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\[ \text{e}^{-} \rightarrow \text{PB}_{\text{ox}} \rightarrow \text{PB}_{\text{red}} \rightarrow \text{O}_{2} \rightarrow \text{OH}^{-} \rightarrow \text{LOx} \rightarrow \text{Lactate} \rightarrow \text{H}_{2}\text{O}_{2} \rightarrow \text{Pyruvate} \]
Cotton Fabric-based Electrochemical Device for Lactate Measurement in Saliva

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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 ($\text{H}_2\text{O}_2$) 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 $\mu\text{A} \text{mM}^{-1}$) 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 ($\text{O}_2$), 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.  

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 Schabmuller 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.4 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 behav iour and
flexible and capable of withstanding various forms of
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 studies11-13 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 investigated14-
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 et 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 technique20 that was first
introduced by Whitesides group21 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 Bat e
Gallery, Kuala Lumpur. Anhydrous sodium carbonate
(Na2CO3) (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 Bate 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 Thew 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−2)
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
Pedioecoccus 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, Silhouette digital craft cutter interfaced with Silhouette Studio software (Silhouette 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\(_2\)CO\(_3\)) 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\(_2\)CO\(_3\) 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 candellilla 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.

![Image](https://example.com/image.png)

**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$_2$O$_2$) 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)).

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.OI125.0U1 (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, thus making it feasible for a wide array of applications such as healthcare, sports, agriculture, environmental, security and food quality monitoring.

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:

$$\text{Fe}^{II}4[\text{Fe}^{II}(\text{CN})_6], + 4\text{K}^+ + 4e^- \leftrightarrow \text{K}_4\text{Fe}^{III}4[\text{Fe}^{III}(\text{CN})_6]$$

(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. More specifically, the diffusion controlled process in this system refers to the diffusion of potassium ions (K\(^+\)) within the cotton fabric platform in the plating phase of the electrode.

3.3 Optimisation of the detection potential for hydrogen peroxide

Prior studies 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 for immobilising LOx enzyme via simple entrapment method, eliminating in this way the additional chemical reagents commonly required in other immobilisation procedures.

The lactate FED reacts as in the following:

$$\text{L-lactate} + \text{O}_2 + \text{H}_2\text{O} \rightarrow \text{Pyruvate} + \text{H}_2\text{O}_2$$

(2)

The LOx enzyme oxidizes lactate in the presence of oxygen (O\(_2\)) and water (H\(_2\)O), and it generates pyruvate and H\(_2\)O\(_2\) (Eq. (2)) (Fig. 2(b)). Since the reaction relies on the detection of H\(_2\)O\(_2\) 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\(_2\)O\(_2\) 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\(_2\)O\(_2\)), 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\(_2\)O\(_2\) 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\(_2\)O\(_2\)) 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\(_2\)O\(_2\).

Generally, catalytic reduction of H\(_2\)O\(_2\) in C-PB electrodes occurs in the range of -0.2 and 0.2 V. 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\(_2\)O\(_2\) 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.

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. 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.

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 ($\sigma$) of the blank as the concentrations that produced the signal at 3$\sigma$ and 10$\sigma$ 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. Dungchait et al. 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 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 and 1 to 11 mM, 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. Our device also showed lower LOD (0.3 mM) compared to 0.36 mM and 1.1 mM 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 for electrodes patterning. Meanwhile, hydrophobic barriers are created using photolithography or wax printing methods that need special commercially available wax printer. 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.
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).](image)

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 H2O2 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.
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.

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Notes and references


