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Lancet-free and label-free diagnostics of glucose in sweat using Zinc Oxide based flexible bioelectronics

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ABSTRACT

We demonstrate a wearable, flexible electrochemical biosensor for the combinatorial label-free detection of glucose in human sweat. The novel device comprises of stacked metal/metal-oxide (gold/zinc oxide) thin films within porous polyamide substrates for low-volume ultrasensitive impedance based detection of glucose and cortisol using non-faradaic electron-ionic charge transfer. In this work, we report the detection of glucose over a concentration range from 0.01–200 mg/dL spiked in synthetic and human sweat. Monoclonal antibodies specific to glucose oxidase were immobilized on thiolated ZnO sensing electrode surfaces resulting in the modulation of charge transfer within the electrical double layer (EDL). Non-Faradaic electrochemical impedance spectroscopy (EIS) was used to calibrate the sensor response with varying dose concentration through measurement of change in impedance. Reliable limit of detection (LOD) of 0.1 mg/dL in human sweat was demonstrated. Correlation of the sensor response with that of a commercial glucose meter TRUEresult™ Sensor (LOD of 20 mg/dL in blood) was found to be 0.9. Combinatorial detection of glucose and cortisol was demonstrated through frequency specific EIS measurements. Sensor variability was found to be within 15% of individual dynamic range for each molecule.

1. Introduction

Biosensors for consumer wearable devices is an exciting field as it facilitates multiplexed physiological monitoring for quantitative assessment of body functions. Highly functional wearable biosensors that can also provide meaningful diagnostics to guide therapeutics would be extremely valuable to end-user consumers or health-professionals [1,2]. In order to make wearable biosensors as successful consumer products it is important to demonstrate enhanced multiplexed functionality, reliability, and ease-of-use through non-invasive monitoring of body fluids [2]. There has been significant research focus on non-invasive body fluids such as sweat, saliva and urine. Sweat is the most widely evaluated body fluid as it contains plethora of medical information and is relatively easier to stimulate, gather, and analyze.

Glucose and cortisol are two valuable molecules that have physiological interconnection [3–6]. Management and control of Diabetes Mellitus requires tight and frequent monitoring of glucose levels daily. Thus glucose biosensors that primarily employ electrochemical-based methods of detection occupy 85% of the commercial handheld biosensors market [6]. Glucose is found in the concentration range of 0.1–50 mg/dL in human sweat [7]. There is currently good correlation established between blood or human serum and sweat glucose levels [8]. Hence, contamination free glucose detection monitored non-invasively from sweat can prove extremely beneficial to assess blood glucose levels. Cortisol is a glucocorticoid hormone that plays an important role in regulation of glucose metabolism. It enhances the expression of enzymes involved in glucogenesis, to maintain the glucose levels in plasma. More importantly, the direct correlation between glucose and cortisol levels in case of Type 2 diabetes patients has been well established [9]. Recent studies have shown that the disturbance in circadian rhythm (influenced by cortisol) may increase both fasting and postprandial plasma glucose concentrations through inadequate pancreatic insulin secretion [9]. Therefore, simultaneous monitoring of these molecules in combinatorial manner can be used to better predict the onset of diabetes and/or stress related symptoms as well as monitoring the diabetic conditions.

Current studies have shown simultaneous detection of multiple electrolytic ions such as Na, K, Cl and metabolites such as glucose and lactate [1,10,11]. Also, most of the techniques that display simultaneous detection are label-based. However, wearable non-invasive biosensors both in the commercial and research domain can primarily detect one protein, enzyme or metabolite

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biomolecule at a time and multiplexing is still one of the challenges in development of sweat based sensors [1,12–15]. Some examples include the tattoo-based biosensors for detecting lactate from sweat using voltammetric or amperometric techniques that require labeling with redox probes [14]; the GlucoWatch for detecting glucose levels using iontophoresis of transdermal interstitial fluid [16], which has been withdrawn from the market due to induced skin irritation problems in patients [17]; the electronic nose or odor system to detect glucose levels in synthetic sweat was formulated using 32 metal oxide semiconductors (MOS) sensors however with a low precision of 66% in the concentration range of 10–15 mg/dL and 40–70 mg/dL in different compositions of synthetic sweat [7]. Hence, the development of wearable non-invasive diagnostics tools, which are capable of quantifying more than one molecule with high precision in a multiplexed manner would be extremely beneficial for managing diseases, provide customized therapies, and reduce the total cost burden on the consumer [1].

The strategy that we have adopted to address these challenges is leveraging the direct charge-electron transfer route between the glucose molecule and the enzyme immobilized on the sensing electrode surface [18]. In this work, we have designed and fabricated 3-D nanostructured semiconducting ZnO sensing elements to establish optimal electron transfer efficacy between immobilized glucose and cortisol molecules and the electrode surfaces. ZnO has been successfully used as electrode material for enzyme immobilization [19] and glucose detection using primarily amperometric based sensing techniques. Study by Wang et al. [20] reported the use of ZnO nanocombs on gold electrodes functionalized using Nafion. The sensor demonstrated detection limit of 0.36 mg/dL glucose in phosphate buffer saline (PBS) buffer solution. Another study by Kong et al. [21] reported the amperometric enzymatic detection of glucose with the detection limit of 0.02 mg/dL in PBS using ZnO nanotubes immobilized with glucose oxidase (GOx). Pradhan et al. [22] demonstrated the growth of ZnO nanowires on gold plated polyster flexible substrate and enzymatic glucose detection with a sensitivity of 19.5 μA/mM cm^2 and detection limit of 0.9 mg/dL. However, all these studies focus on single glucose molecule detection and none of these studies has reported performance in human sweat that has interferents affecting the precision and stability of the sensor.

In our study, we leverage the findings of these researches to focus our efforts in addressing the key challenge in wearable sweat based glucose biosensing, i.e. the ZnO based electrochemical sensor performance in ultralow volumes of synthetic and human sweat. EIS was used to measure the sensor response to the variations in glucose concentrations spiked in synthetic sweat and in human sweat. Additionally, we demonstrate the combinatorial detection of glucose and cortisol in synthetic sweat with EIS by identifying specific frequencies associated with glucose and cortisol biosensing. The sensor performance in human sweat was compared to that with commercial glucose analyzer TRUEresult^{TM} meter [23].

2. Materials and methods

2.1. Sensor fabrication

Fig. 1A shows the sensor fabricated on a flexible nanoporous polyamide substrate with the electrodes and active region of ZnO. Gold measurement electrodes were deposited using shadow mask in e-beam cryo-evaporator. ZnO thin films were sputtered onto patterned in the area between the two gold electrodes to get maximum overlap using an AJA Orion RF magnetron with a 99.999% ZnO target (Kurt J. Lesker) at room temperature. The thickness of the film deposited was measured with Veeco Dektak 8 profilometer. Hall measurements were performed on ZnO deposited glass substrates using 8400 series HMS (Lake Shore Cryotronics, Inc. Carson, CA,

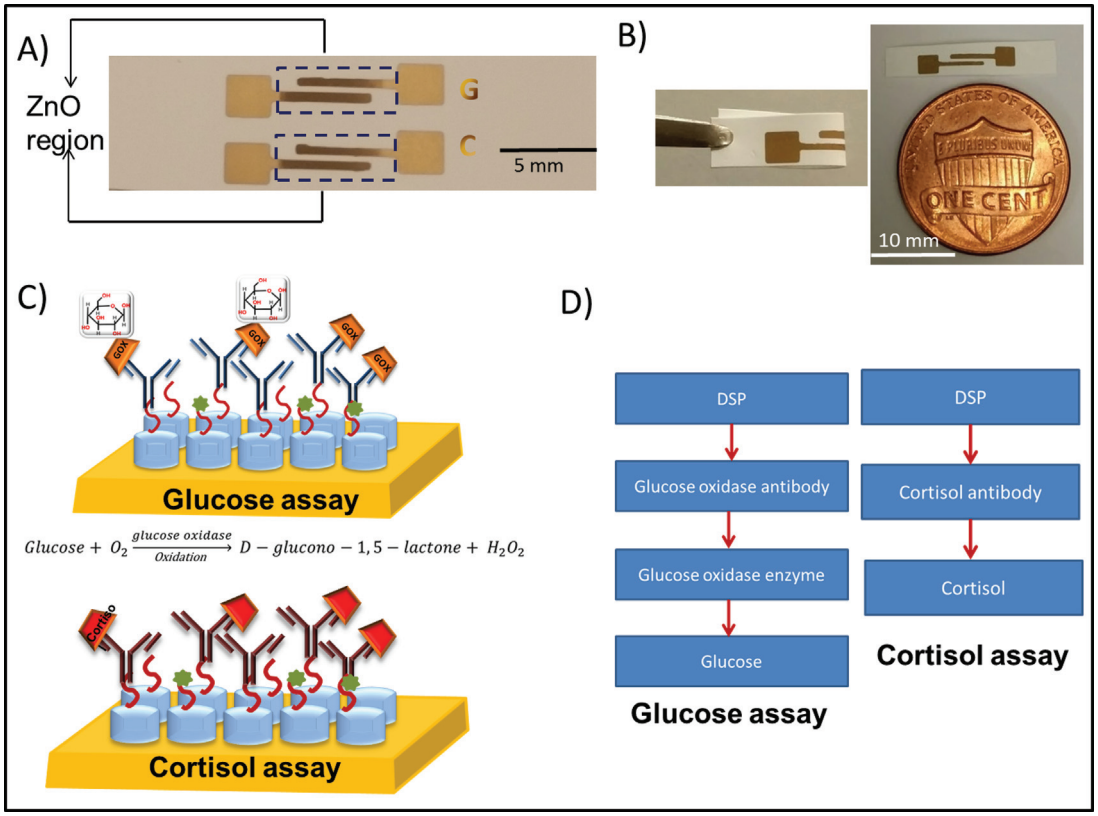

Fig. 1. Schematic of sensor setup and immunoassay A) Image of a sensor array deposited on polyamide substrate B) Flexible sensor and size comparison with a cent C) Schematic of immunoassay for glucose and cortisol detection D) Protocol used for glucose and cortisol detection.