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A low-cost miniaturized potentiostat for point-of-care diagnosis

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ABSTRACT

This paper presents a novel approach of using a miniaturized potentiostat (M-P) chip (LMP91000) to perform full range cyclic voltammetry (CV) measurements for the detection of biomarkers. The LMP91000 evaluation board was reconfigured to perform three-electrode CV measurements in order to achieve electrochemical cortisol immunosensing. The microelectrodes for cortisol estimation were fabricated by immobilizing monoclonal anti-cortisol antibody (Anti-M-Cab) onto self-assembled monolayer (SAM) modified Au microelectrodes. The results obtained using the M-P were compared to those obtained using a conventional potentiostat. The M-P was successful in measuring cortisol levels in the range of pM. The outcomes of the studies suggest that M-P can effectively perform biochemical measurements on three electrode systems, enabling the development of miniature systems for point-of-care (POC) diagnosis.

1. Introduction

Portable miniaturized analytical devices for disease detection at early stages and for monitoring physiological variables at point-of-care (POC) can be useful to personalize health diagnostics for appropriate effectual treatment (Ahn et al., 2004; Choi et al., 2011; Gubala et al., 2011; Justino et al., 2013; Kaushik et al., 2014; Kemmler et al., 2014; Kost, 1995; Kumar et al., 2013; Loncaric et al., 2012; Luppa et al., 2011; Rusling et al., 2010; Soper et al., 2006; Tudos et al., 2001; Wang, 2006). POC diagnostic tools are increasingly being developed for quantifying biomarkers and trends based on time and age. These miniaturized biomedical devices are also being explored to provide fundamental information that could form the basis of health informatics and superior treatment strategies (Ahn et al., 2004; Luppa et al., 2011; Soper et al., 2006).

The existing diagnostic systems are limited to laboratory facilities and are not suitable for POC due to their high costs, the necessity of trained personnel, portability, and long waiting to obtain results (Kaushik et al., 2014). The development of novel methodologies for adequate diagnosis at early stage for healthcare monitoring at POC requires a new class of systems. Such systems must be capable of interacting directly with biological samples in order to retrieve the desired information regarding the patient's metabolism effortlessly and in real-time (Gubala et al., 2011; Rusling et al., 2010).

Electrochemical biosensors have recently been employed for selective, specific and rapid detection of biomarkers within physiological ranges. The sensing performance of such electrochemical biosensors is found to be dependent on the selection of transducers and immobilizing electro-active matrix e.g. nanostructures (Arya et al., 2012; Kaushik et al., 2014; Solanki et al., 2011). The introduction of nanomaterials as immobilization matrices lead to increased sensitivity, linearity, and detection at pico-molar (pM) level (Arya et al., 2012; Huey et al., 2012; Solanki et al., 2011). Electrochemical biosensors enable miniaturization through the use of advanced interdigitated micro-electrodes (IDEs) configurations that enhance sensing performance and reduce form factors (Pasha et al., 2014; Vasudev et al., 2013). IDEs have been used to detect analyte biomarkers in the pM range (Shalev et al., 2013), and also to enhance the sensitivity of the measurements. IDE-based electrochemical sensors, having a series of parallel micro-band electrodes with alternating micro-bands connected together are useful for rapid reaction kinetics, improved sensitivity, large electrode aspect ratio (w/l) and increased signal-to-noise ratio (Arya et al., 2010a). These IDEs can be configured as two-electrode or three-electrode systems, depending on the utilized transduction technique. For example, in electrochemical impedance sensing, two-electrode based IDE systems attain faster steady-state current response to enable easier measurements (Cohen and Kunz, 2000; Iwasaki and Morita, 1995; Morita et al., 1997; Shalev et al., 2013; Yao and Zhu, 2014). Electrochemical biosensors integrated in microfluidic systems have been explored for biomarker detection in order to decrease the probability of human error and the sample volume required. However, such

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systems have limited use at POC detection systems for targets beyond glucose and estrogen (Kim et al., 2012). The area of miniaturized electronics integrated with electrochemical biosensors has great significance for in-field and on-site diagnostics (Huey et al., 2012; Maurer et al., 2006; Park et al., 2002; Poh et al., 2010; Warren et al., 2002; Yao and Zhu, 2014). The junction of laboratory bio-sensing protocols and miniaturized electronics can be explored for mass production with great prospects of commercialization.

To explore a POC electrochemical sensor for a specific target requires an immobilization matrix compatible to the target analyte, and an electronic setup that provides control on the measurements and records, correlates, stores and displays data (Blanco et al., 2006; Huang et al., 2007; Steinberg and Lowe, 2004). The potentiostat is capable of controlling the electrical signals within the electrodes in two, three or four-electrode systems to allow the user to observe multiple electrochemical phenomena. Conventional potentiostats are capable of performing several electrochemical measurements such as chronoamperometry, impedance spectroscopy and CV. However, available potentiostats are costly, bulky, and not practical for POC applications.

In this paper, we present a low-cost and miniaturized potentiostat (M-P) capable of performing CV for electrochemical immunosensing of cortisol. An open source microcontroller unit, along with a micro-power electrochemical sensing integrated circuit has been configured to develop a M-P. This system is capable of performing three-electrode system based electrochemical measurements (Fig. 1) in which the potential is scanned in both positive and negative directions at predefined sweep rates (mV/s) for oxidation and reduction reactions (Blanco et al., 2006; Kwakye and Baeumner, 2007; Steinberg and Lowe, 2004). For cortisol sensing, an immunosensor is fabricated via immobilizing monoclonal Anti-M-Cab onto SAM modified IDEs (Arya et al., 2010a,b; Pasha et al., 2014; Vasudev et al., 2013).

Cortisol, a steroid hormone, is an important biomarker for psychological stress. Thus cortisol detection is gaining prominence for personalized health monitoring (Kaushik et al., 2014; Singh et al., 2014). The obtained sensing parameters using M-P are within the physiological range and are comparable to those obtained using conventional potentiostats.

2. Materials and methods

2.1. Materials

The LMP91000EVM and the BeagleBone microcontroller were purchased from Texas Instruments. Dithiobis (succinimidyl propionate) (DTSP) and sodium borohydride (NaBH4) were purchased from ThermoFisher Scientific. Anti-M-Cab 2330–4809 was procured from Sigma Aldrich. Phosphate buffered saline (PBS) tablets and hydrocortisone (cortisol) were purchased from Sigma Aldrich. All other chemicals were of analytical grade and were used without further purification. PBS solution (10 mM, pH 7.4) was prepared by dissolving 1 tablet in 200 mL of deionized water. Working solutions of hydrocortisone were prepared by dilution in PBS (10 mM, pH 7.4).

2.2. Electrochemical biosensor fabrication to detect cortisol

The interdigitated electrodes (IDEs) were fabricated on an oxidized 4 in. silicon wafer using conventional microfabrication process. First, the electrodes were patterned using UV photolithography. Next, Cr (20 nm) and Au (150 nm) were deposited using an E-beam evaporator. The microelectrode patterns were finally obtained on the silicon wafer by a liftoff process.

Phosphate buffer saline (PBS) solution (10 mM, pH 7.4) was used to prepare the Anti-M-Cab (1 mg/mL) and cortisol solutions. Prior to functionalization, the electrodes were immersed in freshly prepared piranha solution to clean the surface. The electrodes were next immersed in 2 mg/mL solution of DTSP in acetone for 2 h for SAM formation. DTSP was reduced using $NaBH_4$ (10 mg/mL in DI water). The DTSP/Au electrodes were then rinsed with acetone and water to remove any unbound DTSP molecules.

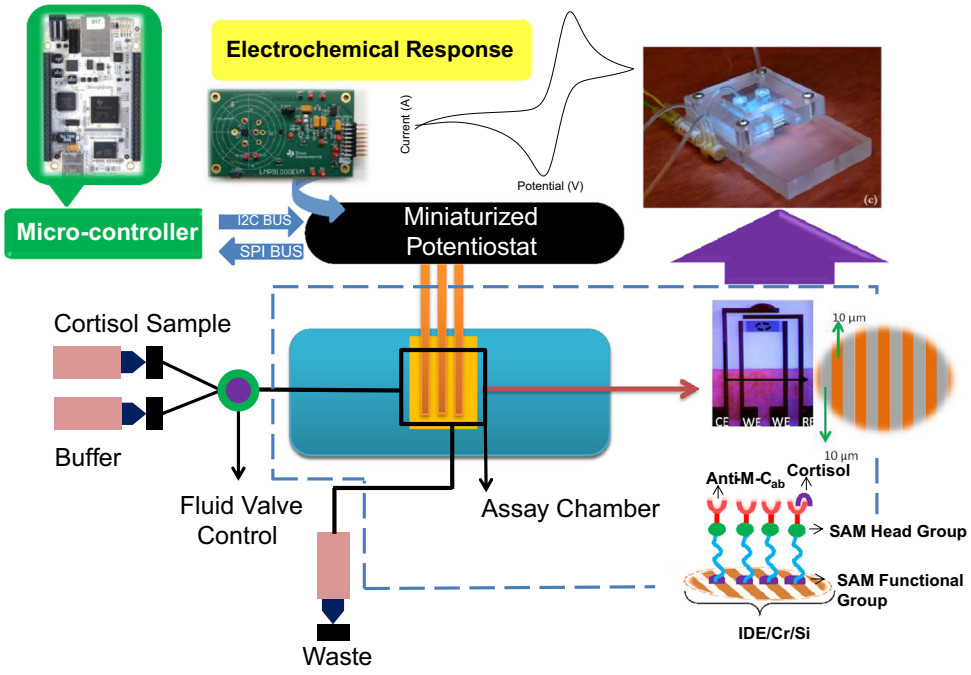

Fig. 1. The diagram above presents a high level representation of the cortisol detection device based on a miniaturized potentiostat (M-P). The system uses a DTSP-SAM based electrochemical cortisol immunosensor, which is integrated to the system through a LTCC microfluidic manifold. The electrochemical sensing is performed using the miniaturized potentiostat, which parameters are configured and controlled by the micro-controller unit.