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PAPER

Organic electrochemical transistor incorporating an ionogel as a solid state electrolyte for lactate sensing

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The bulk of the currently available biosensing techniques often require complex liquid handling, and thus suffer from problems associated with leakage and contamination. We demonstrate the use of an organic electrochemical transistor for detection of lactate (an essential analyte in physiological measurements of athlete performance) by integration of a room temperature ionic liquid in a gel-format, as a solid-state electrolyte.

The detection of lactate (deprotonated form of lactic acid) in blood provides a biochemical indicator of anaerobic metabolism in patients with circulatory failure.[1] In addition to its presence in blood, lactate can be found in sweat (concentration range between 9 and 23 mM), reflecting, in an indirect way, eccrine gland metabolism.[2] It is well known that lactate concentration increases during physical exercise, making it a useful parameter to monitor wellness, physical fitness and the effects of exercise.[3] Detection in sweat offers a less invasive and dynamic way of measuring lactate concentration, particularly during exercise. Current methods of detection of lactate include fibre optics,[4] conducting polyaniline films,[5] carbon nanotubes,[6] screen printed Prussian blue electrodes,[7] and biosensors based on electrochemiluminescent detection.[8] Commercial lactate sensors are also available,[9] based on standard electrochemical methods. One example is the lactate SCOUT (Senslab), which, however, samples from blood, making real-time detection impractical. Therefore, the possibility of a fast, reliable, robust, miniaturised and cheap way of measuring lactate concentration in physiological fluids will open the way to lactate biosensors for health and sport applications. Flexibility plays an important role, here also, as biosensors for lactate sensing in sweat are in demand as wearable sensors, integrated for example on textiles.

Conducting polymers are interesting biosensing materials owing to their low-cost, mechanical flexibility, and ionic conductivity. Such materials have been exploited in the field of organic electronics to fabricate biosensors. One such device is the organic electrochemical transistor (OECT). OECTs have been utilized in a variety of biosensing applications such as the detection of metabolites,[10,11] ions,[12,13] neurotransmitters,[14] cells,[15] antibodies[16] and DNA.[17] The OECT was first described by White *et al.* in 1984.[18] OECTs are three terminal devices containing source and drain electrodes that measure the current across the conducting polymer film (the transistor channel), and a gate electrode. The channel and the gate electrode are in ionic contact *via* an electrolyte. The working mechanism of the OECT relies on changing the doping state of the conducting polymer channel by application of a positive potential at the gate electrode. Such potential forces cations from the electrolyte to penetrate into the channel and decreases the number of charge carriers (holes), consequently decreasing the channel current.[19] The vast majority of OECTs are based on poly(3,4-ethylenedioxythiophene) doped with poly(styrene sulfonate) (PEDOT:PSS), a commercially available polymer with high conductivity, which is also biocompatible.[20]

Room Temperature Ionic Liquids (RTILs) are low temperature molten salts that are entirely composed of cations and anions. Due to their unique properties such as large electrochemical stability window, high conductivity and thermal stability, ionic liquids have received increasing attention from the scientific community for applications in green chemistry[21] and electrochemistry,[22] among others. For instance, RTILs provide an attractive alternative to conventional organic solvents to solubilise and stabilise biomolecules such as enzymes and proteins.[21] There are three main strategies to solubilise biomolecules in RTILs: firstly by direct dispersion, secondly, through surface protein modification by PEGylation (covalent attachment of polyethylene glycol polymer chains to the protein) and thirdly by creating a hydrated RTIL.[22] The last method seems to be the most suitable for biosensors, because the addition of small amounts of water to ionic liquids strongly influences the protein solubility while retaining the properties of the selected ionic liquid. Fujita *et al.*[23] have demonstrated that certain proteins are, in fact, soluble, stable and remain active for up to 18 months in RTILs.

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We have previously integrated an OECT with a RTIL to make a glucose sensor, in which the glucose oxidase enzyme was dispersed in the ionic liquid.[24] In the current paper we report the development of a simple, yet robust biosensor that measures lactic acid, an important metabolite involved in several biological mechanisms. The novelty rests with the use of an ionogel which enables the development of a fully solid state yet flexible sensor, suitable for analysis of lactate in sweat. Ionogels are solid or gel-like polymeric materials that endow room temperature ionic liquids (RTILs) with structure and dimensional stability. Le Bideau *et al.*[25] summarised this new class of hybrid materials, in which the properties of the IL are hybridised with those of various components, which may be organic (low molecular weight gelator, (bio)polymer), inorganic (*e.g.* carbon nanotubes, silica, *etc.*) or hybrid organic–inorganic (*e.g.* polymer and inorganic fillers). These materials are thought to inherit all of the desirable RTIL properties whilst maintaining a gel-like structure. Here, we present the first step towards achieving a fast, flexible, miniaturised and cheap way of measuring lactate concentration in sweat through development of a biosensor based on an OECT that uses an ionogel as a solid-state electrolyte both to immobilise the enzyme and to serve as a supporting electrolyte.

The OECT fabrication started with the deposition of a 2 μm thick sacrificial parylene C layer on a glass wafer. This parylene layer was subsequently patterned by standard lithography followed by a dry etch using O_2 plasma, defining a contact mask for the PEDOT:PSS channel and gate electrode. A 200 nm thick PEDOT:PSS film was then spin-coated from dispersion (PH-500 from H. C. Stark) and annealed at 140 °C for 60 min. To improve the PEDOT:PSS conductivity, 5 ml of ethylene glycol and 50 μl of dodecyl benzene sulfonic acid (DBSA) were added per 20 ml of PEDOT:PSS dispersion. Additionally, 0.25 g of the cross-linker 3-glycidoxypropyltrimethoxysilane (GOPS) was added to the above dispersion to render the PEDOT:PSS film insoluble. Finally the parylene layer was peeled off mechanically to reveal the PEDOT:PSS channel and the gate electrode. A similar process was followed to make transistors on parylene: the glass wafer was coated with a 2 μm thick parylene film (which would become the OECT support), and was treated with a detergent in order to enable the peel-off of the sacrificial parylene layer.

The ionogel consists of two monomeric units: *N*-isopropylacrylamide (NIPAAm) and *N*,*N*′-methylene-bis(acrylamide) (MBAAm) in the molar ratio of 100 : 2, respectively (the chemical structure is shown in Fig. 1a). 1-Ethyl-3-methylimidazolium ethyl-sulfate ionic liquid, $[C_2mIm][EtSO_4]$ (Sigma Aldrich, used as received), was chosen because of its miscibility with water, thus avoiding mixing problems with the phosphate buffer solution (PBS) containing the analyte. The reaction mixture was prepared by dissolving a ferrocene mediator [bis(*n*-5-cyclopentandienyl) iron] (Fc, 10 mM) (Sigma Aldrich) in the IL and subsequently mixing the NIPAAm monomer, the crosslinker MBAAm and the photo-initiator dimethoxy-phenylacetophenone DMPA in 0.8 ml of $[C_2mIm][EtSO_4]$. A significant advantage was found in the solubility of the Fc in the ionic liquid, as Fc shows very poor solubility in aqueous solutions such as PBS. Although it is possible that Fc may not be suitable as a mediator in a wearable device due to toxicity concerns, this may be addressed by ensuring that it is covalently bound to the ionogel and thus will not leach out. Alternative redox mediators also exist and have been used for example for subcutaneous glucose sensors which are FDA approved.[26] The mixture was then sonicated at 45 °C for 10 minutes and a clear and monophasic solution was obtained. Additionally, stock solutions of 100 μM LOx (Sigma Aldrich) and 1 M lactic acid (Sigma Aldrich) were prepared, both in PBS.

By mixing the RTIL mixture and the PBS solution containing the LOx enzyme with a ratio of 4 : 1 (17% w/w of water) a clear liquid was obtained. The hydrated IL completely dissolved the protein and no precipitation was observed. 20 μl of the final solution was placed at the centre of the device where a polydimethylsiloxane (PDMS) well of a diameter of 8 mm was previously attached to avoid solution leakage after drop-casting. Then, the monomers were photo-polymerised within the ionic liquid matrix using a UV irradiation source (three LED arrays at wavelength 365 nm, UV light intensity ≈330 μW cm^{-2}) for 1 minute. It should be noted that UV exposure time was kept short to avoid denaturation of the protein.

Fig. 1b shows the layout of the planar OECT, consisting of two parallel stripes of PEDOT:PSS, with widths of 100 μm and 1 mm, serving as the gate electrode and channel of the OECT, respectively (it has been shown that for enzymatic sensing the area of the channel must be larger than the gate electrode[27]). The hydrated ionogel which contains the LOx enzyme, and the Fc mediator (schematic representation Fig. 1b), covers parts of the channel and the gate of the OECT, as defined by the well.

Measurements were carried out by applying −0.3 V across the channel while triggering the gate electrode at 0.4 V by 3 min long square pulses. Fig. 2a shows the modulation of the drain current before (t < 15 min) and after the introduction of 20 μl of a PBS solution with the desired lactate concentration. The introduction of the analyte is shown to lead to an increase in the modulation of the drain current, consistent with the mode of operation of OECT-based enzymatic sensors.[10] Fig. 3a depicts the series of reactions that take place upon introduction of the lactate. As lactic acid is oxidised to pyruvate, lactate oxidase is reduced and cycles back by the Fc/Ferricenium ion (Fc^+) couple, which carries electrons to the gate electrode. This leads to a decrease in the potential across the gate/electrolyte interface and a concomitant increase of the potential at the channel/electrolyte interface. As a result, more cations from the solution enter and dedope the channel (Fig. 3b) and the modulation of the drain current in response to a voltage pulse at the gate increases.

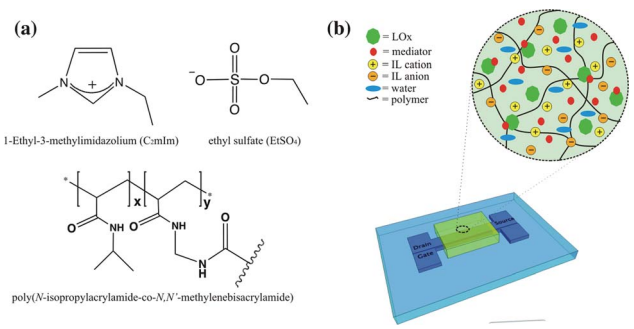

Fig. 1 (a) Ionogel components and (b) a schematic representation of the OECT device with ionogel/enzyme mixture.

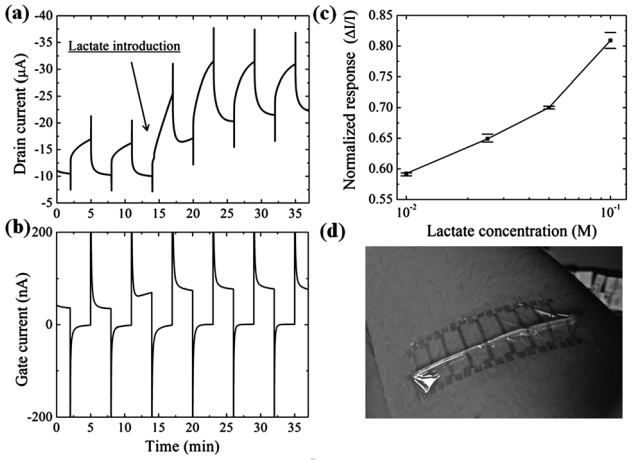

Fig. 2 (a) Drain current *vs.* time with addition of 25 mM lactate indicated by an arrow, (b) corresponding gate current *vs.* time, (c) normalized response of the OECT *vs.* lactate concentration and (d) conformal OECT with gel shown on a forearm.

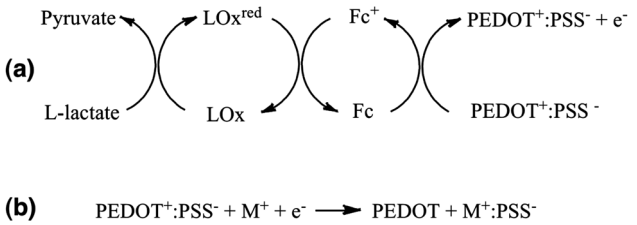

Fig. 3 Reactions at the gate electrode (a) and at the channel (b) of the OECT.

The modulation in the drain current is much larger than the gate current due to the inherent amplification characteristics of the OECT. This is demonstrated in Fig. 2b, where approximately 100 nA of current at the gate results in 11 μA modulation at the drain current. Fig. 2c shows the normalized response of the transistor ($\Delta I/I$) as a function of lactate concentration in the range 10–100 mM. The response of the transistor is defined as the difference in the modulation level of the drain channel during application of a gate voltage in the absence and presence of the analyte. The data shown represent the average of three measurements, and the error bars represent the standard deviation from the mean. The time required for the sensor to reach steady-state after addition of analyte is approximately 10 minutes. This is most likely due to the time it takes for the analyte to diffuse to the enzyme through the gel and may thus be improved by increasing the enzyme concentration, decreasing the gel thickness, *etc.* The data clearly show the detection of lactate in the relevant physiological range, covering the relevant range of lactate present in sweat, and suggesting the potential application of this device in the field of sport science as well as in healthcare. It should be noted that the sensor would also be compatible with the detection of lactate in blood (normal physiological range 0.3–1.3 mM, up to 25 mM during exercise).[28]

Physiological testing is an important tool for athletes and coaches to check the athlete's health and develop individualised training strategies. While laboratory testing may be increasingly widespread, there is a great demand for wearable sensors to be used in the field.[29] Today's wearable technologies are based on physical sensors, such as electrocardiograph (ECG) electrodes, thermistors and accelerometers.[30] These sensors respond to physical changes in their environment *e.g.* heat, movement and light. Wearable chemo-sensors, in contrast, have the potential to measure many more variables relating to the individual's well-being and safety. The integration of chemical sensors (such as lactate) into a textile substrate is a challenging task, as a chemical reaction must happen for these devices to generate a signal and the sensors must be robust, non-invasive, low-power and straightforward to use. The OECT sensor presented here is a step forward towards such devices. Fig. 2d shows a prototype of an array of these sensors (deposited on parylene) in a conformal configuration on a human forearm, demonstrating their wearability. The integration of this prototype with a wireless working platform, previously demonstrated for sweat analysis for non-invasive real time measurements,[29] is currently ongoing.

Conclusions

In summary, we demonstrate the detection of lactate in a relevant physiological range using an OECT sensor with an ionogel solid-state electrolyte. The significance of this work for sensing applications lies in the configuration of the sensor; we show for the first time a solid state electrolyte on a flexible transistor-based biosensor. This has implications for the wearability of the sensor and the storage of the sensor due to the enhanced stability of the enzyme in the ionogel. We envision the use of this sensor as a wearable bandage-type sensor, which can be worn during exercise or health monitoring, allowing sweat to diffuse into the sensor with consequent detection of the lactate analyte. This could also have application for the detection of other sweat components such as pH.

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