to 1.0V.

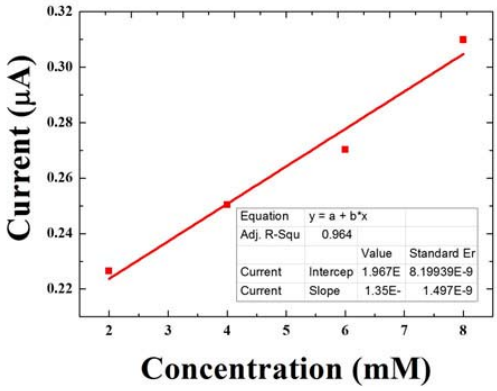

Fig. 4. Current response according to free-cholesterol concentration in 0.1M PBS (pH 7.4) at applied potential of 0.4V and correlation coefficient of current response

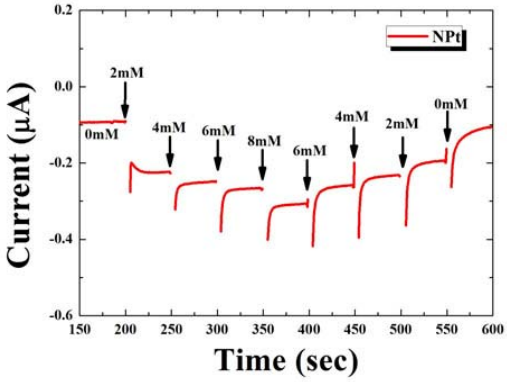

Fig. 5. Recovery characteristic of fabricated micro-needle patch sensor with stepwise change in each free-cholesterol concentration of 0, 2, 4, 6, and 8mM.

Fig. 4 shows current response and linearity as free-cholesterol concentration changes in 0.1M PBS (pH 7.4). The solutions within free-cholesterol concentration of 0, 2, 4, 6 and 8mM were saturated in each beaker before measurement according to the concentration of free-cholesterol. This measurement was performed at potential of 0.4V and by changing the beakers with different concentration after background current was stabilized in constant level. The fabricated cholesterol sensor indicated the sensitivity of 305nA/mM·cm^2 and correlation coefficient of 0.964.

Fig. 5 shows a current-time curve for recovery characteristics of the fabricated micro-needle patch cholesterol sensor for stepwise variation of free-cholesterol. As shown in Fig. 5, the current response is greatly stable in both increasing and decreasing directions in free-cholesterol concentration. Recovery rate for the range of 0 to 8mM was calculated as a difference between the first value and second value was divided into the first value. The recovery rate is more than 89% in whole range.

IV. Conclusion

In this study, non-enzymatic micro-needle patch sensor for continuous free-cholesterol monitoring was developed by using micromachining technique and electroplated NPt. The fabricated free-cholesterol sensor was tested and characterized in 1M sulfuric acid at the potential of -0.4V to 1.0V in order to analyze surface roughness formed by the porous platinum. The roughness factor of NPt on micro-needle array was 195, which was much higher than the sputtered gold. The electrochemical measurements also were carried out in 0.1M PBS (pH 7.4) at the applied potential of 0.4V. The sensitivity and correlation coefficient were 305nA/mM·cm^2 and 0.964, respectively. The recovery rate was more than 89% for stepwise change of free-cholesterol concentration. More characterization and optimization of sensor will be performed for commercial use near future.

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References

[1] G. Li, J.M. Lia, G.Q. Hu, N.Z. Ma, P.J. Wu, "Study of carbon nanotube modified biosensor for monitoring total cholesterol in blood," Biosensor and Bioelectronics, vol. 20, pp. 2140-2144, 2005

[2] Anjan Kumar Basu, Parimal Chattopadhyay, Utpal Roychoudhuri, Runu Chakraborty, "Development of cholesterol biosensor based on immobilized cholesterol esterase and cholesterol oxidase on oxygen electrode for the determination of total cholesterol in food samples," Bioelectrochemistry, vol. 70, pp. 375-379, 2007

[3] Sean Brahim, Dyer Narinesingh, Anthony Guiseppi-Elie, "Amperometric determination of cholesterol in serum using a biosensor

of cholesterol oxidase contained within a polypyrrole-hydrogel membrane," Analytica Chimica Acta, vol. 448, pp. 27-36, 2001

[4] Shyam Aravamudhan, Arun Kumar, Shyam Mohapatra, Shekhar Bhansali, "Sensitive estimation of total cholesterol in blood using Au nanowires based micro-fluidic platform," Biosensors and Bioelectronics, vol. 22, pp. 2289-2294, 2007

[5] Sejin Park, Hankil Boo, Taek Dong Chung, "Electrochemical non-enzymatic glucose sensors," Analytica Chimica Acta, vol. 556, pp. 46-57, 2006

[6] Yanfen Li, Hongyan Bai, Qian Liu, Jianchun Bao, Min Han, Zhihui Dai, "A nonenzymatic cholesterol sensor constructed by using porous tubular silver nanoparticles," Biosensors and Bioelectronics, vol. 25, pp. 2356-2360, 2010

[7] Ahmed El-Laboudi, Nick S. Olver, Anthony Cass, Desmond Johnston, "Use of microneedle array devices for continuous glucose monitoring : A review," DIABETES TECHNOLOG & THERAPEUTICS, vol. 15, pp. 101-115, 2013

[8] Han J. G. E. Gardeniers, Regina Luttge, Erwin J. W. Berenschot, Meint J. de Boer, Shuki Y. Yeshurun, Meir Hefetz, Ronny van't Oever, and Albert van den Berg, "Silicon micromachined hollow microneedles for transdermal liquid transport," Journal of Microelectromechanical Systems, Vol.12, pp. 855-862, 2003

[9] Shawn P. Davis, Benjamin J. Landis, Zachary H. Adams, Mark G. Allen, Mark R. Prausnitz, "Insertion of microneedles into skin : measurement and prediction of insertion force and needle fracture force," Journal of Biomechanics, vol. 37, pp. 1155-1163, 2004

[10] Po-Chun Wang, Seung-Joon Paik, Shuodan Chen, Swaminathan Rajaraman, Seong-Hyok Kim, and Mark G. Allen, "Fabrication and characterization of polymer hollow microneedle array using UV lithography into micromolds," Journal of microelectromechanical systems, vol. 22, pp. 1041-1053, 2013

[11] Arun Kumar, Jajesh, Asha Chaubey, S. K. Brover, and B. D. Malhotra, "Immobilization of cholesterol oxidase and potassium ferricyanide on dodecylbenzene sulfonate ion-doped polypyrrole film," Journal of Applied Polymer Science, vol. 82, pp. 3486-3491, 2001

[12] Sejin Park, Sun Young Lee, Hankil Boo, Hyun-Mi Kim, Ki-Bum Kim, Hee Chan Kim, Youn Joo Song, and Taek Dong Chung, "Three-dimensional interstitial nanovoid of nanoparticulate Pt film electroplated from reverse miceele solution," Chemistry of Materials, vol. 19, pp. 3373-3375, 2007

[13] T. Biegler, D. A. J. Rand, and R. Woods, "Limiting oxygen overage on platinized platinum; relevance to determination of real platinum area by hydrogen adsorption," Electroanalytical Chemistry and Interfacial electrochemistry, vol. 29, pp. 269-277, 1971