IN-SITU SAMPLE PROCESSING AND ELECTROENZYMATIC SENSING FOR RELIABLE DEHYDROGENASE-BASED WEARABLE BIOMARKER MONITORING

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ABSTRACT

Dehydrogenase-based electroenzymatic sensors enable monitoring of a wide panel of clinically important biomarkers (*e.g.*, ketones) in a sample-to-answer manner. To adapt these sensors for non-invasive wearable biomarker monitoring, key challenges remain in terms of achieving low detection limit (due to the analyte dilution in non-invasively retrievable biofluids such as sweat) and mitigating the confounding effect of electroactive interferents. To overcome these challenges, we present an integrated bioanalytical microfluidic device, which features: 1) a sample processing module containing pre-deposited enzymes to efficiently consume electroactive interferents (here, ascorbic acid) within an introduced biofluid sample and 2) a carbon-nanotube-based electroanalysis module for highly sensitive dehydrogenase-based electroenzymatic sensing. We particularly adapted the *in-situ* bioanalytical capabilities of the device for reliable measurement of β-hydroxybutyrate (a major ketone body) at low concentrations. The experimental results support the suitability of our approach for wearable monitoring of β-hydroxybutyrate.

KEYWORDS

wearable biosensors, enzymatic, dehydrogenase-based sensing, in-situ sample processing, ketone

INTRODUCTION

Recent advances in wearable biosensors have presented new opportunities for personal health monitoring and transforming personalized medicine and healthcare. Through frequent harvesting of molecular level biomarker information from non-invasively retrievable biofluids (*e.g.*, sweat), wearable biosensors can track the individuals' dynamic physiological status, enabling timely, personalized, and actionable feedback for health management. In that regard, electroenzymatic sensors are especially useful as they can measure biomarker molecules in a sample-to-answer manner and with a high level of generalizability (*i.e.*, by swapping enzymes). In particular, nicotinamide adenine dinucleotide (NAD)-dependent dehydrogenase-based sensors can be exploited to target a wide panel of biomarkers. Owing to their quinone-based structure, these sensors can detect the reduced form of NAD (NADH)—as the end product of cascaded dehydrogenase-based reactions—and generate electrical signals that are proportional to the target biomarker concentrations.

However, deploying these sensors for non-invasive wearable monitoring of biomarkers is nontrivial. Firstly, they lack the required sensitivity to detect analytes that appear at diluted levels in non-invasively retrievable biofluids—for example, ketone molecules that get diluted by at least ~10× as they partition into sweat. Secondly, since these sensors rely on mediators for electrochemical analysis, their response is susceptible to the background interference stemming from endogenous electroactive species such as ascorbic acid (AA), rendering the measurements inaccurate.

Here, we overcome these challenges by devising an integrated bioanalytical microfluidic device, which features a sample processing module to eliminate the interferent species (specifically, AA) and a carbon-nanotube (CNT)-based electroanalysis module for highly sensitive dehydrogenase-based electroenzymatic sensing (Figure 1). To demonstrate the utility of the devised solution, the sensing interface was adapted to measure β-hydroxybutyrate (HB), which is an important biomarker of body's nutritional/metabolic status and its monitoring is critical for preventing fatal homeostatic disorders such as diabetic ketoacidosis [1].

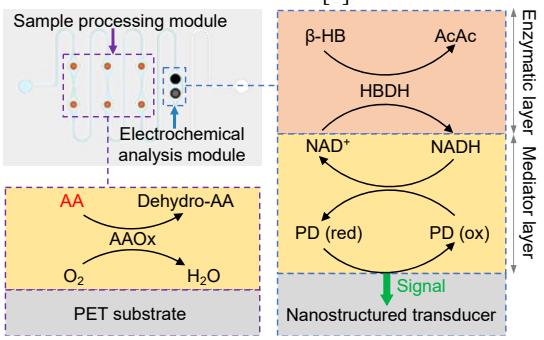

Figure 1: Schematic of the integrated bioanalytical microfluidic device and the corresponding sample processing and electrochemical sensing reactions taking place in the designated modules for reliable dehydrogenase-based sensing of HB. AAOx: ascorbate oxidase. AcAc: acetoacetic acid. HBDH: HB dehydrogenase. PD: 1,10-phenanthroline-5,6-dione.

RESULTS AND DISCUSSION

In our context, the sensitive and selective detection of NADH is the prerequisite for reliable dehydrogenase-based sensing, given that NADH is the measurable end product of the dehydrogenase-based reactions. We utilized 1,10-phenanthroline-5,6-dione (PD), as an electrocatalytic mediator for NADH oxidation, to lower the electrooxidation potential for NADH down to 0.1 V. Operation at this potential minimizes the background reactions stemming from the majority of endogenous electroactive interferents.

To enable fast electron transfer for PD-mediated NADH sensing, we utilized CNT for PD immobilization, since CNT renders high surface area and superior electrocatalytic performance. Accordingly, we fabricated the NADH sensing interface by drop-casting a CNT/PD solution (prepared with Nafion, as a binder) onto a gold electrode patterned flexible substrate (polyethylene terephthalate, PET). Figure 2a shows the response of the developed sensing interface to various NADH concentrations in a range from 10 to 50 μM with an estimated limit of detection of 0.22 μM.

We examined the selectivity of the developed NADH sensing interface, by testing its response toward 10 μM NADH spiked with a variety of common interferents present in biofluids (*e.g.*, ions, metabolites, endogenous electroactive species). As shown in Figure 2b, while most of the tested species had no influence on the NADH readout, the addition of a 10 μM AA generated a 2.5 times larger current response than the case with no interferents. The undesirable response of the NADH sensing interface to AA can be attributed to

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the fact that the incorporated PD can also mediate the oxidation of AA [2], illustrating the importance of mitigating the AA interference.

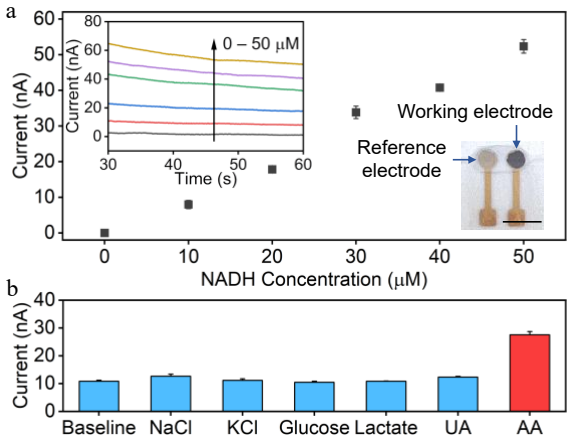

Figure 2: a) Calibration plot of the PD/CNT-based NADH sensing interface. Insets show representative chronoamperometric current responses to NADH for a NADH sensing interface, and an optical image of the NADH sensing interface. Scale bar indicates 5 mm. b) Selectivity study of the NADH sensing interface (all samples contain 10 μM NADH. NaCl: 10 mM; KCl: 2.4 mM; Glucose: 50 μM; Lactate: 5 mM; UA: uric acid, 59 μM). n = 3, error bars indicate standard error, testing buffer: 1× PBS.

We mitigated this interference by integrating a dedicated microfluidic sample processing module, which eliminates the AA in the introduced sample prior to dehydrogenase-based sensing. To this end, we utilized ascorbate oxidase (AAOx) and incorporated it within the microfluidic module (Figure 3a), since it can efficiently consume AA. To illustrate the module's AA consumption capability and its effectiveness for mitigating the AA influence on dehydrogenase-based sensing, we used the developed NADH sensing interface at the downstream of the module. Based on the sensor response and by referring to the sensor's pre-characterized NADH calibration plot, we estimated the NADH content of an introduced sample containing 10 μM NADH and 10 μM AA. We compared the results with that obtained from an unprocessed sample. As shown in Figure 3b, while the NADH sensing interface overestimated the NADH concentration by 119% for the unprocessed sample, it rendered an accurate NADH concentration estimation for the processed sample.

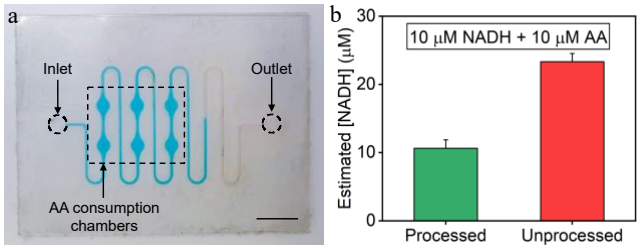

Figure 3: a) Optical image of the microfluidic module for in-situ sample processing. Scale bar indicates 1 cm. b) Estimation of the NADH concentration levels by the PD/CNT-based NADH sensing interface from the microfluidic structure processed and unprocessed samples containing 10 μM NADH and 10 μM AA. (n = 3, error bars indicate standard error, testing buffer: 1× PBS).

To demonstrate the utility of the devised solution for dehydrogenase-based sensing, we constructed a HB sensor based on the NADH sensing interface. Accordingly, we incorporated NAD^+ (cofactor) into the CNT/PD layer, then electrodeposited HB dehydrogenase (HBDH) onto the resultant sensing interface. In this way, the HBDH catalyzes the oxidation of HB and at the same time converts NAD^+ to electro-oxidizable NADH. Figure 4a shows the current response of the developed HB sensors within a concentration range of 50 to 400 μM (representing the clinically relevant concentration range of HB in sweat). To ensure the reliability of the HB sensor in complex biofluids, the sensor was challenged against a mixture of interfering species including KCl, NaCl, glucose, lactate, and UA. As shown in Figure 4b, the sensor had no response toward the interferents mixture. Negligible interference was also observed in the case of an *in-situ* processed sample containing AA (in contrast to the case of unprocessed AA sample). Collectively, the sensor- and device-level results indicate the suitability of the devised approach toward sensitive and reliable HB quantification for non-invasive wearable biomonitoring applications.

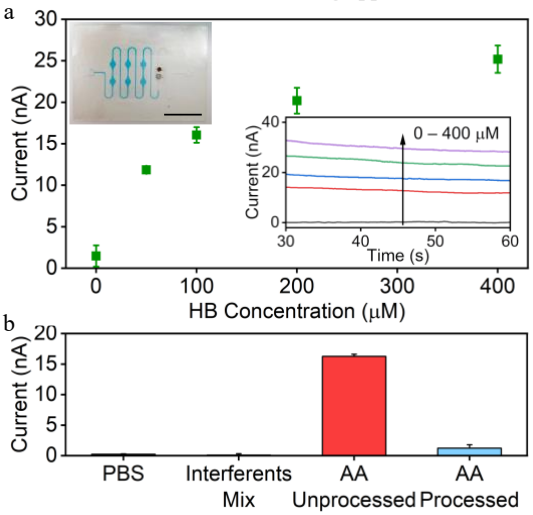

Figure 4: a) Calibration plot of the HB sensor. Insets show representative chronoamperometric current responses to HB for a HB sensor, and an optical image of the integrated bioanalytical microfluidic device. Scale bar indicates 2 cm. b) Selectivity study of the HB sensor. The interferents mix contains 10 mM NaCl, 2.4 mM KCl, 50 μM glucose, 5 mM lactate, and 59 μM UA. AA processed and unprocessed samples originally contain 10 μM AA. n = 3, error bars indicate standard error, testing buffer: 1× PBS.

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