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A Wearable Multisensing Patch for Continuous Sweat Monitoring

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Abstract

In sport, exercise and healthcare settings, there is a need for continuous, non-invasive monitoring of biomarkers to assess human performance, health and wellbeing. Here we report the development of a flexible microfluidic platform with fully integrated sensing for on-body testing of human sweat. The system can simultaneously and selectively measure metabolite (e.g. lactate) and electrolytes (e.g. pH, sodium) together with temperature sensing for internal calibration. The construction of the platform is designed such that continuous flow of sweat can pass through an array of flexible microneedle type of sensors (50 μm diameter) incorporated in a microfluidic channel. Potentiometric sodium ion sensors were developed using a polyvinyl chloride (PVC) functional membrane deposited on an electrochemically deposited internal layer of Poly(3,4-ethylenedioxythiophene) (PEDOT) polymer. The pH sensing layer is based on a highly sensitive membrane of iridium oxide (IrOx). The amperometric-based lactate sensor consists of doped enzymes deposited on top of a semipermeable copolymer membrane and outer polyurethane layers. Real-time data were collected from human subjects during cycle ergometry and treadmill running. A detailed comparison of sodium, lactate and cortisol from saliva is reported, demonstrating the potential of the multi-sensing platform for tracking these outcomes. In summary, a fully integrated

sensor for continuous, simultaneous and selective measurement of sweat metabolites, electrolytes and temperature was achieved using a flexible microfluidic platform. This system can also transmit information wirelessly for ease of collection and storage, with the potential for real-time data analytics.

Keywords: Flexible Patch; Microfluidic; Sensors; Exercise; Saliva

1. Introduction

One of the unique challenges in sport, exercise science and healthcare, is the need for continuous, non-invasive monitoring of biomarkers for assessing human performance, health and wellbeing (B. Lo 2011). For example, the monitoring of hydration status and other vital signs during sporting activities can provide a wealth of information regarding one's physiological capacity and efficiency under stress. Alternatively, it may assist in personalising programmes for optimal training gains and recovery. Biochemical markers, while being important for physiological and pathological characterisations, are typically measured with blood assays; however, these can be problematic due to the invasive nature of sample collection and associated risks, along with poor compliance rates among subjects where blood collection is undesirable and difficult.

Sweat has been recognised as an easily accessible bodily fluid that can provide important diagnostic information (Mena-Bravo and de Castro 2014; Raiszadeh et al. 2012). For example, certain genetic disorders (e.g. cystic fibrosis) can be diagnosed in infants from their sweat composition (Rock et al. 2014). Sweat also contains other proteins and metabolites linked to disease and infection (Mena-Bravo and de Castro 2014; Raiszadeh et al. 2012). Humans have two types of sweat glands; eccrine glands cover the entire body and produce sweat to regulate body temperature, whereas apocrine glands are mainly found in the armpits and have a limited

role in body cooling. Sweat is a clear, hypotonic and odourless fluid often described as an ultrafiltrate of plasma. It contains mainly ions such as sodium, potassium, calcium, magnesium, chloride and lactate. Sweat is easily accessible, with a typical sweat rate of human males measuring $0.85 \text{ mg cm}^{-2} \text{ min}^{-1}$ at the lower back (Patterson et al. 2000).

Several key biomarkers relevant to human health and performance can be assessed in human sweat. Sodium, for instance, is a marker for electrolyte imbalance and important for monitoring athletic performance in hot and humid environments, where sweat (and electrolyte) losses can impair physiological function. In healthcare, a significant loss of sodium in patients with cystic fibrosis (Mena-Bravo and de Castro 2014) can also cause hyponatremia. Sweat pH is another indicator of health and wellness. Lactate concentration in sweat can also indicate the energy pathways contributing to physical activity or general metabolic efficiency. A recent study reported a correlation between lactate levels in blood and sweat during exercise (Sakharov et al. 2010). This biomarker has additional potential for examining oxygen supply to tissue in diseased states, as a fall in oxygen delivery will produce a concomitant rise in anaerobic metabolism and thus, sweat lactate concentration. One such example is the testing of oxidative metabolism under pressure-induced ischemia (Arena and Sietsema 2011).

Electrochemical sensors present a promising technique for continuous monitoring of localised biological changes in sweat, due to inherent advantages of miniaturisation and non-invasive sample collection (Gao et al. 2016; Kim et al. 2015; Lisak et al. 2015; Matzeu et al. 2015). One difficulty faced when developing wearable biosensors is the need for physical contact and continuous analyte sampling. Unlike spot measurements, where large quantities of sweat are collected in a time-lapse manner, continuous monitoring requires the management of continuous sweat flow. In addition, this must be achieved under those dynamic environments (e.g. exercise, training) that active a sweat response. Previous biosensors have some limitations, as they can only monitor a single analyte and do not have on-site signal processing

circuitry and sensor calibration mechanisms (Bandodkar and Wang 2014; Jia et al. 2013; Rose et al. 2015). Recently, Gao et al. (2016) reported a fully integrated system that could simultaneously and selectively measure different analytes, as well as skin temperature for real-time sensor calibration. We hoped to extend this work by achieving simultaneous and selective measurements using several integrated sensors, plus temperature control for internal calibration, along with additional capabilities to allow a constant sweat flow for analysis and wireless data transmission to make the platform robust, wearable and easy to use.

In this paper, we present the development of a wearable electronic sensor, complete with microfluidic sampling and wireless readout electronics, which simultaneously measures the concentrations of hydrogen and sodium ions, as well as lactate in human sweat. Additional temperature and humidity sensors were used for internal calibration. The proposed platform is tailored for sweat monitoring during exercise of extended periods by incorporating paper microfluidic channels and reservoirs (Thuvo et al. 2014). Sweat enters the paper channel via a small window (10 x 10 mm) through capillary attraction. The small window size ensures that even a small amount of sweat is sufficient for detecting the parameters of interest. The natural liquid wicking properties of paper facilitates sweat sampling, circumventing the problem of sweat accumulation, which often leads to inaccurate acquisition during longer experimental periods (Gao et al. 2016). Sweat is continuously absorbed at the entry window of the smart microfluidic system while sweat collected at the exit ports (reservoirs) are evaporated. This provides a continuous flow through the sensor surface and avoids sweat accumulation.

2. Materials and Methods

This study was conducted as a two-step process. First, a microfluidic platform was designed to create a constant flow of sweat within a robust and flexible embodiment for the chemical transducers. Low power wireless electronics were also developed and successfully

integrated. The miniature electrochemical sensors were calibrated in the laboratory and checked against standard equipment. Second, the sensors were validated on human volunteers during running and cycling activities of increasing intensities. Saliva samples were also collected across exercise for tracking and comparing sodium, lactate and cortisol in this fluid, with an expected trend towards elevated biomarker levels with exercise intensity.

2.1. Microfluidic patch design

Several in vitro experiments were conducted with a novel thin flexible wearable patch that incorporates a paper microfluidic channel, with embedded flexible microneedle-based sensors connected with a wireless potentiostat. The advantage of using a microfluidic system is the possibility of using micro volumes of sweat for analyte testing. The fluid system is simple and the size can be modified and tuned depending on the attachment site on the body. An integrated sensing system inside the microfluidic channel also gives the advantage of minimum delay in sampling and subsequent analysis of the sample fluid.

The microfluidic patch consists of layers of polymer, paper microfluidics, and flexible sensors. A CO₂ laser fabrication system (Model VLS 6.6, Universal Laser System) was used to cut out structures on the various polymer layers, which includes a 80 µm thick layer of pressure sensitive adhesive (PSA) (layer 2 from the bottom, Figure 1) that was laminated onto a layer of 50 µm thick Poly (methyl methacrylate) (PMMA) (layer 1 and 4 from the bottom, Figure 1). The sensors were placed inside the microfluidic channel (Figure 1) which draws a constant flow of fresh sweat passing through them. The continuous delivery of fresh sweat towards the biosensing area is essential for the correct operation of the biosensors during prolonged periods of exercise. However, the use of conventional or miniaturised pumping system (e.g. peristaltic pumps) is not practical for wearable devices. Therefore, a hybrid microfluidic device was produced by simply incorporating paper structures with specific

absorption rates inside the microchannel of the platform. The use of paper channels ensures fresh sweat from the skin surface are driven through the microfluidic channel by capillary action towards the electrochemical sensors for measurements. Flow control was achieved by incorporating two different grades of paper; Whatman 4 is a fast absorbing paper while Whatman grade 113 is a high capacity absorbing paper. The Whatman 4 was employed in the straight section of the microfluidic platform (see Figure 1). Conversely, four round pads of the Whatman 113 were located at the terminal points of the channel to collect the analysed sweat and to drive more fresh sweat from the skin through the microchannel. The initial time for the sweat to cover all three sensors is estimated to be 15 μ l (Fu et al. 2011).

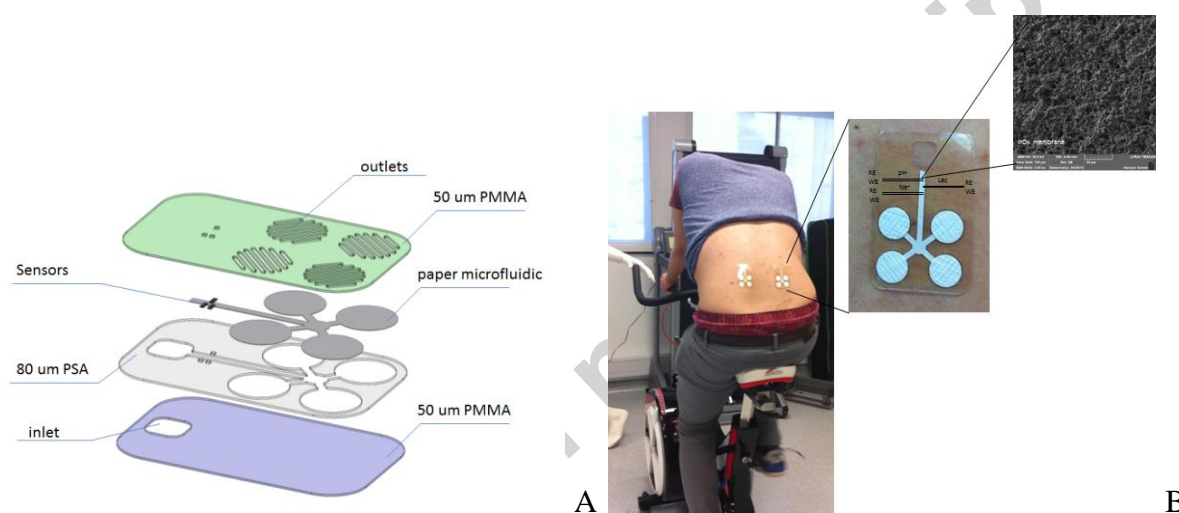


Figure 1. (A) Schematic representation of the fabrication steps of the micro-fluidic chip; (B) photo of the platform attached to the body and scanning electron microscopy (SEM) image (magnification 2.00 kx) photo of IrOx pH sensor membrane on top of a 50 μ m Pt wire.

To enhance subject comfort during the exercise experiments, the overall thickness of the microfluidic patch was limited to 180 μ m and a layer of stick-to-skin® double-sided adhesive were used to ensure the device is completely adherent to the skin when exercising. No delamination of the device was observed during the physical exercise of the subjects, while fresh sweat is sampled through the inlet port of the patch (Figure 1). The small transmission circuit is connected with all the sensors on the back of the platform.

2.2. Sensor design and fabrication

Platinum and silver wires (diameter 50 μm , Goodfellow Corp.) were used in the fabrication process. The electrode surface was polished sequentially using 1, 0.3, and 0.05 μm aluminium oxide films. The polished electrodes were cleaned by ultrasonication for 2 min in acetone, ethanol, and water and dried at 40 $^{\circ}\text{C}$ for 30 minutes. Cyclic voltammetry, including electrode cleaning and electropolymerisation, was performed in electrode mode using a commercial Ag/AgCl reference electrode and a platinum counter in N_2 purged solution. For validation, all of the electrochemical measurements were made with a PalmSens potentiostat (Palm Instruments BV, Netherlands) and Ivium potentiostat (Ivium Technologies, Netherlands) interfaced with a personal computer with electrochemistry software (PS Trace and Ivium).

Double-distilled (DI) water was used for the preparation of all solutions. Iridium oxide pH electrodes were achieved through electrochemical oxidation during potential cycling as described (Yamanaka 1989). Potentiometric sodium ion selective electrode (ISE) membranes were prepared using 250 mg 2-Nitrophenyl octylether, 125 mg PVC, 6.5 $\text{mmol}\cdot\text{kg}^{-1}$ 4-*tert*-Butylcalix [4] arenetetraacetic acid tetraethyl ester (Sigma420484) and 2.7 $\text{mmol}\cdot\text{kg}^{-1}$ potassium tetrakis(4-chlorophenyl) borate dissolved in dry tetrahydrofuran (THF) and evaporated slowly (Morris et al., 2008). This solution was drop-casted layer by layer using 20 μl of Na^+ selective membrane on the top of Pt/PEDOT electrode gasket. PEDOT was electrochemically deposited from 3,4 ethylenedioxythiophene (EDOT) monomer through potentiostatic deposition applying a constant potential of 1.0 V vs Ag/AgCl reference electrode for 714 s. Reference electrode membranes for ion sensing were prepared in line with other work (Anastasova-Ivanova et al. 2010). A reference electrode for the lactate sensor was dipped into a potassium dichromate reference solution (BASi, US) for 3 s, and then into a solution of diluted 37% hydrochloric acid for 20 s, to remove the oxide layer from the

working and auxiliary electrodes. Cyclic voltammetry was used to assess the working electrode surface.

2.3. Enzymatic membrane transducer

Lactate oxidase from *Pediococcus*, lyophilized powder, and all common chemicals were obtained from Sigma (UK). Polyurethane membrane was based on a proprietary prepolymer Trixene SC7602 (Baxenden Chemicals Ltd, Accrington, UK). The cleaned electrode was drop-casted with an enzyme crosslinked to bovine serum albumin using glutaraldehyde. To achieve high selectivity for lactate, an internal copolymer of sulphonated polyester ether sulphone – polyether sulphone (SPEES/PES) membrane is used as internal layer of the electrode. External 10% polyurethane coatings prepared in tetrahydrofuran were applied.

2.4. Participants

Six healthy males (aged 25-40 years) were recruited for this study. Pre-screening indicated that they were physically fit and healthy, with no known medical disorders or conditions that would either confound the study results or prevent them from exercising safely. Written consent was sought prior to commencement of the study and ethical approval was provided by the National Research Ethics Service, UK (Reference 10/H0808/124).

2.5. Saliva collection and analysis

Saliva samples (~1 mL) were taken across exercise using a passive drool method without stimulation. Samples were provided before, immediately after each exercise bout, and 10 minutes into the recovery period. Saliva was collected into plastic vials and stored at -80°C until assay. After thawing and centrifugation, the samples were analysed in duplicate for cortisol concentrations using a commercial immunoassay kit (Salimetrics, Europe). All

samples were analysed within a single assay plate, so no inter-assay variation is reported. Intra-assay CV's on duplicate samples are typically <5% for this assay.

2.6. Miniature wearable wireless instrumentation

For data acquisition, custom wireless sensing electronics based on the nRF51822 (Nordic semiconductors) IC with Blue-Tooth Smart wireless transmission were employed. Analogue sensor interfaces were designed with a LT1638 (Linear Technologies) operational amplifier for measurements from the lactate sensor and a LMP7721 (Texas instruments) amplifier for voltage measurements from the ionic sensors. The system developed also caters for impedance and temperature measurements when connected to a thermistor (Epcos B57891M0333K000). An Android application was developed to capture the data from the wearable sensors. The electronics were housed in a 3D printed case (Objet Eden). The overall size of the wearable package measures 12mm x 30mm x 30mm.

3. Results

We carried out independent validation of the pH sensor for sweat monitoring during the exercise testing of human subjects (Figure 2) against spot measurements taken with a commercial pH sensor (Hanna). Our results showed good agreement with the commercial sensor during a maximal run with a sustained speed of 11 km/h and an increase in inclination of 1.1 %, where a change between 5.3 to 6.8 pH units was observed (Figure 2A). Further testing was conducted during a treadmill running protocol, with the sensor pH measurements showing good agreement with the expected exercise-induced trends (Figure 2B), which aligns with other results reported in the literature (Bandodkar 2014).

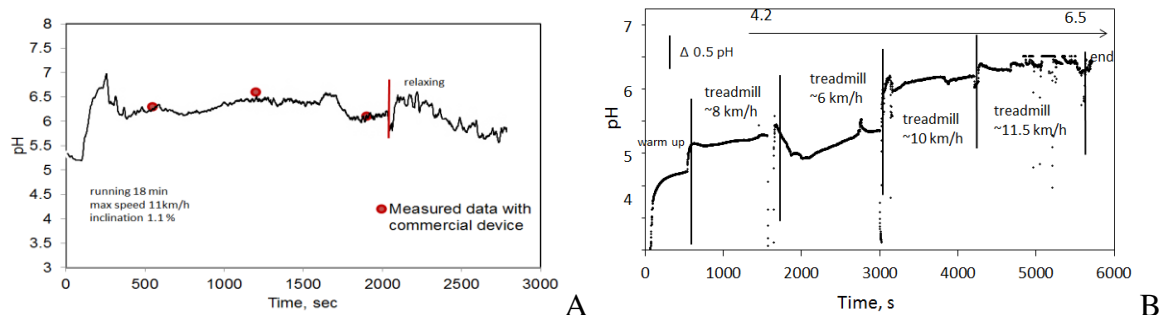


Figure 2. Changes of pH during running for 18 min at max speed of 11 km/h and inclination of 1.1 % on a treadmill. The red dots showed the measurements of commercial skin based pH Hanna meter (A); gradually increase of the running speed and real-time measurements done in second volunteer.

Subsequent cycling experiments were carried out with the volunteers wearing the custom-made sensor patch, where the changes in pH, sodium and lactate concentrations are given in Figure 3. The lactate concentration initially increased and then decreased, owing to the dilution effect caused by an increase in sweat rate (Figure 4B) (Gao et al. 2016). A maximum lactate value of 25 mM was achieved during cycling (Figure 4B), compared to 18 mM baseline level. During recovery, the level dropped to 20 mM and further still once the subject slowed down, which corresponds with prior literature (Gao et al. 2016). The sensor response time is rapid (~90 sec). Sweat (Matzeu et al.) increases at the onset of perspiration (Figure 3A), a result shown in ex situ using collected sweat samples (Patterson et al. 2000) and in other human studies (Matzeu et al. 2016). The measured sodium concentration varied during exercise and reached a level of 42 ± 1.2 mM (see Figure 3A). The pH measure showed a real-time response that varied from 5 to 6.5, but generally increased as the time of exercise increased.

During cycling exercise, followed by subsequent running on a treadmill, the flexible platform was placed against the skin in the lumbar region of the lower back. As shown in Figure 5C, the sensor readings are presented following different exercise phases from warming up to intense spinning, followed by intense running. The data from the remaining 4 volunteers are summarised in Table 1. These results showed that the concentration of lactate,

sodium, pH varied between the volunteers. In general, we noted a rise in the mean values of pH, sodium and lactate across each exercise over time, followed by a slight reduction during the recovery period. The reliability of these measures were acceptable for this study, with CV's ranging from 6% (Na), 7% (pH) and 9% (Lactate).

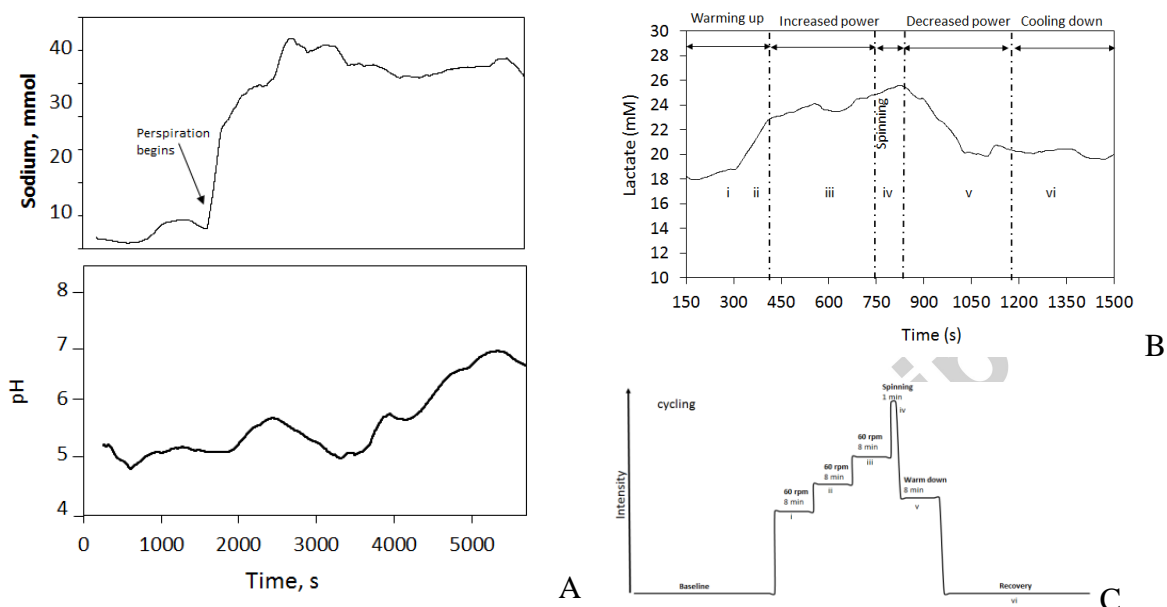


Figure 3. Previous experiments of real-time lactate and sodium traces measured wirelessly placed on the back of human body during exercise (A, B), on platform without paper microfluidics; Experimental protocol (C). Sweat levels were monitored during cycling at 4 levels of increasing rpm (i-v), a level of warming down (vi), and a final period of resting (vi). The wireless potentiostat used for this experiment consists of MSP430 and CC2420 (Texas instruments) wirelessly linked to a laptop, as detailed elsewhere (Bembnowicz et al. 2013).

Table 1. Measured average (from 4 volunteers) and standard deviation levels of pH, sodium, lactate during cycling and running.

	Running					Cycling					
	6	7	8	5	6	6	4	8	5	7	Recovery
	rpm	rpm	rpm	rpm	rpm	km/h	km/h	km/h	km/h	km/h	
pH	5.50	5.30	6.03	5.88	6.30	5.90	5.03	5.13	5.28	5.78	5.10
Sodium	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0
Lactate	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0

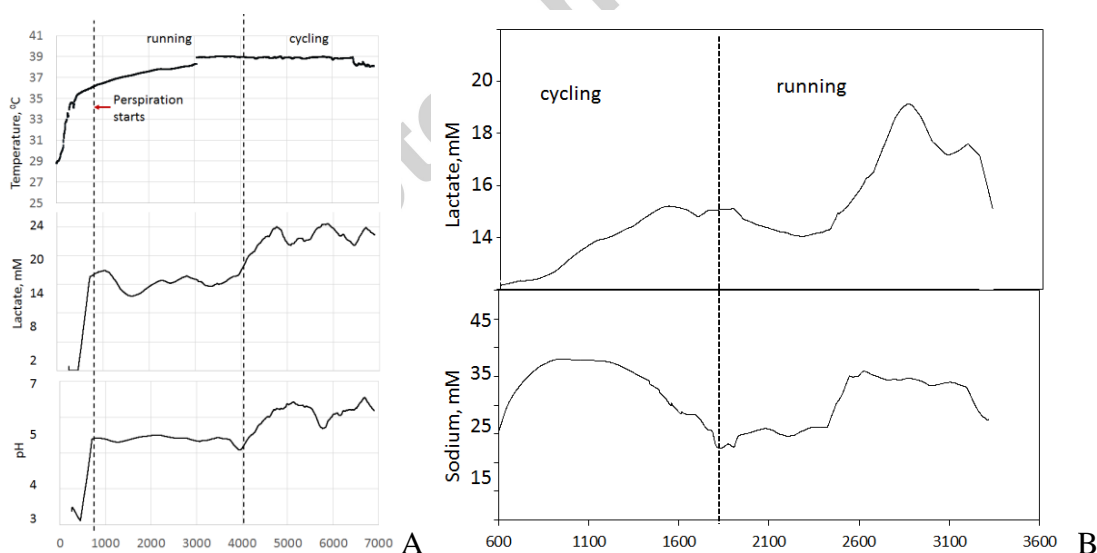
	D	36	.24	.59	.2	.42	48	13	.15	.45	.61	.48	76
					5								
					4								
N		4	4	4	4.	4	4	31	3	4	4	4	4
a		3.0	1.5	2.0	5	4.3	5.8	.3	8.3	0.0	4.3	3.0	1.8
					1								
		1	1	1	0.	1	1	7.	3	5	1	1	1
D		5.6	4.8	3.4	4	0.2	1.6	5	.9	.8	2.2	4.7	3.8
L					2								
acta		1	1	2	0.	2	1	16	1	1	1	1	1
te		8.8	8.5	0.0	3	1.8	9.8	.3	7.5	7.8	9.0	8.0	7.1
					1								
		3.	3	4	.7	2	2.	1.	3	4	4	2	2.
D		68	.11	.69	1	.87	99	71	.32	.50	.29	.45	04

An increase in muscle heat conductance to the skin causes an increase in skin temperature. To account for this, the sensors were placed inside the microfluidic channel and on the body where the sensors were kept in conditioning solutions before use. Depending on the individual, perspiration started during the initial 10-15 min of the warm up. Initially, the sweat on the skin is slightly acidic, but as exercise continues the sweat rate increases due to thermoregulation and, in this period, we observed a reduction in the reabsorption process (Magalhaes et al. 2010); therefore, sweat pH increases along with sodium concentration (Figure 4). Sodium levels increased with the increased intensity of the physical activity. The level went up to $35 \pm 1.5 \text{ mmol}\cdot\text{L}^{-1} \text{ Na}^+$ (Figure 7B) and dropped down to $15 \text{ mmol}\cdot\text{L}^{-1}$ during recovery. These results are consistent with previous studies (Matzeu et al. 2016). A correlation between lactate and sodium levels in sweat was also observed. Lactate concentration (Figure 4 A, B) increased with a higher level of exercise effort. Sweat lactate is a function from the eccrine gland energy metabolism. As a result, increasing exercise intensity leads to an increase production of sweat lactate (Bandodkar and Wang 2014).

The sensor output was stable during exercise testing and the results were reproducible with a maximum standard deviation of ± 0.215 for pH sensor and ± 0.312 for the sodium based on 6 measurements. The differences in sensor readings between volunteers are consistent with

expected variation due to factors such as sweat rates and fitness levels (Havenith and Vanmiddendorp 1990). Lactate concentrations also varied between subjects, which is probably related to differences in lactate excretion and sweat rates with increasing workloads (Buono et al. 2010). The stabilisation of our readings (Figure 4A) is likely to correspond to the stabilisation of the physiological responses (Derbyshire et al. 2012). Interestingly, further lactate elevations were observed (Figure 4A and 4B) during the next stage of experimental testing (completed consecutively) and again followed by a slow decline. In general, we noticed a rise in sweating as the cycling workload increased and this was followed by a rise in skin temperature, sweat lactate and sodium levels (Schabmueller et al. 2006).

Temperature readings were taken for re-calibration of the sensors as this affects enzyme activity in the amperometric (lactate) sensors, as well as (to a lesser extent) the potentiometric pH and sodium sensors. We recorded temperature changes between 20 to 40°C across the volunteers during exercise. This information was used to compensate for the sensor readings.



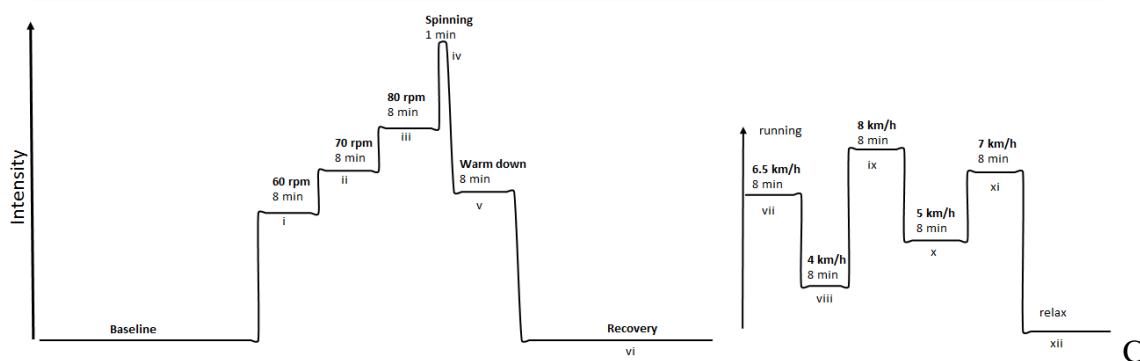


Figure 4. Changes in temperature, lactate, pH measurements (A) and lactate, sodium (B) levels during cycling and running experiment (C).

In addition to the sweat sensing devices, we tested salivary cortisol as a key indicator of physiological stress. Intuitively, this hormone should follow the sweat biomarker responses as they change with higher exercising intensities. It has been reported that the levels of sodium and lactate found in saliva are also correlated with blood measurements (Sonner et al. 2015). Figure 5 presents the spot measurements of salivary lactate, sodium and cortisol concentrations during exercise testing. As expected, lactate levels increased during and after exercise from baseline values (Figure 5 A), reflecting a rise in anaerobic metabolism across exercise. Sodium (Figure 6 B) followed the same pattern of change and for both measures the highest values were obtained during exercise itself. Compared to lactate and sodium measurements, salivary cortisol levels exhibited the largest variation across the subjects, suggesting that cortisol might be a more sensitive marker for subject-specific stress.

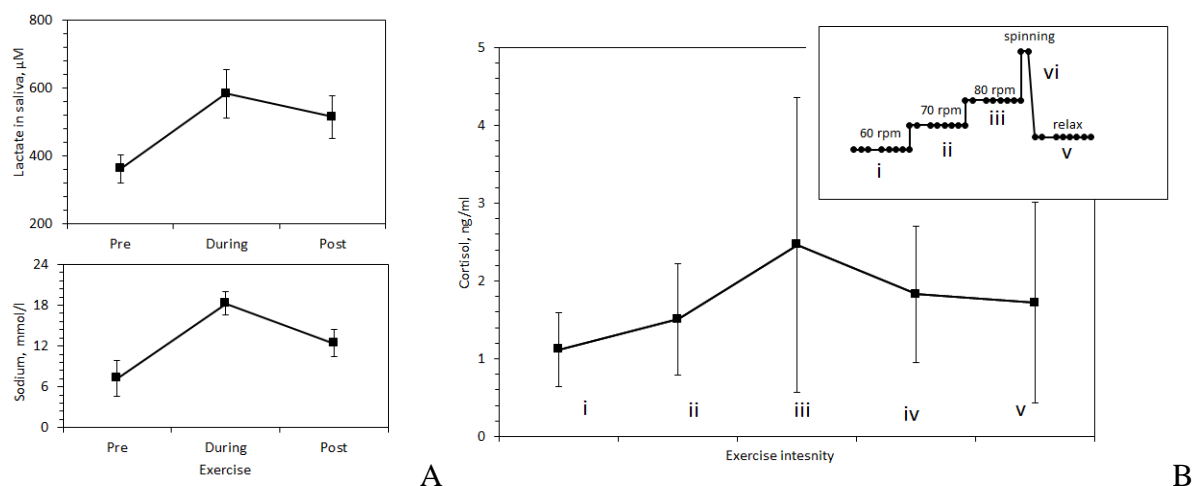


Figure 5. Exercise testing of salivary lactate (A) and sodium (B).

4. Discussion

This paper presents novel work on the development, validation and application of a flexible microfluidic platform with incorporated sensors for direct contact onto the human body, thereby allowing prolonged and continuous measurements of key analytes during exercise. To achieve platform delivery, several areas of specialisation were merged including human physiology, electrochemistry, material engineering, microfluidics, electronics and computer science.

Compared to the previously reported non-invasive sweat sensors (Jia et al. 2013; Rose et al. 2015; Schazmann et al. 2010), we developed a fully integrated system for continuous and simultaneous detection of several physiological parameters inside a passively-activated microfluidic system. A constant potential of +0.65 V (so that to detect hydrogen peroxide, a product of enzymatic reaction of lactate with lactate oxidase) was used for calibrating the lactate sensors, which was performed in vitro in phosphate-buffered saline solution. The linear range (up to 28 mM) covered the physiological values of interest. The internal SPEES/PES layer of the sensor is negatively charged, which makes it highly selective for targeted sensing. The outer biocompatible membrane protects the sensing layer and prevents enzyme leaching.

Dry storage of IrOx electrodes led to noticeable sensitivity decrease of typically 10–20%. Soaking these electrodes for 1 h in buffer solution restore their sensitivity to the original value (Ges et al. 2005). Na⁺ and pH sensors had relative standard deviations (R.S.D.) ~ 0.6 % and the lactate sensor has RSD of ~4 % in sensitivity.

Sensitivity of the pH sensor is 71.90 ± 0.8 mV/unit and for [Na⁺] is 56 ± 1 mV/unit. Long-term studies showed stability and repeatability of over 8 months for the pH sensor, over 5 weeks for the sodium sensor and over 4 weeks for the enzymatic sensor if kept properly (Modali et al. 2016). The signals from both types of sensors reach steady state within 10 s of starting any measurements. In this work, the sensors were tested both inside and outside the channels, but we observed no differences to suggest that cross-contamination or accumulation occurred. The lactate and sodium sensors also showed excellent selectivity in the presence of varying amounts of different analytes, such as 100 μ l glucose, 50 μ l uric acid, 50 μ l ascorbic acid, 10 mM NaCl for lactate sensor and 10 mM NaCl, 5 mM KCl, 5 mM NH₄Cl, 0.5 mM MgCl₂, 0.5 mM CaCl₂ for sodium sensors.

Overall, we have developed a flexible platform that can house several sensors simultaneously, with extended capabilities for wireless data collection. Real-time, multi-parameter analysis of sweat during exercise provides valuable information about the health and fitness level of an individual, as well as subject-specific responses. In addition, we have shown that the concurrent measurement of lactate, sodium and cortisol can be achieved non-invasively (across a relatively intense bout of exercise) with little or no discomfort to the subjects being tested. This type of monitoring overcomes many of the problems associated with blood sample collection, with much higher compliance and adherence rates among different populations (e.g. athletes, patients).

We do acknowledge some of the shortcomings of this study. For example, the small number of men tested to date using the wearable sensors and the relatively short duration of

exercise monitoring. To achieve a better understanding of the health and clinical meaning of the data collected, more studies are needed to examine individuals under conditions likely to promote both physiological and pathophysiological changes. Other directions for research include the deployment of more biomarkers inside the microfluidic platform and sensor validation in other non-physical environments associated with an increase in sweat production (e.g. stress induction and anxiety, hyperhidrosis, temperature and humidity changes).

5. Conclusions

A highly sensitive wearable system was developed for the continuous measurement of clinically important parameters in human sweat. This integrated system can also transmit information wirelessly for ease of collection and storage, with the potential for real-time data analytics. To advance this work, a large scale population-based exercise study using our wearable platform will be performed.

Acknowledgments

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References

- Anastasova-Ivanova, S., Mattinen, U., Radu, A., Bobacka, J., Lewenstam, A., Migdalski, J., Danielewskic, M., Diamond, D., 2010. *Sensors and Actuators B-Chemical* 146(1), 199-205.
- Arena, R., Sietsema, K.E., 2011. *Circulation* 123(6), 668-680.
- Lo B., B. Crewther, A.M. Spehar-Deleze, S. Anastasova, P. Conway, C. Cook, S. Drawer, P. Vadgama, and G-Z Yang, 2011. *Delivering London 2012: ICT Enabling the Games*, The IET special interest publication, 53-62.
- Bandodkar, A.J., Wang, J., 2014. *Trends in Biotechnology* 32(7), 363-371.

- Bandodkar, M.J., Molinnusa, D., Mirza, O., Guinovart, T., Windmiller R. J., Valdés-Ramírez, G., Andrade, J. Fr., Schöning, J. M., Wang, J., 2014. *Biosensors & Bioelectronics* 54, 603-609.
- Bembnowicz, P., Yang, G.Z., Anastasova, S., Spehar-Deleze, A.M., Vadgama, P., IEEE, 2013. *Buono, M.J., Lee, N.V.L., Miller, P.W., 2010. Journal of Physiological Sciences* 60(2), 103-107.
- Derbyshire, P.J., Barr, H., Davis, F., Higson, S.P.J., 2012. *Journal of Physiological Sciences* 62(6), 429-440.
- Fu, E.L., Ramsey, S., Kauffman, P., Lutz, B., Yager, P., 2011. *Microfluidics and Nanofluidics* 10(1), 29-35.
- Gao, W., Emaminejad, S., Nyein, H.Y.Y., Challa, S., Chen, K.V., Peck, A., Fahad, H.M., Ota, H., Shiraki, H., Kiriya, D., Lien, D.H., Brooks, G.A., Davis, R.W., Javey, A., 2016. *Nature* 529(7587), 509-+.
- Ges, I.A., Ivanov, B.L., Schaffer, D.K., Lima, E.A., Werdich, A.A., Baudenbacher, F.J., 2005. *Biosensors & Bioelectronics* 21(2), 248-256.
- Havenith, G., Vanmiddendorp, H., 1990. *European Journal of Applied Physiology and Occupational Physiology* 61(5-6), 419-427.
- Jia, W.Z., Bandodkar, A.J., Valdes-Ramirez, G., Windmiller, J.R., Yang, Z.J., Ramirez, J., Chan, G., Wang, J., 2013. *Analytical Chemistry* 85(14), 6553-6560.
- Kim, J., de Araujo, W.R., Samek, I.A., Bandodkar, A.J., Jia, W.Z., Brunetti, B., Paixao, T., Wang, J., 2015. *Electrochemistry Communications* 51, 41-45.
- Lisak, G., Arnebrant, T., Ruzgas, T., Bobacka, J., 2015. *Analytica Chimica Acta* 877, 71-79.
- Magalhaes, F.C., Passos, R.L.F., Fonseca, M.A., Oliveira, K.P.M., Ferreira, J.B., Martini, A.R.P., Lima, M.R.M., Guimaraes, J.B., Barauna, V.G., Silami-Garcia, E., Rodrigues, L.O.C., 2010. *Journal of Physiological Anthropology* 29(1), 1-12.
- Matzeu, G., Florea, L., Diamond, D., 2015. *Sensors and Actuators B-Chemical* 211, 403-418.
- Matzeu, G., O'Quigley, C., McNamara, E., Zuliani, C., Fay, C., Glennon, T., Diamond, D., 2016. *Analytical Methods* 8(1), 64-71.
- Mena-Bravo, A., de Castro, M.D.L., 2014. *Journal of Pharmaceutical and Biomedical Analysis* 90, 139-147.
- Modali, A., Vanjari, S.R.K., Dendukuri, D., 2016. *Electroanalysis* 28(6), 1276-1282.
- Patterson, M.J., Galloway, S.D.R., Nimmo, M.A., 2000. *Experimental Physiology* 85(6), 869-875.
- Raiszadeh, M.M., Ross, M.M., Russo, P.S., Schaepper, M.A., Zhou, W.D., Deng, J.H., Ng, D., Dickson, A., Dickson, C., Strom, M., Osorio, C., Soeprono, T., Wulfkuhle, J.D., Petricoin, E.F., Liotta, L.A., Kirsch, W.M., 2012. *Journal of Proteome Research* 11(4), 2127-2139.
- Rock, M.J., Makhölm, L., Eickhoff, J., 2014. *Journal of Cystic Fibrosis* 13(5), 520-527.
- Rose, D.P., Ratterman, M.E., Griffin, D.K., Hou, L.L., Kelley-Loughnane, N., Naik, R.R., Hagen, J.A., Papautsky, I., Heikenfeld, J.C., 2015. *Ieee Transactions on Biomedical Engineering* 62(6), 1457-1465.
- Sakharov, D.A., Shkurnikov, M.U., Vagin, M.Y., Yashina, E.I., Karyakin, A.A., Tonevitsky, A.G., 2010. *Bulletin of Experimental Biology and Medicine* 150(1), 83-85.
- Schabmueller, C.G.J., Loppow, D., Piechotta, G., Schutze, B., Albers, J., Hintsche, R., 2006. *Biosensors & Bioelectronics* 21(9), 1770-1776.
- Schazmann, B., Morris, D., Slater, C., Beirne, S., Fay, C., Reuveny, R., Moyna, N., Diamond, D., 2010. *Analytical Methods* 2(4), 342-348.
- Sonner, Z., Wilder, E., Heikenfeld, J., Kasting, G., Beyette, F., Swaile, D., Sherman, F., Joyce, J., Hagen, J., Kelley-Loughnane, N., Naik, R., 2015. *Biomicrofluidics* 9(3).
- Thuo, M.M., Martinez, R.V., Lan, W.J., Liu, X.Y., Barber, J., Atkinson, M.B.J., Bandarage, D., Bloch, J.F., Whitesides, G.M., 2014. *Chemistry of Materials* 26(14), 4230-4237.

Yamanaka, K., 1989. Japanese Journal of Applied Physics Part 1-Regular Papers Short Notes & Review Papers 28(4), 632-637.

Hihglights

- Novel results on the development of multisensing flexible platform.
- A wearable patch with incorporated microfluidic channel and fast responding sensors.
- Robust structure allowing an undisturbed sweat flow and huge implications for continuous monitoring.

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