Chemical and biological sensors

Detection of Lactate via Amperometric Sensors Modified With Direct Electron Transfer Enzyme Containing PEDOT:PSS and Hydrogel Inks

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Abstract—In this letter, we present amperometric sensors based on direct electron transfer (DET) enzyme for the detection of lactate, which is an important medical parameter present in blood and interstitial dermal fluid (ISF). For measurement in blood, we present a planar screen-printed biosensor with carbon working electrodes, whereas for the intended measurement in ISF, we investigated platinum-metallized epoxy microneedle sensors. On both sensor types, a bioink was applied, consisting of a DET enzyme mixed with poly(3,4-ethylenedioxythiophene) polystyrene sulfonate. As a second layer, a hydrogel layer is deposited to hold the enzyme on site. Local modification of the platinum microneedle sensors was performed by non-contact spotting. The developed modification enables the detection of lactate at a potential of 0 V with response times of 500–700 s. For carbon sensors, a limit of detection of 0.12 mM lactate was determined, and two linear ranges of 0.3–5 and 10–50 mM were observed with sensitivities of 319 and 9.6 nA/(mm^2·mM), respectively. For locally modified platinum microneedle sensors, two linear ranges of 0.3–2.5 and 5–30.5 mM were observed with sensitivities of 322.5 and 3.7 nA/(mm^2·mM), respectively. Given the low sensitivities in the higher concentration range, saturation for carbon sensors and locally modified platinum microneedle sensors starts at 10 and 5 mM lactate, respectively. Thus, both sensors allow sensitive measurements in the lower concentration range. Current densities at saturating lactate concentration are higher on freshly prepared carbon electrodes with 1.80 μA/mm^2 (10 mM) compared to platinum microneedle electrodes with 0.75 μA/mm^2 (10 mM) with full electrode modification. For platinum microneedle electrodes with optimized, dried local microneedle modification, a current density of 0.95 μA/mm^2 (5 mM) was measured. Detection of lactate in whole blood was demonstrated on carbon sensors, showing increasing currents after exercise, correlating with higher blood lactate levels, measured with a test strip reference system.

Index Terms—Chemical and biological sensors, microneedles, biofunctionalization, lactate sensing, non-contact spotting.

I. INTRODUCTION

L-lactate is an important marker in healthcare [1] and sports medicine [2], [3]. For sports medical purposes, a broad concentration range needs to be covered for lactate in blood, with levels at rest in the range of 0.5–1.5 mM and up to 25 mM under extreme exercise conditions [1], [3]. To allow for widespread use, mobile diagnostic devices suitable for blood lactate detection at the point-of-care are required. Electrochemical sensors [4] are well suited for this purpose, as they combine low production costs such as screen-printing with the possibility of small read-out devices. With the trend for non- or minimally invasive diagnostics, other body fluids are also of interest for lactate detection, such as interstitial dermal fluid (ISF), which can be accessed via microneedle technology [5], [6], [7], [8]. Early devices for lactate detection were based on lactate oxidase, requiring potentials of 0.7 V to detect generated hydrogen peroxide [5], [6]. With the development of direct electron transfer (DET) enzymes [9], [10], detection at significantly lower potentials can be performed, making the sensors more robust against electrochemical interferences.

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Amperometric sensors using DET lactate enzymes were reported in the literature [8], [11]. Freeman et al. [8] deposited DET lactate mixed with carbon inks on gold microneedles. Hiraka et al. [11] immobilized the DET-enzyme via polyethylene imine and polyethylene glycol (PEG) diglycidylether on planar MWCNT-modified gold electrodes. Detection of lactate was possible in these two reports at potentials of 0.2 V and 0.15 V versus AgCl, respectively. In both cases, a cellulose acetate film on top of the enzymatic layer was necessary to widen the linear range. Examples of DET lactate immobilization on environmentally more friendly platinum [12] electrodes were not found in the literature. To address the diagnostic needs for the detection of lactate in blood and ISF and reduce the environmental impact, we aim for two amperometric biosensors: 1) carbon screen-printed sensors for a fast single measurement of lactate in blood and 2) platinum-metallized microneedle-based sensors for minimally invasive continuous monitoring of lactate in ISF. Functionalization should be done with a DET enzyme poly(3,4-ethylenedioxythiophene) polystyrene sulfonate (PEDOT:PSS) bioink covered with a protective hydrogel layer [15], which should enable biocompatible measurements at low potentials [13], [14]. We also report on an up-scalable dispensing process method for local microneedle functionalization: a topic often neglected in biosensing publications.

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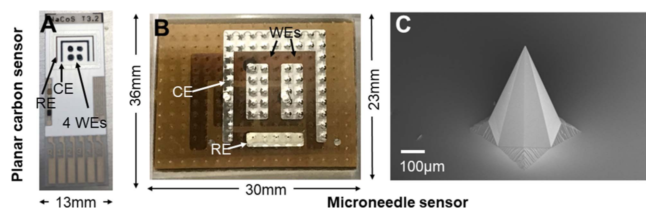

Fig. 1. Images of sensor types used in this work. (a) Screen-printed planar sensor. (b) Microneedle sensor with ten microneedles per WE. (c) SEM image of platinum metallized microneedle.

II. MATERIALS AND METHODS

A. Materials

Lactazyme (DET), a recombinant enzyme derived from the previously characterized DET enzyme fcb2 [9], [10], was provided by DirectSens GmbH as stock solutions in phosphate buffer and stored at −20 °C. Proof of DET properties of the used enzyme were previously reported by Freeman et al. [8]. PEDOT:PSS (1.1wt%, neutral pH, high conductivity), cysteamine hydrochloride, glycerol, PEG dimethacrylate (PEG-DMA) (10 kDa), diethyleneglycol vinylether (DEGVE), lithium-phenyl-2,4,6-trimethylbenzoylphosphinate (LAP), and hypochlorite solution were purchased from Sigma Aldrich. ARcare7759 was purchased from Adhesives Research. AgCl paste Loctite EDAG 7019 was purchased from Henkel.

B. Sensors

Fig. 1 shows the two sensor types used in this letter. Screen-printed planar sensors with carbon counter electrode (CE), Ag/AgCl reference electrode (RE), and four carbon working electrodes (WE) (area: 0.74 mm^2) were provided by SCIO Holding GmbH, Linz, Austria. Metallized epoxy microneedle sensors (height 500 μm) were provided by Tyndall National Institute, Cork, Ireland. Front-to-back connections ($<$10 Ω) were enabled by filling the via holes with AgCl paste from Henkel. WEs, CE, and backside connection areas were metallized with platinum. REs were metallized with silver and then chlorinated by pipetting a 15%(v/v) sodium hypochlorite aqueous solution on the silver layer using an incubation time of 10 s, followed by a water rinsing step for cleaning.

C. Ink Preparation

The DET-PEDOT:PSS ink was prepared by mixing PEDOT:PSS 16.1%(v/v), water 20.7%(v/v), and a DET solution in different concentrations (63.2%(v/v)) in low protein binding Eppendorf vials to obtain 1–7.2 mg/mL DET enzyme in the ink. The hydrogel precursor ink was prepared by dissolving PEG-DMA in water (166.7 mg/mL) and mixing 30%(v/v) of this solution with DEGVE (60%(v/v), water (8%(v/v)), and LAP solution (2%(v/v)). LAP solution was prepared by dissolving 10 mg LAP in a mixture of 50 μL water and 50 μL ethanol under ultrasonication. After deposition of the hydrogel precursor ink on the sensor surfaces, the sensors were placed in a UV crosslinker (UVP CL-1000), (365 nm) and irradiated with 1 J/cm^2. After cross-linking, the sensors were washed with physiological PBS for 5 min and again for 10 min with fresh PBS.

D. Sensor Preparation

For the screen-printed sensors, a volume of 0.5 μL DET-PEDOT:PSS ink was manually drop cast twice on each WE. After each application, the layers were dried. Then, 15 μL of the hydrogel precursor ink was pipetted on the whole WE array. In the case of the microneedle sensors, the platinum WEs were treated with cysteamine hydrochloride solution (104 mg in a mixture of 7.6 mL physiological PBS and 2.4 mL glycerol) for improved adhesion of the DET-PEDOT:PSS. The microneedle sensors were modified on the WEs either by pipetting or by non-contact spotting with a BioDOT AD1520 dispenser (ceramic tip, orifice 190 μm). For sensors modified by pipetting, 18.7 μL of DET-PEDOT:PSS were manually drop cast twice on each WE, letting the ink dry after each layer. Then, 20 μL hydrogel ink was applied on each WE and cured and washed as described. For BioDOT dispensing, either the full WE or only the microneedles were modified. In the case of the full WE modification, 18 μL of DET-PEDOT:PSS were dispensed twice on each WE, letting the ink dry after each layer. Then, 20 μL hydrogel precursor ink was dispensed on each WE, cured, and washed as described. For the local modification of the microneedle sensors, they were first passivated using an ARcare7759 passivation film [7]. The film had a regular hole pattern (x, y distance 1.75 mm, average hole diameter 764 μm) to leave the microneedles non-passivated. This hole pattern was fabricated with a laser cutter Speedy 360 from Trotec, Marchtrenk, Austria. For local modification, four layers of DET-PEDOT:PSS (4 × 150 nL per needle, with 10 min drying between the layers) were deposited on the WE microneedles. In a second step, the hydrogel precursor ink was dispensed on the WE microneedles and the microneedle row between (two layers of 2 × 250 nL per microneedle, in total 25 μL). After curing and washing the hydrogel layer with ultrapure water, the sensors were dried in a vacuum for 1 h and kept in a closed desiccator for a further 2 h. The sensors were then stored in aluminum bags with desiccant at 4 °C until measurement.

E. Electrochemical Lactate Measurement

The sensors were characterized by a lactate concentration series in physiological PBS. Chronoamperometric measurements in PBS and blood were performed at 0 V versus AgCl with the sensors connected to an Emstat3 4WE potentiostat from PalmSens. Measurements were performed at an interval of 0.5 s with the Multitrace 4.4 software. For the screen-printed sensors, measurements were started with 80 μL physiological PBS. After 500 s, the PBS buffer was replaced with lactate standards in 500 s intervals by carefully pipetting off the previous solution. For the microneedle sensors, measurements were started with 500 μL physiological PBS. After 700 s, the PBS buffer was replaced with lactate standards by careful pipetting off the previous solution. Current values were obtained by averaging the last 100 s before applying the next lactate standard. For measuring lactate in blood on screen-printed sensors, first, a calibration curve was generated by measuring lactate standards in physiological PBS (0.3, 1, 2.5, 5, 10, and 20 mM, one standard/sensor). To characterize the sensors, anonymized blood samples were collected at AIT-Molecular Diagnostics from finger pricks of volunteers, before and after light exercise. The collected blood was pipetted on dry sensors. Measurements were performed with 8 s equilibration time, 10 s run time, and averaging the last 5 s. For comparison, collected blood was also measured with the Roche Accutrend Plus lactate detection system.

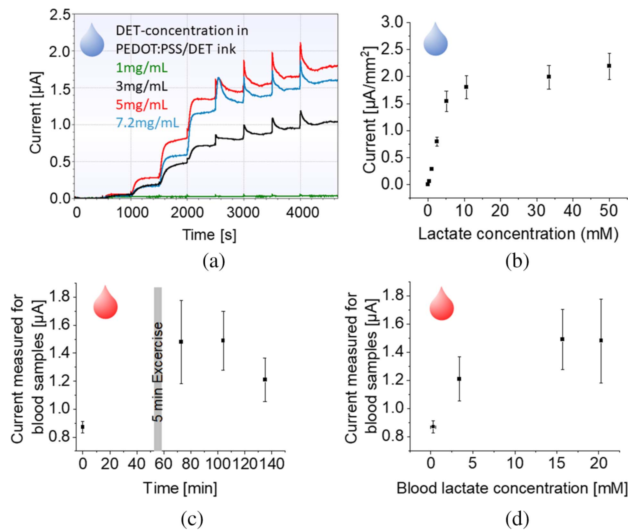

Fig. 2. (a) Chronoamperometry (0 V versus AgCl) with different DET concentrations and 0-50 mM lactate in PBS. (b) Averaged current values ($n = 4$) versus lactate concentration with 5 mg/mL DET. (c) Time evolution of averaged currents ($n = 2$) for measured blood samples before and after exercise. (d) Correlation of currents measured versus blood lactate concentrations detected with the Roche Accutrend Plus.

III. RESULTS

A. Screen-Printed Sensors: Ink Formulation and Optimization

In the first step, the DET-PEDOT:PSS ink was optimized by determining the highest enzyme concentration that still gave a stable ink formulation. Inks with more than 7.2 mg/mL DET enzyme started to precipitate very fast. Therefore, inks with 1, 3, 5, and 7.2 mg/mL were prepared and quickly screened by measuring lactate concentrations ranging from 0–50 mM on freshly prepared sensors with one WE/DET concentration [see Fig. 2(a)] at 0 V versus AgCl. The current curves for 3, 5, and 7.2 mg/mL of DET enzyme exhibited two quasi-linear ranges, the first from 0.3–5 mM reaching a maximum of ~2 μA/mm^2 and the second from 10–50 mM. Sufficient current densities with short equilibration times could be achieved with DET-PEDOT:PSS inks comprising ≥5 mg/mL of the DET enzyme, although sensors prepared with a DET enzyme concentration of 7.2 mg/mL performed slightly poorer than with 5 mg/mL. For sensors with 1 mg/mL DET concentration, only very low currents (50 mM gave 47.5 nA/mm^2) were measured. Due to the high currents of the 5 mg/mL ink and the instability of the 7.2 mg/mL ink (which started to precipitate a few minutes after mixing), the 5 mg/mL ink was chosen for further studies. Measurements with the 5 mg/mL ink averaging four WEs [see Fig. 2(b)] confirmed the quick screening results with the two quasi-linear ranges with sensitivities of 319 nA/(mm^2·mM) for 0.3–5 mM and 9.6 nA/(mm^2·mM) for 10–50 mM. Given the standard deviation in the higher concentration range, sensor saturation starts at 10 mM lactate. A limit of detection (LOD) of 0.12 mM was calculated. Measurements of 10 mM lactate on screen-printed sensors with and without hydrogel showed currents in the range of ~1 μA and 2 nA, respectively, proving that the hydrogel is crucial to keep the enzyme immobilized and prevent washout. The storability of the sensors was tested by measuring 10 mM lactate using freshly prepared sensors and using sensors that were dried and then stored for 0–8 weeks at 4 °C. After 1 week of storage, the current dropped to 46% of the current of freshly prepared sensors. A further decrease in current is seen over 4 weeks to 32%. After 4 weeks, the current stays constant for at least up to 8 weeks. The initial steep current drop was mainly caused by the drying process, which could be confirmed by measuring freshly prepared sensors before and after drying.

B. Measurement of Lactate in Blood

For measurements in blood, a quick measurement procedure was adopted. The sample liquid was applied to the dried sensors and the measurement was run for 10 s. First, a calibration curve was created by measuring a concentration series (six lactate standards, 0.3–20 mM) with one lactate standard per sensor. A saturation of the signal could be found for a lactate concentration around 5–10 mM. For one volunteer, currents on screen-printed sensors and blood lactate concentrations via test strips were measured with blood samples collected before and starting 15 min after 5 min of light exercise. Baseline levels of blood lactate were below the detection limit of the test strip system (<0.6 mM). Blood lactate levels up to 20.3 mM were measured after exercise. The current increased after exercise and then decreased, although not reaching the baseline level in the recorded time frame [see Fig. 2(c)]. A correlation between measured currents and blood lactate levels is observed; however, a distinction between blood lactate concentrations of 15 and 20 mM was not possible due to the lower sensitivity in the higher concentration range [see Fig. 2(d)]. Still, lactate measurements in whole blood could be demonstrated, showing the compatibility of the biofunctionalization with the complex body fluid, especially the developed hydrogel that prevents electrode blocking by cellular components in whole blood.

C. Microneedle Sensor Modification

In the first step, it was investigated if manually drop cast layers and layers applied with a BioDOT dispenser exhibited similar characteristics to ensure that the ink was not impacted by the deposition process. The measurement of lactate concentrations (0–80 mM lactate) showed a good match between the different processes. Currents for lactate concentrations of 10–80 mM were on average 15% higher for dispensed layers compared to manually drop cast layers. Current densities measured for 10 mM lactate on a freshly prepared microneedle sensor (WE area of 25 mm^2) were in the range of 0.75 μA/mm^2 and, thus, lower compared to current densities on screen-printed carbon sensors with 1.80 μA/mm^2. Since only the microneedles will enter the skin and the modification of the area between the microneedles is prone to interaction with sweat under in vivo conditions, which would result in interference currents, we investigated a local functionalization of the microneedle tips. To define the microneedle area for modification, the sensor was passivated with a biocompatible film as described in Section II-D. Four layers of 150 nL DET-PEDOT:PSS ink were dispensed in a 5 × 2 drop pattern on the needles of each of the two WEs per sensor. This resulted in a clean drop pattern, with the ink kept at the microneedle location with only low amounts of ink outside of the hole in the passivation film [see Fig. 3(a)]. For the hydrogel layer, 1000 nL of precursor ink were dispensed on each needle of the WE, resulting in a hydrogel film that covered the entire area and prevented a washout of the DET enzyme from the PEDOT-PSS matrix.

D. *In Vitro* Measurements of the Locally Functionalized Microneedle Sensor

After washing and drying, sensors were characterized by measuring lactate concentrations of 0–30.5 mM in PBS [see Fig. 3(b)]. A baseline stabilization for the platinum microneedle sensors was achieved in the

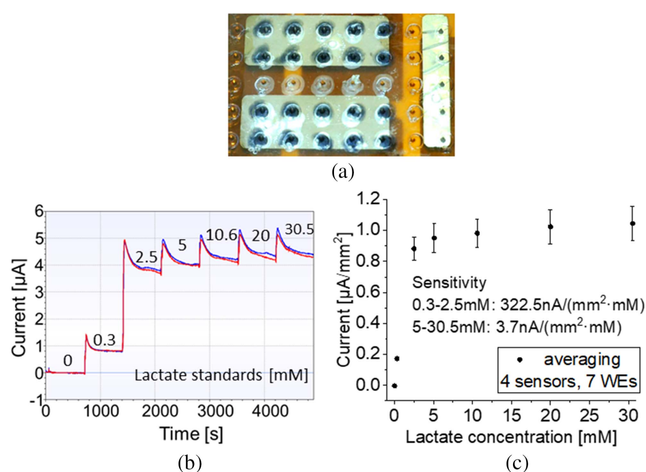

Fig. 3. (a) Passivated, locally functionalized microneedle sensor (ten microneedles/WE), WEs and RE shown, rotated 90°. (b) Chronoamperometric curves for a microneedle sensor with two WEs at 0 V versus AgCl. (c) Calibration curve averaging seven WEs from four sensors, 0–30.5 mM lactate in PBS.

range from 500 to 1200 s. The current stabilization was improved compared to the modification covering the full electrode area, especially for higher lactate concentrations, and could be performed within 700 s. Similar to the screen-printed sensors, two linear ranges were found with different sensitivities, the first ranging from 0.3 to 2.5 mM and the second ranging from 5 to 30.5 mM [see Fig. 3(c)]; however, given the low sensitivity and the standard deviation for this second range, saturation at 5 mM can be stated. Current densities for 5 mM are in the range of 0.95 μA/mm^2 for the local modification and thus higher than for sensors with full electrode modification at a concentration of 10 mM, which we attribute to the optimized hydrogel washing with ultrapure water instead of PBS. Averaging seven WEs measured on four sensors [see Fig. 3(c)] gave a calibration curve with coefficients of variations for the measurement signal of 0.075–0.107, showing good reproducibility of the dispensing process, which is a promising result for the intended *in vivo* measurements.

IV. CONCLUSION

Two amperometric enzyme sensors based on direct electron transfer for lactate detection were investigated. The modification of screen-printed sensors and microneedle sensors allowed lactate detection at even lower potentials than previously reported sensors using the same enzyme [8]. The results achieved on the microneedle sensors are promising for the realization of a wearable sensor patch for lactate monitoring.

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This work involved human subjects in its research. Anonymized blood samples were collected at AIT-Molecular Diagnostics from finger pricks of volunteers, having signed the informed consent. As stated by the ethical commission of the City of Vienna upon AIT's request, no specific ethical commission approval is necessary in such cases.

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