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

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The bulk of currently available biosensing techniques often require complex liquid handling, and thus suffer from problems associated with leaking and contamination. We demonstrate the use of an Organic Electrochemical Transistor (OECT) for detection of lactate (an essential analyte in physiological measurements of athlete performance) by integration of a RTIL in a gel-format, as a solid-state electrolyte.

The detection of lactate (deprotonated form of lactic acid) in blood is 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 to 23 mM), reflecting, in an indirect way, eccrine gland metabolism[2]. It is well known that lactate concentration increases during physical exercise, being 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 electro-chemiluminescent 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, 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 bio-sensing applications such as the detection of metabolites (glucose and lactate)[10], ions [11-12], neurotransmitters [13]and DNA [14]. The OECT was first described by White *et al* in 1984[15]. In general, OECTs are three terminal devices; source and drain electrodes that measure the current across the conducting polymer (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 the cations of the electrolyte to penetrate into the channel and drop the number of charge carriers (holes), consequently decreasing the channel current[16]. 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[17].

Room Temperature Ionic Liquids 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 their potential applications in green chemistry[18] and electrochemistry [19], among others. For instance, RTILs have been an attractive alternative to conventional organic solvents to solubilise and stabilise biomolecules such as enzymes and proteins[18]. There are three main strategies to solubilise biomolecules in ILs: 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 IL[19]. 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.*[20] have demonstrated that certain proteins are, in fact, soluble, stable and remain active for up to 18 months in certain RTILs.

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[21]. In this paper we report the development of a simple, yet robust biosensor that measures lactic acid, an important metabolite involved in several biological mechanisms. We also chose to take advantage of an ionogel to develop a fully solid state but 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.*[22] 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 favourable IL properties whilst maintaining a solid, semi-solid gel like structure. 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.

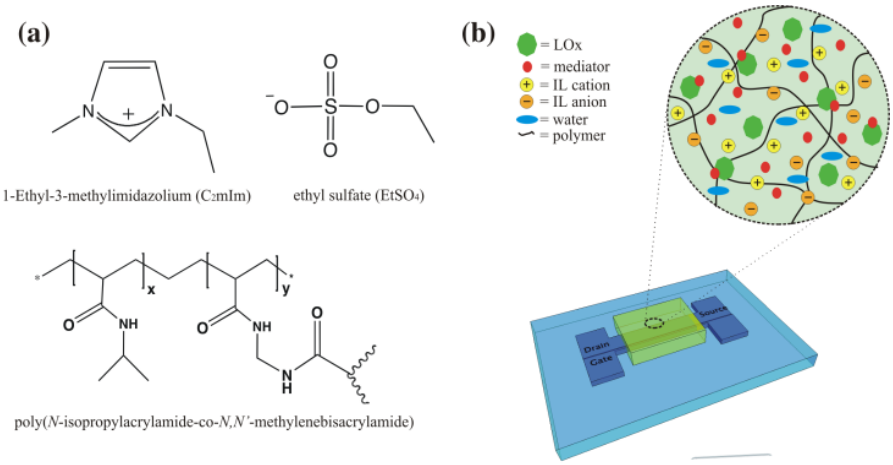

Fig 1: (a) Ionogel components and (b) a schematic representation of the OECT device with ionogel / enzyme mixture.

The OECT fabrication begins with deposition of a 2 µm thick Parylene C layer patterned with a standard lithographic process. This was followed by a dry etching process using O_2 plasma to define

the OECT pattern. The PEDOT:PSS was then spin coated from dispersion (PH-500 from H.C. Stark) and annealed at 140° C for 60 min to yield a 200 nm thick film. 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 PEDOT:PSS insoluble. Finally the parylene layer was peeled off mechanically to reveal the PEDOT:PSS channel and the gate electrode. 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. 1-a). 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 and the enzyme. The reaction mixture was prepared firstly by dissolving a ferrocene mediator [bis (n5-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 allowable as a mediator 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 [23]. The mixture was then sonicated at 45 °C for 10 minutes and a clear and monophasic solution was obtained. Additionally, a stock solution of 100 µM LOx (Sigma Aldrich) and 1 M of 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 array at wavelength 365 nm, UV light intensity ~ 330 μWcm^{-2}) for 1 minute. It should be noted that UV exposure time was kept short to avoid denaturation of the protein.

Figure 1-b 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 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 [24]). The hydrated ionogel which contains the LOx enzyme, water and the Fc mediator (schematic representation Fig. 1-b), covers parts of the channel and the gate of the OECT as defined by the well.

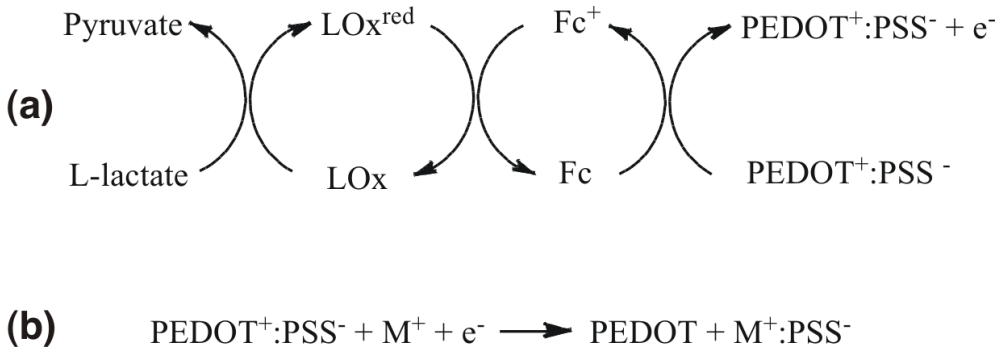

Fig 2: Reactions at the gate electrode (a) and at the channel (b) of the OECT

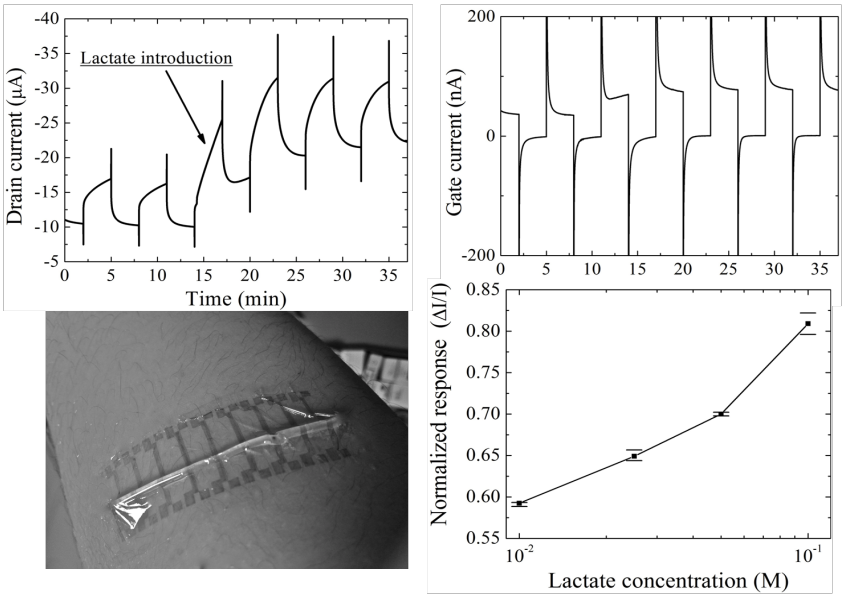

Fig 3: (a) Drain current vs. time (b) Corresponding Gate current vs. Time (c) Conformal OECT with gel shown on forearm (d) Normalized response of the OECT vs. lactate concentration

The experiment was carried out by applying -0.3V across the channel while triggering the gate electrode at 0.4V by a 3 min long square pulse, providing sufficient time for the lactate (when added) to diffuse into the ionogel. When the drain current of the system reached a steady state, 20 μL of a PBS solution with the desired lactate concentration was added. Figure 2-a depicts the series of reactions that take place upon introduction of the lactate analyte. Figure 3-a shows the drain current before (0s<t<900s) and after introduction of the analyte, with the characteristic decrease in the drain current upon gating when the enzymatic reaction takes place. 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. Simultaneously, the drain current dynamic increases in response to a pulse at the gate, due to more dedoping of the PEDOT: PSS, as a result of the redox cycles shown in Figure 2-b, where cations from the solution enter and dedope the channel. Such current variation is much larger than gate current owing to the in-built amplifier characteristics of OECTs. This is demonstrated in figure 3-b where approximately 100nA of current at the gate results in 11 μA modulation at the drain current.

Figure 3-d shows the normalized response of the transistor ($\Delta I/I$) as a function of Lactate concentration in the range 10 -100mM. 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 is the average of five data points, and the error bars represent the standard deviation from the mean. The data clearly shows the detection of lactate in the relevant physiological range, covering the entire range of lactate present in sweat, suggesting its application in the field of sport science as well as healthcare.

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 great demand for wearable sensors to be used in the field [25]. Today's wearable technologies are based on physical sensors, such as electrocardiograph (ECG) electrodes, thermistors and accelerometers [26]. 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 and lactate based Ionogel sensor leads the way to such devices. Figure 3c shows a prototype of the sensor in a conformal configuration on a human forearm, demonstrating the wearability of the sensor. The integration of this prototype with a wireless working platform, previously demonstrated for sweat analysis for non invasive real time measurements [25], 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 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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