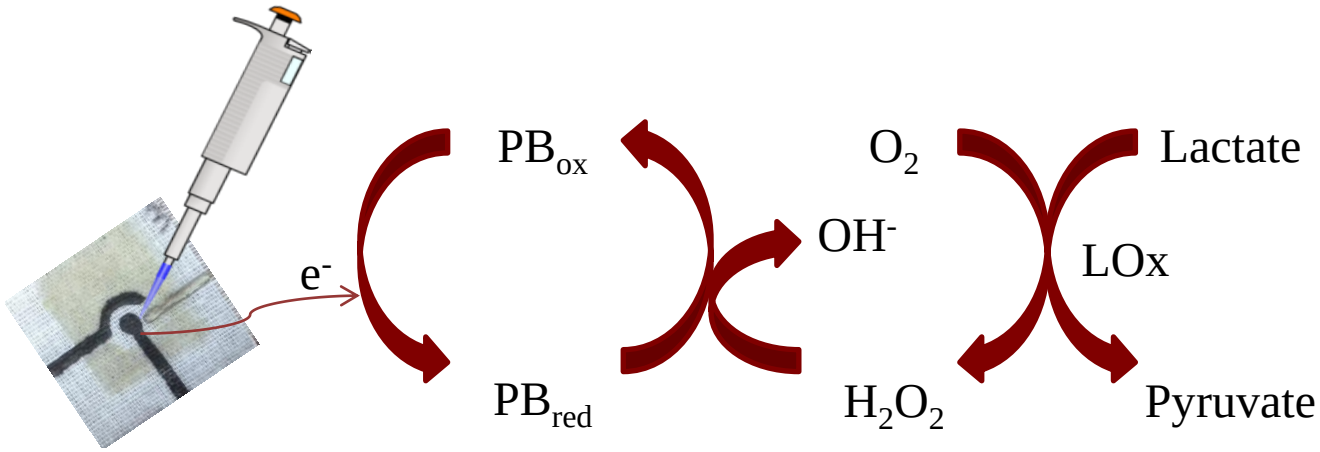
Analyst

Accepted Manuscript

ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/analyst



Journal Name

RSCPublishing

ARTICLE

Cotton Fabric-based Electrochemical Device for Lactate Measurement in Saliva

Cite this: DOI: 10.1039/xoxxoooooox

Radha S.P. Malon,[a] K.Y. Chua,[a] Dedy H.B. Wicaksono[a,b] and Emma P. Córcoles[a*]

Received ooth January 2012,
Accepted ooth January 2012

DOI: 10.1039/xoxxoooooox

www.rsc.org/

Lactate measurement is vital in clinical diagnostics especially among trauma and sepsis patients. Recent years have shown saliva samples to be an excellent applicable alternative for non-invasive measurement of lactate. In this study, we describe a method for the determination of lactate concentration in saliva samples by using a simple and low-cost cotton fabric-based electrochemical device (FED). The device was fabricated using template method for patterning the electrodes and wax-patterning technique for creating the sample placement/reaction zone. Lactate oxidase (LOx) enzyme was immobilised at the reaction zone using a simple entrapment method. The LOx enzymatic reaction product, hydrogen peroxide (H_2O_2) was measured using chronoamperometric measurements at the optimal detection potential (-0.2 V vs. Ag/AgCl), in which the device exhibited linear working range between 0.1 to 5 mM, sensitivity (slope) of 0.3169 μA mM^{-1}) and detection limit of 0.3 mM. The low detection limit and wide linear range were suitable to measure salivary lactate (SL) concentration, thus saliva samples obtained under fasting conditions and after meals were evaluated using the FED. The measured SL varied among subjects and increased after meals randomly. The proposed device provides a suitable analytical alternative for rapid and non-invasive determination of lactate in saliva samples. The device can also be adapted to a variety of other assays that requires simplicity, low-cost, portability and flexibility.

1. Introduction

When aerobic metabolism of glucose shifts to anaerobic metabolism due to the absence of oxygen (O_2), lactic acid is produced as a primary by-product. During high concentrations of lactic acid in blood, a significant drop in the blood pH occurs, which is a physiological condition known as lactic acidosis. Hence, it is important to monitor capillary blood lactate (CBL) concentration, especially among critical-care patients as lactic acidosis can lead to muscle damage that may result in heart attack.[1] It is also essential to measure CBL concentration among diabetics due to the close metabolic relationship between glucose and lactate.[1, 2] In addition, analysis of CBL concentration is of high interest in sports medicine for athletes to tailor their exercise training in order to optimise their performance.[3, 4]

Lactate can also be detected in saliva due to the passive diffusion of lactate from blood and secretion from salivary glands.[5] Since salivary lactate (SL) has a high correlation to CBL concentration; typically a 1: 4 saliva/blood ratio, SL is suitable for non-invasive CBL analysis[6] especially for critical-care patients, diabetics and athletes.[3, 7] The measurement of analytes in saliva is highly advantageous because it has a simple collection and storage method, minimal or no risk of cross-contamination among patients and prevents health care personnel from contracting infectious agents such as HIV and hepatitis. Furthermore, it is also useful for patients who require frequent clinical monitoring and those with difficulty in extracting blood samples such as haemophiliacs, neonates, elderly people and disabled people among others.[8-10] A test for routine SL analysis was developed by GLAD (Gesellschaft für labordiagnostische Entwicklung GmbH), in which saliva samples were collected by untrained personnel and sent to the laboratory for analysis. The interpretation of the results was provided in a physician's report. However, the test requires an expensive lactate analyser that additionally lacks automated results interpretation. Hence, there is a need for miniaturised SL

sensors that allow rapid, location independent and real-time measurement of SL concentration.[3]

Although lactate sensors have been extensively reported in literature, there are limited published results on the application of the sensors for lactate measurements in saliva.[1, 3, 7, 8] Palleschi *et al.*[7, 8] introduced a surface-type electrochemical biosensor that was manufactured on Plexiglas, in which the effects of meals and physical exercise towards SL concentration were studied, while Schabmueller *et al.*[3] fabricated a cavity-type electrochemical biosensor using silicon microfabrication technologies to continuously monitor SL concentration variations during physical exercise. This was followed by the development of a disposable optical biosensor, where the electrochemiluminescent properties of the device were used to measure SL concentration.[1] The feasibility of the aforementioned biosensors to monitor SL concentration evinced the potential of saliva as an attractive non-invasive analysis alternative for determining CBL concentration. However, the material and fabrication process involved in the development of these SL biosensors were relatively expensive, complicated and inappropriate for use in the developing world and in resource-limited settings.

In recent years, sensors and biosensors using textiles have gained attention due the fact that they are low-cost, lightweight, flexible and capable of withstanding various forms of mechanical strain and deformation. These properties make textile a favourable substrate especially in the field of wearable sensors.[11-13] Since electrochemical detection provides a versatile and quantitative detection method, Wang's group integrated carbon electrodes through direct screen-printing into briefs as a model garment. This was the first example of electrochemical sensing on textile substrate.[13] The electrochemical measurements were performed using the textile-based printed carbon electrode as working electrode (WE), while platinum wire and silver/silver chloride (Ag/AgCl) electrode were used as the counter (CE) and reference electrode (RE), respectively in a bulk solution. The printed textile electrode exhibited favourable electrochemical behaviour and mechanical or adhesion properties.[13] In a subsequent study, all three-electrodes were printed directly on textile, resulting in a textile-based screen-printed electrochemical sensor. The electrochemical measurements were performed by directly placing the sample on the printed electrode surface, where nitroaromatic explosives were detected.[11] However, the aforementioned studies[11-13] did not investigate the possibility of incorporating an enzyme layer within the textile-based electrochemical sensor for specific analyte detection. On the other hand, the use of cotton fabric as an immobilisation matrix using various modification techniques has been investigated[14-17], but its capability for electrochemical sensing in real samples has not been studied. This has led towards our interest to investigate the use of cotton fabric as the structural material for the fabrication of SL assay. We named this device as fabric-based electrochemical device (FED).

Generally, the common methods for patterning electrodes within electrochemical devices are metal deposition or thick-film technologies such as electroless deposition, evaporation, sputtering and screen printing as reviewed by Fiorini *et al.*[18] Nevertheless, there is still a need for simpler and inexpensive techniques that can be easily performed by novice personnel. To this end, we report the use of self-adhesive vinyl templates as a novel alternative for patterning electrodes. In order to highlight the merits of this new fabrication strategy, we have used the template method in a wide variety of rigid and flexible substrates, demonstrating its capability to address the limitations of screen-printing technology for patterning electrodes on non-planar and oversized substrates as raised by Windmiller e*t al.*[19]

In this study, a novel platform for constructing SL assay is presented. Template method was used for patterning the electrodes, while wax-patterning technique[20] that was first introduced by Whitesides group[21] was adapted for depositing hydrophobic walls of wax layer on the hydrophilic cotton fabric substrate to create the sample placement/reaction zone. The bio-recognition molecule, lactate oxidase (LOx) enzyme was immobilised via a simple matrix entrapment by the hierarchical structure of the cotton fabric. The increase of CBL concentration after meals and during physical exercise has been reported to be proportional to the increase in SL concentration.[3, 7, 8] Therefore, SL concentrations before and after meals were measured amperometrically using the fabricated lactate FED to demonstrate the capability of the device for lactate measurements in saliva.

2. Experimental section

2.1 Materials, chemicals and equipment

White plain weave cotton fabric was purchased from Jadi Batek Gallery, Kuala Lumpur. Anhydrous sodium carbonate (Na_2CO_3) (Sigma Aldrich, Malaysia) was purchased for scouring treatment of the cotton fabric. Self-adhesive vinyl papers and A4 papers were used to prepare the templates for electrodes patterning and sample placement/reaction zone, respectively. Local wax was obtained from Jadi Batek Gallery, Kuala Lumpur and candellila wax was purchased from Sigma Aldrich, Malaysia. Carbon graphite paste modified with Prussian Blue (C-PB) and silver/silver chloride (Ag/AgCl) paste were purchased from The Gwent Group, United Kingdom. The supporting electrolyte solution used was 0.1 M phosphate buffer solution (PBS) that was prepared using appropriate dilution of 1.0 M PBS (pH 7.4, 25°C) (Sigma Aldrich, Malaysia)[22] with ultrapure water (18 MΩ cm^{-1}) obtained from Millipore Milli-Q purification system. A stock solution of 0.1 M sodium L-lactate (Sigma Aldrich, Malaysia) was prepared in the supporting electrolyte and more dilute standards (0.1 - 25 mM) were prepared by appropriate dilution with 0.1 M PBS. 100 U lactate oxidase (LOx) enzyme from *Pediococcus species* (Sigma Aldrich, Malaysia) were diluted in 500 µL of 0.1 M PBS. After dissolution, aliquots of 100 µL (20 U) of the LOx enzyme solution were stored into 5 separate

Eppendorf tubes and kept in the freezer at -20 °C. For interference study, D-(+)-glucose, L-ascorbic acid and uric acid were purchased from Sigma Aldrich, Malaysia. Solutions containing lactate-to-interferent concentration ratio of 1:1 was then prepared in 0.1 M PBS. All chemical reagents were of analytical grade and used as received without further purification.

The equipment used in this experiment includes hot plate magnetic stirrer, Silhoutte digital craft cutter interfaced with Silhoutte Studio software (Silhoutte America, Inc), soldering iron and convection oven. The electrochemical measurements were carried out using µSTAT400 portable potentiostat with built-in DropView software (DropSens, Spain) for data handling and Igor Pro software was used for graphing and data analysis.

2.2 Design and fabrication of the device

First, scouring of the cotton fabrics were carried out by using the previously reported method[20] that utilizes anhydrous sodium carbonate (Na_2CO_3) treatment in order to produce a sufficiently hydrophilic cotton fabric. Briefly, 1 L of ultrapure water was boiled until it reached ~100 °C. 20 g of Na_2CO_3 were dissolved in the boiling ultrapure water and 10 pieces of cotton fabrics, cut into equivalent sizes (10 x 10 cm) were soaked in the boiling mixture for 10 min. After the treatment, the treated cotton fabrics were rinsed with plenty of ultrapure water until the pH reverted to neutral range (pH ~6 to 7). The fabrics were left to dry overnight at room temperature prior to use.

Next, all necessary electrodes for a three-electrode configuration system were integrated on the treated cotton fabric by using template method. The template for patterning electrodes was designed using AutoCAD 2010a software, in which the CE was designed with a substantially larger surface area compared to the WE and RE. The three-electrodes were designed with minimal distance from each other to allow optimal charge transfer within the device. The template was printed on self-adhesive vinyl paper using a digital craft cutter (Fig. 1(b)). The printed template was adhered on the cotton fabric surface (or onto other surfaces such as common lab supplies), then the template openings were filled with C-PB paste for the WE and CE, while Ag/AgCl paste was used for the RE (Fig. 1(c)). After removing the template, the cotton fabric was cured at 60°C for 30 min in the oven as recommended by the electrodes paste manufacturer (Fig. 1(d)).

The hydrophilic sample placement/reaction zone was patterned on the previously electrode-embedded cotton fabric using wax-patterning technique.[20] First, wax-impregnated papers were prepared by dipping 10 x 10 cm pieces of plain paper into a melted wax mixture (3:1) of local and candellila wax. Next, the template for the sample placement/reaction zone was designed using AutoCAD 2010a software and printed on the wax-impregnated paper (Fig. 1(e)). The wax-impregnated paper template was placed accordingly on the cotton fabric and the wax was transferred by heat treatment using a soldering iron at an operating temperature of 150°C similar to previous work[20] (Fig. 1(f)). When the wax on the printed wax-impregnated paper template melts, it spreads in both vertical and lateral directions within the cotton fabric. The vertical spreading creates a hydrophobic barrier across the thickness of the fabric, but careful attention needs to be given as lateral spreading of molten wax occurs rapidly resulting in wider hydrophobic barriers compared to the original printed pattern, consequently affecting the resolution of the design. The FED was ready to use after removing the template and allowing it to cool at room temperature (Fig. 1(g)). All the electrochemical measurements were performed after cutting the fabric into 15 x 15 mm strips, each containing the three-electrode set (Fig. 2(c)). The overall fabrication process of the FED is illustrated in Fig. 1.

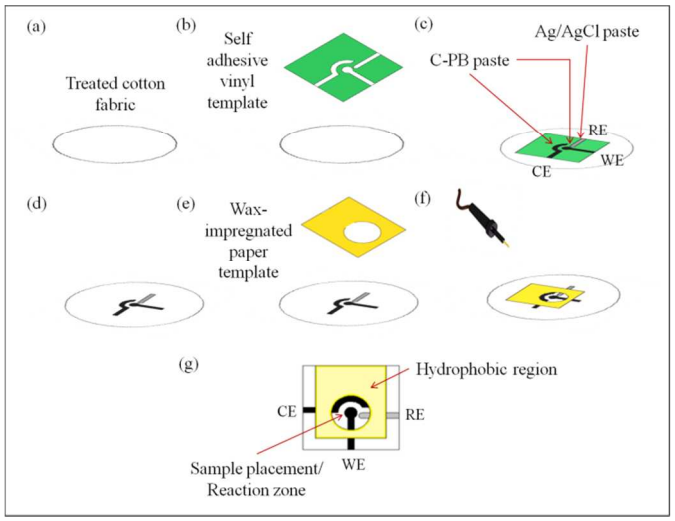


Fig.1 Schematic illustration of the fabrication process of the FED. (a) The platform for FED is treated cotton fabric. (b) For patterning the electrodes, self-adhesive vinyl template was used. (c) C-PB paste was applied for both, the WE and CE, while Ag/AgCl paste for the RE. (d) After the template was removed, the substrate was cured at 60°C for 30 min in the oven. (e) The template for patterning the sample placement/reaction zone was printed on wax-impregnated paper. (f) The wax-impregnated paper template was placed accordingly and heat treatment was used to transfer the wax onto the substrate at 150°C using a soldering iron. (g) The ready-to-use device. RE, reference electrode; WE, working electrode; CE, counter electrode.

2.3 Electrochemical measurement of the device

The three electrodes on the device were connected to the µSTAT400 portable potentiostat (Dropsens, Spain) using the provided connector clips and the electrochemical signals were measured and displayed using DropView software provided by the manufacturer (Fig. 2(a)).

In order to characterise the electrochemical behaviour of PB, a redox-active compound within the fabricated FED, 4 µL of 0.1 M PBS was spotted at the sample placement/reaction zone and cyclic voltammetry (CV) was performed at various scan rates.

For quantitative measurement of lactate, 3 µL of LOx enzyme solution (0.6 U) from the 100 µL aliquot was pre-loaded at the reaction area of the FED and allowed to dry at room temperature for 30 min. For analysis, 4 µL of standard or

sample solution was dropped at the sample placement area. The solution wicks through the cellulose fibres within the cotton fabric and reacts with the entrapped LOx enzyme molecules, hence generating hydrogen peroxide (H_2O_2) that can be detected electrochemically. The reaction that takes place at the C-PB/LOx electrodes of the FED in the presence of lactate is illustrated in Fig. 2(b)).

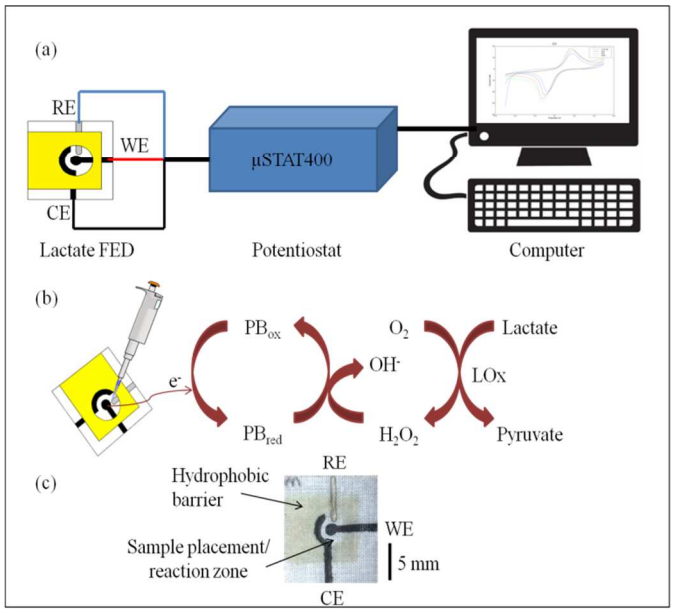


Fig.2 Overview of FED technology. (a) The instrumental setup for lactate determination. (b) The reaction that occurs at the C-PB/LOx electrodes of the FED. (c) Picture of the fabricated FED (15 x 15 mm). RE, reference electrode; WE, working electrode; CE, counter electrode.

Prior to SL measurement, lactate aliquots using both 0.1 M PBS and saliva samples were prepared to obtain concentrations of 0, 2 and 4 mM of lactate, respectively. The lactate aliquots were analysed by pipetting 4 µL of each prepared solution at the sample placement area of the lactate FED. The calibration curves in the absence (0.1 M PBS) and presence of saliva were compared to study the possible interference effect due to the saliva complex matrix.

2.4 Saliva sample collection

Six healthy volunteer subjects were chosen for this study. The inclusion criteria were age 18 and over and self-identified as healthy. For fasting samples, the subjects were asked to refrain from eating, drinking or smoking prior to their appointment for saliva collection. The subjects were asked to rinse their mouth with water and wait at least 10 min before collecting saliva samples to avoid sample dilution. The saliva samples were collected using passive drooling, in which the subjects were instructed to refrain from swallowing their saliva for a short duration (~ 30 s) and the accumulated saliva was collected by drooling down a plastic drinking straw (5 cm) into a disposable test tube. The subject was asked to repeat drooling down the straw as often as necessary until sufficient sample was collected. Saliva samples were collected again from the six subjects, 30 min after food intake. Both before and after meal saliva samples were labelled and stored at 4°C until they were used for the assay in order to arrest bacterial metabolism and avoid degradation of lactate. For analysis, the saliva samples were used directly without previous processing or alteration. The experimental protocols were approved by the Research Ethics Committee of Universiti Teknologi Malaysia (UTM) (Ref no: UTM.J.45.O1l25.0U1 (9)).

3. Results and discussion

3.1 Electrodes patterning using template method

The template method was used to pattern a single conventional three-electrode sensor and a three-electrode array onto commonly available lab supplies as shown in Fig. 3. It is evident that the template method produces rapid and high-quality transfer of the electrode patterns regardless of the uneven and non-planar surface of the substrates, thus making it suitable to extend the fabrication technique to a variety of substrates that are incompatible with standard screen-printing protocols. Additionally, the method does not require any sophisticated instrumentation. Basically, it needs a computer equipped with graphical software and an electronic cutting tool to produce the templates. The resulting devices could be easily interfaced with an electrochemical analyser using a similar protocol as described in literature,[19, 23] thus making it feasible for a wide array of applications such as healthcare, sports, agriculture, environmental, security and food quality monitoring.

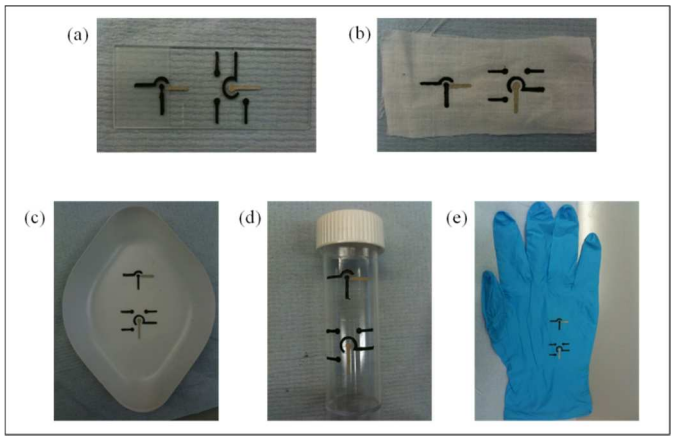


Fig.3 Electrodes patterned on: (a) Glass microscope slide. (b) Cotton fabric. (c) Plastic weighing boat. (d) On the outer surface of a polypropylene centrifuge tube. (f) Nitrile glove.

3.2 Electrochemical characterisation of the device

The electrochemical characterisation of the C-PB electrodes on the FED was performed using CV within the potential limits of

-0.5 and 0.5 V at different potential scan rates (10, 25, 50, 100 mV/s) in 0.1 M PBS as depicted in Fig. S1. The anodic and cathodic peaks correspond to the following redox reaction:

$$Fe^{III}_4[Fe^{II}(CN)_6]_3 + 4K^+ + 4e^- \leftrightarrow K_4Fe^{II}_4[Fe^{II}(CN)_6]_3 \quad (1)$$

It can be observed that the anodic peak potential shifts more towards the positive potential, while the cathodic peak potential shifts in the opposite direction with increasing potential scan rates (Fig. S1). The redox peak currents show a linear relationship with the square root of the potential scan rate, $mV/s^{1/2}$ (Inset in Fig. S1). This indicates that the electrochemical process that takes place is a diffusion controlled electron transfer process similar to conventional electrochemical cells.[24-28] More specifically, the diffusion controlled process in this system refers to the diffusion of potassium ions (K^+) within the PB lattice in the plating phase of the electrode.[24, 27, 28]

3.3 Optimisation of the detection potential for hydrogen peroxide

Prior studies[14-17] have shown cotton fabric to be an excellent choice of substrate for enzyme immobilisation due to its attributes that include high porosity (> 95%), large specific surface area and excellent mechanical properties. In addition, cotton fabric is hydrophilic but insoluble in water, stable to chemicals and biocompatible. Therefore, in this study we utilised the hierarchically structured cellulose fibres within the cotton fabric platform[29] for immobilising LOx enzyme via simple entrapment method, eliminating in this way the additional chemical reagents commonly required in other immobilisation procedures.[14-17]

The lactate FED reacts as in the following:

$$\text{L-lactate} + O_2 + H_2O \rightarrow \text{Pyruvate} + H_2O_2 \quad (2)$$

The LOx enzyme oxidizes lactate in the presence of oxygen (O_2) and water (H_2O), and it generates pyruvate and H_2O_2 (Eq. (2)) (Fig. 2(b)). Since the reaction relies on the detection of H_2O_2 for quantification of lactate in the sample solution, it is important to determine the optimum detection potential of the lactate FED to achieve reliable H_2O_2 detection at the C-PB/LOx electrodes.

First, the C-PB/LOx electrodes were characterised using CV within the potential limits of -0.4 V and 0.4 V at 10 mV/s in 0.1 M PBS (absence of H_2O_2), which resulted in the characteristic redox peaks of PB. It can be observed that in both, C-PB (Fig. 4(a) (i)) and C-PB/LOx (Fig. 4(a) (ii)) electrodes, the cathodic peak potential occurs at -0.09 V and -0.08 V, respectively. However, the cathodic peak current of C-PB/LOx electrodes increased ~1.26 fold compared to the C-PB electrodes, from -15.9 µA to -20 µA, which implies that there is an increase in efficiency of C-PB electrodes after modification with LOx enzyme.

Next, the catalytic capability of the C-PB/LOx electrodes to the reduction of H_2O_2 was studied; in which a larger cathodic peak current (-26.1 µA) was observed in the presence of 5 mM lactate solution (presence of H_2O_2) in comparison to the background electrolyte, 0.1 M PBS (-20 µA) (Fig. 4(b)). However, since the cathodic peak potential shifted towards less positive potential, from -0.08 V to -0.12 V in the presence of lactate, a more detailed investigation was required to determine the optimum detection potential for H_2O_2.

Generally, catalytic reduction of H_2O_2 in C-PB electrodes occurs in the range of -0.2 and 0.2 V.[24] Therefore, the effect of applied potential towards the signal (5 mM lactate solution) and background electrolyte (0.1 M PBS) was investigated in the particular region from the CV data shown in Fig. 4(b), resulting in Fig. 4(c). The data points are the mean and the error bars are the standard error of the mean (SEM) for three separate lactate FEDs, respectively. From Fig. 4(c), it can be observed that the cathodic current for both signal and background increases continuously with increasing cathodic potential until -0.1 V, but significantly drops at -0.4 V. Fig. 4(d) shows the signal-to-background (S/B) ratios at each of the potential and the highest S/B ratio was obtained at -0.2 V. Therefore, a detection potential of -0.2 V was chosen for further studies. This detection potential offers a better selectivity for H_2O_2 as it is relatively low, hence it reduces the signal of electroactive interfering substances such as ascorbic acid and uric acid often present in real matrices.

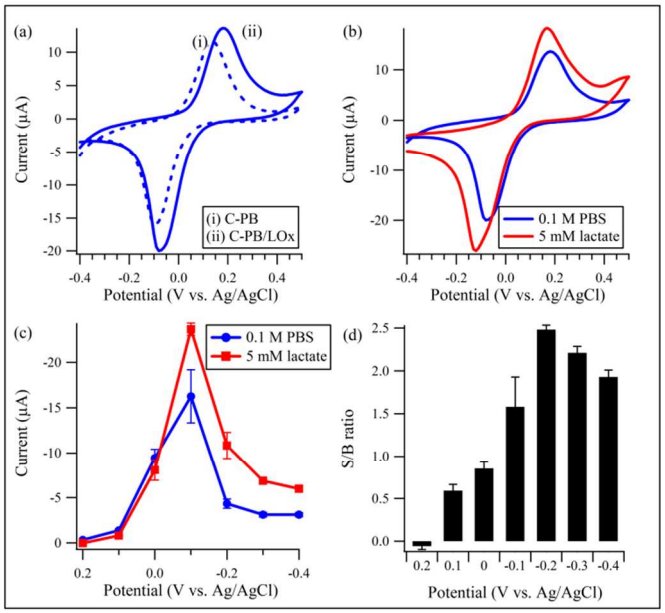


Fig.4 FED detection potential optimisation for H_2O_2 (a) CV of the: (i) C-PB electrodes and (ii) C-PB/LOx electrodes for the lactate FED in 0.1 M PBS with potential scan rate of 10 mV/s. (b) CV of the C-PB/LOx electrodes in the absence (0.1M PBS) and presence of H_2O_2 (5 mM lactate solution) with potential scan rate of 10 mV/s. (c) The effect of applied potential towards the signal (5 mM lactate solution) and background electrolyte (0.1M PBS) extracted from the data shown in part (b). (d) Signal-to-background (S/B) ratios calculated from the data shown in part (c). Error bars = ± SEM (n = 3).

3.4 Calibration of the FED for lactate measurement

After determining the optimum detection potential, CVs of the C-PB/LOx electrodes of the lactate FED were performed at different concentrations (2 to 10 mM) of lactate standard solutions as shown in Fig. 5(a). The calibration curve (Inset of Fig. 5(a)) was obtained from the cathodic currents of the CV at potential of -0.2 V vs. Ag/AgCl. The calibration curve for lactate FED that was obtained via CV measurement presents a linear range for lactate detection from 2 to 5 mM with correlation coefficient (r^2) of 0.997.

Subsequently, chronoamperometric measurements were performed as it offers better sensitivity (higher signal-to-noise ratio) and lower detection limits. Therefore, they are more reliable for long-term applications compared to CV measurements.[24] The chronoamperometric measurements of lactate (0.1 to 25 mM) using the lactate FED were carried out at -0.2 V vs. Ag/AgCl, in which anodic current was recorded for 300 s and the calibration curve was plotted from the apparent steady state current (Fig. 5(b)). The inset shows the linear calibration plot of the anodic current as a function of lactate concentration. The data points and the error bars for both, Fig. 5(a) and Fig. 5(b) are the mean and SEM for three separate lactate FEDs, respectively. Since the lactate FED confines the sample solution within the reaction zone, it minimizes noises contributed by random motion, vibration, thermal or other disturbance sources that are common in electrochemical experiments performed in bulk solution.[30]

Based on the inset of Fig. 5(b), it can be deduced that the lactate FED have linear working range for lactate detection from 0.1 to 5 mM with r^2 of 0.997, which is in agreement with the calibration results obtained previously (Fig. 5(a)). The sensitivity of the device was determined from the slope of the linear calibration curve that is 0.3169 μA mM^{-1}. The limit of detection (LOD) and limit of quantitation (LOQ) of the device was calculated from standard deviation (σ) of the blank as the concentrations that produced the signal at 3σ and 10σ of the blank (n=10) for three separate devices. The LOD and LOQ were 0.3 mM and 1.0 mM, respectively. The repeatability of the device was calculated using the same lactate FED three times for each lactate concentrations and the relative standard deviation (%RSD) obtained was 4.0% for 0.5 mM lactate and 1.0% for 25.0 mM lactate, while the reproducibility was measured using three different devices for 0.5 mM and 25.0 mM lactate concentrations and the %RSD attained was 5.3% and 6.8%, respectively.

To highlight the contribution of the proposed work, we compared several analytical parameters of the fabricated lactate FED with other low-cost paper-based electrochemical assays reported in literature for lactate measurement. Dungchai *et al.*[24] reported the first successful integration of electrochemical detection and paper-based microfluidic devices. Although the device comprises of a microfluidic channel that diverges into three different reaction areas, it requires a small sample volume of 5 μL only. On the other hand, micro-paper-based analytical devices (μPADs) combined with a commercial hand-held glucometer[31] require a large sample volume of 1.2 to 1.5 mL due to its design to mimic the format of commercial test strips. Our lactate FED requires a small sample volume of 4 μL only. This makes our device suitable for the analysis of biological fluids produced in small quantities. Both of the mentioned devices also demonstrated wide linear range for lactate detection of 0 to 50 mM[24] and 1 to 11 mM,[31] compared to our device that has a linear range from 0.1 to 5 mM only. Regardless, our lactate FED is still a comprehensive device to measure lactate in a variety of biological matrices since the typical concentration of lactate is 0.7 to 1.7 mM in blood, 0.5 to 1.7 mM in serum, 5.5 to 22 mM in urine and 0.1 to 2.5 mM in saliva.[3, 32] Our device also showed lower LOD (0.3 mM) compared to 0.36 mM[24] and 1.1 mM[31] of the paper-based lactate assays. Moreover, we could reduce the LOD further by improving the enzyme loading on the cotton fabric.

Commonly, electrochemical paper-based assays are fabricated using screen-printing methods[24, 31] for electrodes patterning. Meanwhile, hydrophobic barriers are created using photolithography[24] or wax printing methods that need special commercially available wax printer[31]. These techniques involve expensive and highly complex equipments that need substantial maintenance and in some cases, clean room facilities. These drawbacks make it financially impractical for the mass production of low-cost biomedical assays compared to our simple and economical fabrication approach.

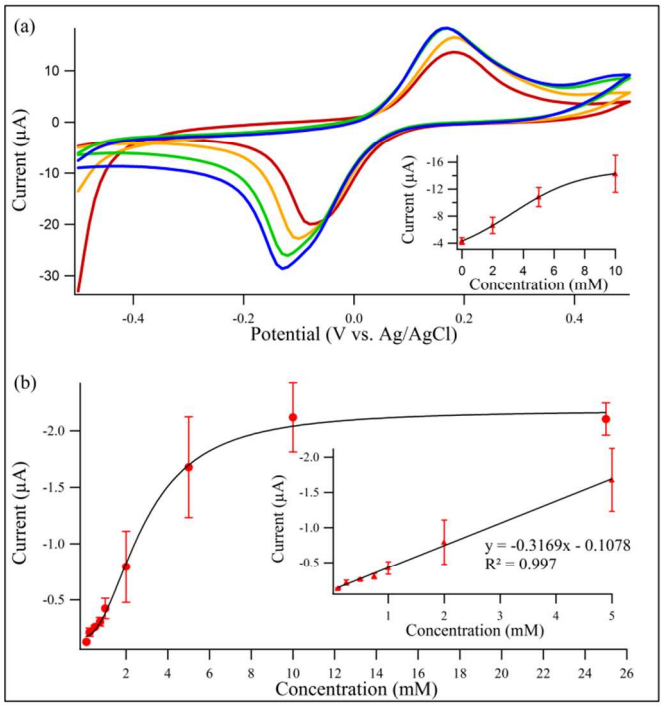


Fig. 5 Lactate calibration of the FED (a) CV of the C-PB/LOx electrodes for the lactate FED in 0.1M PBS and lactate standard solutions (2 to 10 mM) at 10 mV/s. *Inset* shows calibration curve for the lactate FED obtained from the CV data. (b) The calibration plot of the anodic current as a function of lactate concentration using chronoamperometric measurement for the lactate FED. *Inset* shows the linear calibration plot for lactate concentration from 0.1 to 5 mM. Error bars = ± SEM (n = 3).

In this study, the use of cotton fabric as the structural material for the fabrication of analytical device has several

advantages: (i) it is ubiquitous and low cost, hence suitable for single-use and disposable devices; (ii) it is lightweight, flexible and foldable, simplifying the logistics of transport and storage; (iii) it is highly porous and biocompatible, making it useful as an enzyme immobilisation matrix; (iv) it has good absorbency and wicking properties that require microvolumes of reagent and sample solution; (v) it is flexible and mechanically durable towards bending and stretching stresses, thus suitable to be integrated in clothing;[11-13] (vi) it is washable, hence suitable for wearable sensors that are subjected to traditional laundry cycle;[11] and (vii) environmentally sustainable and biodegradable. Overall, the fabricated FED is in accordance to six out of seven criteria outlined by the World Health Organization for low-cost diagnostic platforms that are summarized by the acronym 'ASSURED', which stems from affordable, sensitive, specific, user-friendly, rapid and robust, equipment-free and deliverable to end-users.[33] The FED does not meet the equipment-free criteria because it requires an electrochemical analyser for quantitative measurement.

3.5 Stability monitoring of the FED for lactate measurement

Fig. 6 displays the operational stability of the lactate FED that was evaluated over a 3 hour period with repetitive measurements of 0.5 mM lactate standard solution carried out every 15 min. The data points and the error bars are the mean and SEM for three separate lactate FEDs, respectively. The data indicates that there is a slight decline of the device sensitivity over time. However, this loss of sensitivity, which is most probably due to the fact that the free enzyme, entrapped within the cellulose fibres, leaked out with the repeated application of sample solution, is minimal (~ 27.8%). This shows that the device can be used for both one-off use as a disposable device and also for extended use. Nevertheless, further experiments to increase the stability of the extended use would be necessary, such as improving the enzyme loading and immobilisation method.

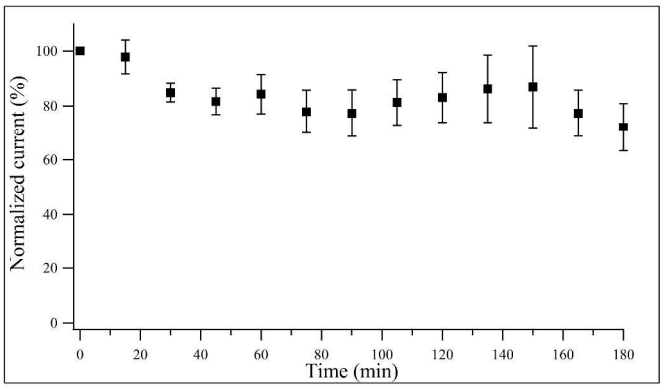


Fig.6 Stability of the lactate FED to 0.5 mM lactate over a 3 h operation with repetitive measurement carried out at 15 min intervals. The initial current at t=0 min is normalised to 100%. Error bars = ± SEM (n = 3).

3.6 Selectivity of the FED for lactate measurement

In order to evaluate the selectivity of the lactate FED towards lactate measurement, substances tested as potential interferents for lactate were glucose, ascorbic acid and uric acid. The data (Fig. S2) indicate that these interference substances have a negligible effect upon the lactate response. This is due to the specificity of the entrapped LOx enzyme and the low detection potential for H_2O_2 provided by the PB layer.

3.7 Measurement of lactate in saliva

The fabricated lactate FED presented a relevant working range for the typical concentrations of SL (0.1 to 2.5 mM)[3] (Fig. 6). In addition, the current response to lactate aliquots prepared with either 0.1 M PBS or saliva samples showed no significant difference at 95% confidence level (P value = 0.285) (Fig. 7(a)). Based on these results, the device was deemed suitable for lactate measurements in saliva. Thus, saliva samples that were obtained under fasting conditions and after meals were evaluated using the lactate FED as a model assay to exhibit the potential of the proposed device as an analytical tool. Pre-calibration of the lactate FED and ongoing calibrations with 1 mM lactate standards as a control between each measurement, without a significant decrease in sensitivity, demonstrated the reliability and reusability of the immobilised enzyme within the device. SL levels were observed to vary among the subjects and the concentration increased after meal randomly for all the subjects (Fig. 7(b)). The results obtained could not be compared with blood lactate measurements due to the non-availability of a micro-scale blood lactate test. Nevertheless, the results were in good agreement with previous studies.[7, 8]

Although various commercialised portable lactate meters have been introduced, it has been limited for CBL measurement in whole blood samples only. SL is normally measured using enzymatic colorimetric assay kit using laboratory-based equipment such as spectrophotometer/fluorometer. This procedure requires a relatively large volume of reagent/sample solution (500 µL) and involves multiple steps of sample acquisition, labelling, freezing, transportation, processing in the laboratory (centrifugation, sorting, aliquotting and loading into the analyser) and finally, results reporting. It is tedious, time-consuming and usually requires skilled laboratory personnel for equipment handling and analysis. However, SL analysis can be carried out in our lactate FED using a small volume of reagent/sample solution (4 µL), hence making it feasible for SL determination even among xerostomic individuals. It does not require sample pre-treatment or dilution steps and provides immediate results with a sample drop. Both the lactate FED and electrochemical analyser are also transportable and portable, hence suitable for point-of-care testing.

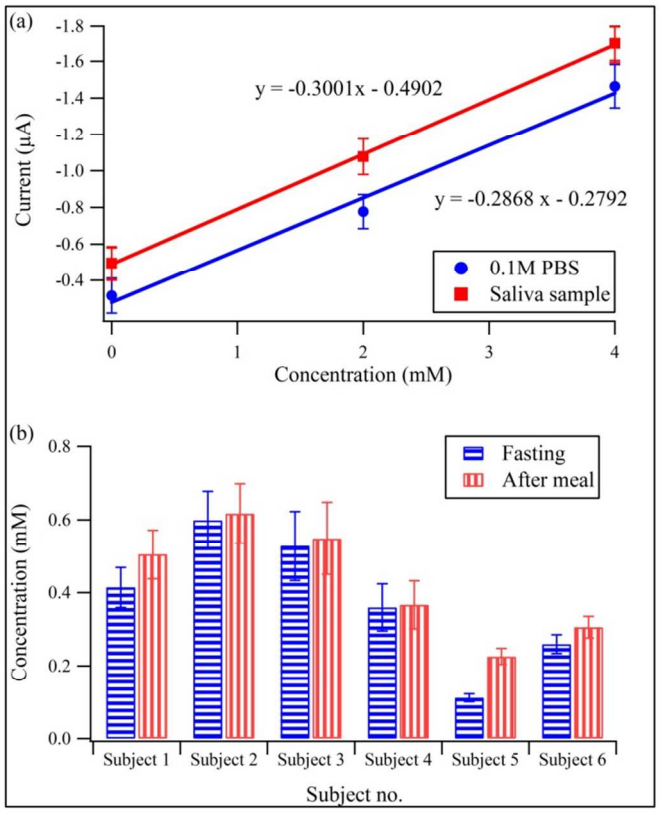


Fig.7 (a) The linear calibration plot for both, 0.1 M PBS and saliva samples. (b) The determination of lactate in saliva during fasting and after meal using the fabricated lactate FED. Error bars = ± SEM (n = 3).

4. Conclusion

The proposed device holds a great potential as a viable analytical tool for non-invasive quantitative determination of lactate using saliva samples. The device is especially useful for clinical diagnostics and sports monitoring. The FED combines the advantages of cotton fabric (easily available, low-cost, lightweight, flexible, biocompatible, requiring minute volume of reagent and sample solution, mechanically durable and environmental friendly) with the benefits of electrochemical detection (fast and reliable quantitative analysis). In summary, the FED is a miniaturized and robust analytical device that has the potential to be adapted for the determination of a variety of assays in a wide array of applications. Since capillary forces control the fluid flow in the cotton fabric platform, we envision that the usage of pipettes can be eliminated by integrating a hydrophilic cotton thread as the liquid transporting channel towards the reaction zone within the FED. This could lead towards a device that incorporates both sample collection and quantitative analysis within a single device. In addition, future applications would benefit from the incorporation of electronic components, display and other functionalities within cotton fabric interfaced with a wireless, portable electrochemical reader to nurture it into a more practical platform technology for real-world applications.

Acknowledgements

We would like to thank Universiti Teknologi Malaysia (GUP Grant Vote No. 09J32, 03H30) and the Ministry of Higher Education (MOHE) for the funding of this research work. We would also like to express our gratitude to Professor Martyn G. Boutelle from the Department of Bioengineering, Imperial College London for his invaluable suggestions on the work of this manuscript.

Notes and references

a Faculty of Biosciences and Medical Engineering (FBME), Universiti Teknologi Malaysia (UTM), 81310 Skudai, Johor, Malaysia.; Email: emmacorcoles@gmail.com, emma@biomedical.utm.my
b Medical Implant Technology Group (MediTeg), Materials and Manufacturing Research Alliance (MM-RA), Universiti Teknologi Malaysia (UTM), 81310 Skudai, Johor, Malaysia.

1. J. Ballesta Claver, M. C. Valencia Miron and L. F. Capitan-Vallvey, *The Analyst*, 2009, 134, 1423-1432.
2. T. S. Ching and P. Connolly, *International journal of nanomedicine*, 2008, 3, 211-223.
3. C. G. Schabmueller, D. Loppow, G. Piechotta, B. Schutze, J. Albers and R. Hintsche, *Biosens. Bioelectron.*, 2006, 21, 1770-1776.
4. K. Svedahl and B. R. MacIntosh, *Canadian journal of applied physiology = Revue canadienne de physiologie appliquee*, 2003, 28, 299-323.
5. T. Ohkuwa, H. Itoh, Y. Yamazaki and Y. Sato, *Scandinavian journal of medicine & science in sports*, 1995, 5, 285-290.
6. R. V. Santos, A. L. Almeida, E. C. Caperuto, E. Martins, Jr. and L. F. Costa Rosa, *Comparative biochemistry and physiology. Part B, Biochemistry & molecular biology*, 2006, 145, 114-117.
7. G. Palleschi, M. H. Faridnia, G. J. Lubrano and G. G. Guilbault, *Anal. Chim. Acta*, 1991, 245, 151-157.
8. G. G. Guilbault, G. Palleschi and G. Lubrano, *Biosens. Bioelectron.*, 1995, 10, 379-392.
9. L. F. Hofman, *J. Nutr.*, 2001, 131, 1621S-1625S.
10. T. Pfaffe, J. Cooper-White, P. Beyerlein, K. Kostner and C. Punyadeera, *Clin. Chem.*, 2011, 57, 675-687.
11. M.-C. Chuang, J. R. Windmiller, P. Santhosh, G. V. Ramírez, M. Galik, T.-Y. Chou and J. Wang, *Electroanal.*, 2010, 22, 2511-2518.
12. J. R. Windmiller and J. Wang, *Electroanal.*, 2013, 25, 29-46.
13. Y. L. Yang, M. C. Chuang, S. L. Lou and J. Wang, *The Analyst*, 2010, 135, 1230-1234.
14. J. J. Karimpil, J. S. Melo and S. F. D'Souza, *Int. J. Biol. Macromol.*, 2012, 50, 300-302.
15. M. Monier and A. M. El-Sokkary, *Int. J. Biol. Macromol.*, 2012, 51, 18-24.
16. E. Ondul, N. Dizge and N. Albayrak, *Colloids Surf., B*, 2012, 95, 109-114.
17. B. Y. Şahinbaşkan and M. V. Kahraman, *Starch - Stärke*, 2011, 63, 154-159.
18. G. S. Fiorini and D. T. Chiu, *Biotechniques*, 2005, 38, 429-446.
19. J. R. Windmiller, A. J. Bandodkar, S. Parkhomovsky and J. Wang, *The Analyst*, 2012, 137, 1570-1575.

20. A. Nilghaz, D. H. Wicaksono, D. Gustiono, F. A. Abdul Majid, E. Supriyanto and M. R. Abdul Kadir, *Lab Chip*, 2012, 12, 209-218.
21. E. Carrilho, A. W. Martinez and G. M. Whitesides, *Analytical chemistry*, 2009, 81, 7091-7095.
22. P. Salazar, M. Martin, R. D. O'Neill, R. Roche and J. L. González-Mora, *Int. J. Electrochem. Sci.*, 2012, 7, 5910-5926.
23. K. Malzahn, J. R. Windmiller, G. Valdes-Ramirez, M. J. Schoning and J. Wang, *The Analyst*, 2011, 136, 2912-2917.
24. W. Dungchai, O. Chailapakul and C. S. Henry, *Anal. Chem.*, 2009, 81, 5821-5826.
25. X. He, R. Yuan, Y. Chai, Y. Zhang and Y. Shi, *Biotechnol. Lett*, 2007, 29, 149-155.
26. C. Hong, R. Yuan, Y. Chai and Y. Zhuo, *Electroanal.*, 2008, 20, 2185-2191.
27. D. Moscone, D. D'Ottavi, D. Compagnone, G. Palleschi and A. Amine, *Anal. Chem.*, 2001, 73, 2529-2535.
28. N. F. Zakharchuk, B. Meyer, H. Henning, F. Scholz, A. Jaworksi and Z. Stojek, *J. Electroanal. Chem.*, 1995, 398, 23-35.
29. S. J. Kadolph, *Textiles*, Prentice Hall, 11th edn., 2010.
30. Z. Nie, C. A. Nijhuis, J. Gong, X. Chen, A. Kumachev, A. W. Martinez, M. Narovlyansky and G. M. Whitesides, *Lab Chip*, 2010, 10, 477-483.
31. Z. Nie, F. Deiss, X. Liu, O. Akbulut and G. M. Whitesides, *Lab Chip*, 2010, 10, 3163-3169.
32. N. W. Tietz, *Clinical Guide to Laboratory Tests*, W.B. Saunders Company, Philadelphia, PA, 3rd edn., 1995.
33. D. Mabey, R. W. Peeling, A. Ustianowski and M. D. Perkins, *Nat Rev Micro*, 2004, 2, 231-240.