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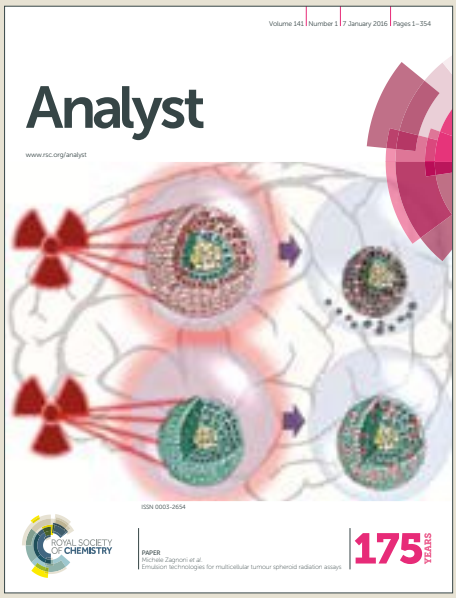

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An electrochemiluminescence cloth-based biosensor with smartphone-based imaging for detection of lactate in saliva

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Abstract

Cloth fabrics and smartphones have become the two things people are most familiar with. Especially, smartphones are used as portable personal computers, revolutionizing communication and life types. Here, the screen-printing technology is applied to fabricate carbon electrodes and electrochemical chambers on a single hydrophilic cloth, while the cloth-based electrochemiluminescence (ECL) signals are read out by an inexpensive smartphone. Therefore, the ECL detection is available in both low-cost disposable sensors and portable format, which may be very suitable to be used as non-invasive monitoring tool for medical diagnostics. As the proof-of-principle, lactate oxidase is immobilized onto the working electrode for the lactate measurement. Under optimized conditions, the lactate levels can be quantified over the range of 0.05-2.5 mM, with a detection limit of 0.035 mM and the relative standard deviations of 4.7%, 5.2% and 5.0% for 0.05, 0.5 and 2.5 mM lactate (n = 5). In addition, the proposed biosensor has an acceptable stability and selectivity. Finally, the ECL biosensor is successfully applied for determination of lactate in human saliva. These results show that the presented ECL platform has the potential to be applied to non-invasively detect a variety of analytes of medical interest.

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Introduction

Lactate is one of the important metabolites during the anaerobic phase of glycolysis, where lactic acid is produced from pyruvate by the enzyme lactate dehydrogenase and then dissociated into lactate at physiologic pH ranges. Lactate plays significant roles in many fields such as clinical diagnosis, sport physiology and food analysis [1]. As we know, lactate is associated with several diseases (such as shock, respiratory insufficiency, heart failure, systemic disorder, drugs or toxins intake, and hereditary disorders) [2], and thus the measurement of its concentration in physiological fluids is essential for diagnosis of patient conditions. It is also useful in sport medicine, especially for estimation of physical conditions of athletes who are engaged in strenuous physical activity for a prolonged time. In food industry, lactic acid is produced by the fermentation of lactose because of the microbial activity of lactic acid bacteria, and the lactate level depends on the total amount of bacteria and determines the freshness, stability and preservation quality of food products.

Up to now, various methods have been developed for lactate determination, such as nuclear magnetic resonance [3, 4], liquid chromatography [5], spectrophotometry [6], fluorimetry [7], colorimetry [8, 9], electrochemistry [10-19], chemiluminescence (CL) [20-25], and electrochemiluminescence (ECL) [26, 27]. Among these detection methods, both electrochemical and chemiluminescent methods have recently attracted more and more attentions. Especially, the ECL technique possesses some intrinsic advantages of both electrochemistry and CL, such as low background signal, low detection limit, wide dynamic range, simple instrumentation, and as well great selectivity and controllability. However, ECL detection has rarely been used in both disposable sensors and portable format, possibly

because the commonly-used photomultiplier tube (PMT) detector needs a high voltage supply and has a bulky size, and moreover the reaction cell/electrodes are relatively expensive actually.

In recent years, the smartphone technologies are increasingly improved, and as a result its range of applications is unexpectedly expanded. In particular, the great advancements of complementary metal oxide semiconductor (CMOS)-based photo-cameras have enabled its use as an imaging device in optical sensors [28-30]. The smartphone-based imaging device can provide several attractive advantages: (1) smartphones are potentially more accessible and cheaper than portable analytical laboratory devices, and thus these low-cost and portable devices may be highly desirable for point-of-care diagnostics even in resource-limited settings lacking adequate healthcare facilities; (2) smartphones usually have high resolution screens for result display, and powerful memories and processors for the storage and analysis of imaging results; and (3) smartphones are generally equipped with powerful data transmission capabilities, thus allowing short- or long-distance communication between a remote test site and centralized laboratory (or professional specialist). In spite of these advantages, however, there are few examples of ECL coupled with smartphone-based imaging. Doeven *et al.* have described a smartphone-based ECL using the glassy carbon disc working electrode (WE) and gold counter/reference electrode (CE/RE) [31].

Nowadays, the application of microfluidic chip is well-developed and attracts more attention in chemistry and biology [32-35]. However, the wide use of conventional microfluidic technologies is often limited by some factors including complexity and high cost of pressure control systems and fabrication techniques. To partly circumvent these limiting

factors, some simple and low-cost disposable microfluidic paper-based analytical devices (μPADs) have been developed for ECL detection [36-38]. Recently, our group [39] and others [40, 41] have coupled μPADs with bipolar or three-electrode ECL, where the emitted signals are read with a smartphone camera. Although these two μPADs are characterized by the simpleness and feasibility of fabrication and functionalization approaches, they still have some limitations such as bad "wet-strength"; low mechanical strength, durability and flexibility; too narrow choice for paper substrates (thus a relatively high cost); and relatively less interstitial spaces of the paper. To partially solve these limitations, cloth has recently emerged as an interesting alternative substrate for low-cost disposable microfluidic cloth-based analytical devices (μCADs) [42]. To our knowledge, however, no reports on coupling cloth-based ECL with smartphone-based imaging have been published.

The development of non-invasive procedures for the determination of lactate in matrices other than blood is very interesting, especially for critical-care patients, for people who have problems with blood extraction, and for those who need to frequently control the lactate level (such as diabetics and athletes). In this way, saliva is considered extremely attractive for non-invasive lactate monitoring [43], in part due to its continuous and convenient availability, simplicity of collecting samples that can be performed by untrained staff, and high correlation to blood lactate (typically a 1:4 saliva/blood ratio [44]). Nowadays, the non-invasive lactate detection methods include colorimetry [8], electrochemistry [10-13, 18], CL [20], and ECL [27]. Although these reported analyses can exhibit some acceptable performances, they still have some important limitations, such as high detection limits (>0.1 mM) [8, 12, 20], large sampling volumes [27], expensive signal detectors [27], complex

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preparation procedures (including device fabrication, material synthesis and sensor functionalization) [8, 10-13, 18]. Therefore, it is necessary to explore novel techniques and methods for lactate monitoring in saliva.

With this in mind, we have tried to integrate the cloth-based microfluidics, electrogenerated luminescence and smartphone-based imaging to achieve a low-cost and disposable lactate sensor associated with a portable detection system. The carbon ink screen-printing is applied to pattern the carbon electrodes onto the hydrophilic cloth, while the wax screen-printing is used for direct fabrication of ECL chambers onto the electrodes-containing cloth. For the presented biosensor, hydrogen peroxide (H_2O_2) is generated by the oxidizing enzyme on the WE, and under an electrocatalysis it then reacts with luminol to produce the ECL emissions that are captured by an inexpensive smartphone and wirelessly transmitted onto personal computer (PC). Under optimized conditions, the cloth-based biosensor can obtain the acceptable detection performances for the lactate assay. Finally, the proposed method is successfully applied for the oral fluid lactate determination.

Experimental section

Chemicals and materials

Lithium lactate (99%), lactate oxidase (LOx) (from Aerococcus viridans, ≥20 units/mg), and potassium ferricyanide were purchased from J&K Chemical Ltd. (Beijing, China). Phosphate buffer solution (PBS, 20×), potassium chloride (KCl) (>98.5%), ammonium chloride (NH_4Cl), uric acid (UA) (98%), L-ascorbic acid (AA) and citric acid (CA) (anhydrous, ≥99.0%) were bought from Sangon Biotech Co., Ltd. (Shanghai, China). Luminol and α-D-glucose

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(anhydrous, 96%) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Potassium thiocyanate (KSCN) (99.99% metals basis) was bought from Shanghai Aladdin Biochemical Technology Co., Ltd. (Shanghai, China). Calcium chloride ($CaCl_2$) (anhydrous, ≥96.0%) was purchased from Chengdu Kelong Chemical Reagents Co., Ltd. (Chengdu, China). Sodium hydroxide (NaOH) and hydrochloric acid (HCl) was provided by Guangzhou Chemical Reagent Factory (Guangzhou, China). The lactate assay kit was bought from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). Conductive carbon ink (solid content–39.8%, fineness–5 μm, viscosity–10 Pa.s, sheet resistance–<60 Ω/square) was purchased from Bohui New Material Technology Co., Ltd. (Xuzhou, China). White plain weave cloth (100% cotton, ~10.1 mg/cm^2, ~10.7 mg/m of thread, ~102 threads per inch of width) was bought from Guangzhou Haiyin Cloth Confluence Co., Ltd. (Guangzhou, China). The crayons (Detong Co., Ltd., Guangzhou, China) and a smoothening utensil were obtained from a local department store.

Design and assembly of the portable ECL system

The proposed ECL system is schematically shown in Fig. 1. It mainly consists of the portable potentiostat (CHI 1024B, Shanghai CH Instruments, China), smartphone (Meizu MX3, Meizu Telecom Equipment Co., Ltd., Zhuhai, China), instrument container, cloth-based device and PC. Here, the instrument container acts as a black box, with an appropriately-sized hole drilled on its cover and a well-designed support installed on its right side. The smartphone is fixed on the cover of instrument container, and its CMOS camera is aligned to the hole and placed right above the WE of cloth-based device. Through two

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specified cables, the potentiostat is connected with the PC and three screen-printed electrodes (SPEs). Due to the wide field-of-view of the smartphone and the unique design of the system, the ECL image can be well captured, without any complicated mechanical movements. In a wireless fidelity (WiFi) way, the ECL images can be transmitted to the PC that can rapidly process these images to obtain the desired analytical graphs. Therefore, the entire ECL system is portable, low-cost and interface-friendly.

Screen-printing fabrication of the cloth-based devices

For fabricating the cloth-based devices, the screen's patterns were designed by Adobe Illustrator (Adobe Systems Inc.), and then delivered to the Lianchang Printing Equipment Shop (Guangzhou, China) to produce the electrode-screen and chamber-screen (300 mesh of polyester with an aluminum frame). As shown in Fig. S1, the cloth was cut into an appropriate size (approximately 75 mm × 100 mm), and then placed below the electrode-screen (Step 1). Subsequently, a squeegee was used to screen-print the carbon ink onto the cloth (Step 2), followed by separating the cloth from the electrode-screen (Step 3) and curing the ink-patterned cloth for 10 min in a 100 ^{o}C oven (DHG-9035A, Tensuclab Instruments Manufacturing Co., Ltd., Shanghai, China) (Step 4). After that, the chamber-screen was placed on the back of the SPEs-containing cloth, and it was rubbed with an orange red crayon, followed by being pressed and rubbed with a smoothening utensil to allow more waxes to print onto the cloth (Steps 5-6). Next, the cloth together with the chamber-screen was placed onto the heating board (YH-946B, Guangzhou Yihua Electronic Equipments Co., Ltd., Guangzhou, China), and heated at 85 ^{o}C for about 2 s to melt the wax

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into the cloth (Step 7). Finally, the chamber-screen and cloth were taken away and separated from each other, and the desired cloth-based devices could be formed (Step 8). And, the microscopic analysis of the devices could be carried out by using a scanning electronic microscope (SEM) (ZEISS Ultra 55, Carl Zeiss, Germany).

ECL assay on the cloth-based devices

Before applying the cloth-based devices for the ECL assays, the EC behavior of SPEs on cloth could be characterized by cyclic voltammetry (CV), where potassium ferricyanide (5.0 mM) in KCl solution (0.5 M) was used as a model electroactive species and the scan rate of 5-40 mV/s was applied to the WE (*versus* the carbon pseudo-reference electrode). When the ECL assays were performed, the device's electrodes were connected to the potentiostat with the connection clips, and then the device was fixed onto the support in the black box. Subsequently, 3 μL of enzyme solution (0.02 unit/μL) was spotted to enzymatically modify the WE. After that, the lactate-containing test solution was added into the ECL chamber for the production of H_2O_2 based on the enzymatic reaction. Next, the luminol-containing substrate solution was dropped onto the chamber, and an appropriate step potential was applied to trigger the ECL reaction. And, the possible mechanism of the luminol/H_2O_2 ECL reaction is as follows [45]:

$$\text{Luminol} - e^- - H^+ \rightarrow \text{Diazoquinone} \quad (1)$$

$$H_2O_2 + \text{Diazoquinone} \rightarrow \text{Luminolendoperoxide} \quad (2)$$

$$\text{Luminolendoperoxide} \rightarrow N_2 + \text{3-aminophthalate}^* \quad (3)$$

$$\text{3-aminophthalate}^* \rightarrow \text{3-aminophthalate} + h\nu \quad (4)$$

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Data acquisition and analysis

During ECL reaction, the imaging function of the smartphone was turned on to capture the ECL images, and the image capture settings were set as follows: shutter speed–1.5 s, ISO–800, exposure compensation-4. The captured images were stored in the JPEG format, and then transferred to the PC by a WiFi network. Each desirable image was cut to be a single picture of 16 mm × 16 mm (length × width) using Adobe Photoshop CS6 software (Adobe Systems Inc.), and it contained the whole light-emitting zone at the WE of the cloth-based device. To perform the quantitative analysis, the intensity of each pretreated image was expressed as the average gray value of the light-emitting region, which was completed by the Matlab R2012a software (Math Works company). Finally, the acquired ECL data were imported into Origin 7.0 (MicroCal Software Inc.) for further analysis.

Results and discussion

Device fabrication and characterization

Based on two facile screen-printing processes, the cloth-based devices could be well fabricated. In the presented case, the array containing six sets of ECL units could be screen-printed on a single cloth piece (Fig. S2), and thus the proposed method could provide a relatively high throughput of device fabrication. It should be noted that the use of the smoothening utensil could cause more waxes to be screen-printed into the cloth and thus the cloth-based devices could be successfully constructed (Fig. S3). Based on the chosen materials, the cost of each cloth-based ECL device was estimated to be about $0.013 (Table

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S1). Note that in this calculation the equipment costs (smartphone, potentiostat, instrument container and PC) were not included. In addition to these features, the sequential screen-printing procedures using the carbon ink and wax could allow the carbon ink-patterned cloth to be rapidly dried in the oven. As a result, the entire screen-printing fabrication process could be completed in less than 20 min, which was much faster than that in our two recent works where the time length of about 5-6 h was required to fabricate the paper- or cloth-based electrochemical chambers [46, 47].

Fig. 2 shows the elements of a typical cloth-based ECL device and their respectively corresponding SEM images. Obviously, the working zones of the as-fabricated device included an approximately toroidal hydrophobic zone (as a wax barrier), a hydrophilic chamber and three electrodes, indicating that the presented fabrication method could provide an acceptable hydrophilic/hydrophobic contrast to define the cloth-based device (Fig. 2A). As shown in Fig. 2B, the gaps between the cotton fibers in the unwaxed zone could be seen clearly, and thus the bare cloth could provide the ECL assay with an excellent hydrophilic microenvironment. However, it is observed in Fig. 2C that these gaps and the surface of individual fibers were covered by wax, indicating that the hydrophobic wax barrier was well formed in the cloth. Besides, a continuous and dense carbon layer on the surface of the cloth was observed (Fig. 2D), showing a good coverage of conducting materials on the surfaces of the cloth cellulose fibers was achieved.

Further, the CV curves of potassium ferricyanide were used to characterize the electrode reactions in the cloth-based devices. Potassium ferricyanide was chosen because it is one of the representative redox species and has been often applied for electrochemical

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characterization of the new-developed device. Characteristic CV voltammograms at various scan rates are shown in Fig. 2E. As can be seen, the peak shapes of the obtained voltammograms showed a typical reversible electrochemical reaction, and moreover the peak current ratios were close to 1 at each scan rate, indicating that no side reactions occurred. The insert in Fig. 2E shows that the cathodic and anodic peak currents varied linearly with the square root of the scan rates ranging from 5 to 40 mV/s, and there was a negligible different (~1.2%) in the slopes of the forward and reverse sweeps, suggesting that the mass transfer in the presented cloth-based ECL devices is a diffusion-controlled process.

Cloth-based ECL assay optimization

In the presented ECL analysis, luminol is electrochemically oxidized and produces the light emission in alkaline solutions at positive potentials when oxygen or H_2O_2 is in the medium, and thus the applied potential should have a significant impact on the luminescent performance. To evaluate this effect, the applied potential of 0-1.2 V was investigated, as shown in Fig. 3A. It has been shown that the ECL intensity increased with increasing the applied potential from 0.3 to 0.9 V, possibly due to the increase in the electrochemical oxidation rate of luminol to form 3-aminophthalate* [39]. When the applied potential was lower than 0.3 V, the ECL intensity was very weak, as similar to a previous report [27]. However, after application of a potential higher than 0.9 V, the ECL signals dropped rapidly, possibly because the electrochemical oxidation of H_2O_2 took place (the oxidation potential of H_2O_2 is typically 1.12 V at pH 7.4 [27]). Therefore, the applied potential of 0.9 V was chosen for the following ECL experiments.

As general knowledge about luminol ECL, high pH (up to 12) in basic solutions is of benefit for higher yielding of luminol radicals (thus ECL intensity), while the enzyme might be deactivated at such high pH [48]. To solve this problem, the proposed ECL assay involves the enzymatic reaction between the enzyme LOx and the test solution for production of H_2O_2 (here, pH was maintained at ~7.4 to maintain the optimal enzyme activity), followed by adding the substrate solution for the electrocatalytic luminol luminescence reactions. As a result, the pH of the substrate solution has to be optimized for the best ECL response. As seen from Fig. 3B, the ECL intensity increased with an increase in the PH value from 8.0 to 10.0, because the luminol ECL reaction is more efficient in basic media [39, 49]. However, a weaker ECL signal was obtained when the pH value was higher than 10.0, since the highly alkaline surroundings would cause the decrease of the fluorescent quantum yield of the phthalate ion [50]. Therefore, pH 10.0 was optimized for the later experiments.

Luminol is used as the luminescence reagent, and its concentration has a great effect on the ECL response. In this study, such an effect has been investigated in the range between 0.5 and 2.5 mM (Fig. 3C). It has been demonstrated that the ECL intensity almost linearly increased with an increase in the concentration of luminol from 0.5 to 2.0 mM. Further increase in the concentration of luminol did not cause any enhancements on the ECL intensity, inversely decreasing lightly. This fact can be explained by the dominating self-quenching effect at higher concentrations of luminol [49]. Therefore, 2.0 mM luminol was used for further experiments.

As we know, the fiber material-based ECL chamber is greatly different from that in traditional ECL assays, in part because it is open and can hold different volumes of reaction

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solution [51]. So, it is necessary to evaluate the effect of the reaction solution's volume on the ECL response. In this analytical case, the test solution and the substrate solution were applied in the same volume to form the assay solution. Such an assay volume was studied, showing that the ECL signal grew when the volume increased (Fig. 3D). An increase from 15 to 35 μL meant an increase of 68.6% in the ECL intensity, because an increase in the volume meant an increase in the amounts of reagents and consequently in the ECL signal. However, higher volume (40 μL) did not cause more enhancements on the ECL signal, possibly because of the dispersion resulted from the lens shape of the deposited drop. So, 35 μL was selected as the optimal volume for this study. This result also indicates that the proposed ECL cloth-based devices may be suitable for volume-tunable biochemical assays which are usually difficult to be performed in traditional ECL microdevices.

Analytical performance

Analytical function and detection limit

Under optimized conditions, the analytical function was obtained by a calibration set composed of 9 standards with 5 replicates. It has been shown that the ECL signals were directly linearly related to the lactate concentrations in the range of 0.05-2.5 mM (Fig. 4). Such an analytical function may be superior to that achieved in some enzymatic lactate biosensors where the signal intensity shows a nonlinear dependence on the lactate concentration in a relatively wide range of concentration [12, 14, 17, 20, 22]. The linear fitting equation could be expressed as $Y = 5.7876X + 3.7918$, with a squared correlation coefficient of 0.9900 ($R^2 = 0.9900$), where Y and X represent the ECL signal and the lactate

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concentration. According to the calibration function, the limit of detection (LOD), which was defined as the concentration that produced a signal three standard deviations higher than the blank, was estimated to be 0.035 mM. This value, as compared with salivary lactate concentration in normal people at rest (about 0.11-0.56 mM) [10], shows that the presented biosensor has the potential to be applied for detection of lactate in saliva in the relevant range of concentrations.

Reproducibility and stability

In our case, the assay reproducibility could be defined as the one within the device of two sets of ECL units and between three different devices, where each cloth-based device was fabricated at three different time intervals and five independent measurements were taken on such devices. It has been shown that the relative standard deviations (RSDs) of five repeated measurements of 0.05, 0.5 and 2.5 mM lactate were 4.7%, 5.2% and 5.0%, respectively. Thus, the reproducibility of the presented assay was acceptable.

The stability of the ECL devices was investigated within 22 days by using a series of as-prepared biosensors and checking their ECL response to a lactate concentration of 1.0 mM. The LOx-modified cloth-based devices were protected from light and preserved at 4 ^{o}C in a dry environment, and were then measured at intervals of two days. In this way, the ECL intensity varied slightly during this period (Fig. 5). After 6, 12 and 18 days, the devices could retain 99.4%, 97.6% and 96.4% of the initial response, respectively. So, the proposed ECL devices had acceptable storage stability and might be suitable for analytical applications in remote areas.

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Selectivity

Since the developed cloth-based devices are expected to be exposed to complex salivary samples, they should offer the selective responses to lactate in the presence of potential interferents in saliva. In the presented work, a coexisting species was considered not to interfere if it caused a relative error of less than 5% compared to the response associated with 1.0 mM lactate. As shown in Fig. 6, all tested species did not lead to any observable interference with measurement of lactate if they were maintained at appropriate concentrations. Actually, the tolerant concentrations of these species were much higher than their normal levels in many samples. For example, as reported previously, the salivary samples from 51 healthy volunteers had an UA concentration of 184.9 ± 78.4 μM [52], and the AA concentration in mixed submandibular and sublingual saliva samples collected from 37 subjects after a 12 h fast was found to be 4.03 ± 3.18 μM [53]. These results showed that the proposed ECL assay had an acceptable anti-interference ability.

Analysis of saliva samples

To verify the feasibility of the proposed method, the ECL cloth-based biosensor was used to detect lactate in human saliva samples. Meanwhile, the commercial spectrophotometer (UV-T6, Beijing Purkinje General Instrument Co., Ltd., Beijing, China), associated with the lactate assay kit, was also used to measure the lactate levels in these samples in parallel. The salivary samples from four volunteers were obtained under fasting conditions and after meals, respectively. As shown in Fig. 7, the salivary lactate levels obtained by two methods were

observed to vary among the subjects, and were randomly increased after meal for all the subjects. These results were in good agreement with some previous studies [12, 54, 55]. Interestingly, the concentration of salivary lactate measured using the proposed method was found to be in acceptable agreement with that obtained on the spectrophotometer. Further, the similarity of the results between these two methods is shown in Fig. S4. The regression equations of $Y = 1.0077X - 0.0120$ ($R^2 = 0.9933$) and $Y = 0.9586X + 0.0196$ ($R^2 = 0.9905$) were obtained for salivary lactate measurements under fasting conditions and after meals, respectively. It has been shown that the statistically significant difference estimated by the common *F*-test showed no significant difference (at the 95% confidence level) between the results of the two methods. And, the difference in error bar of each data point and the difference of the slope from unity might result from differences in preparation of the standards. Notably, the presented ECL assay did not require any sample pretreatment or dilution steps, and the obtained salivary samples were directly applied for their lactate measurements. Therefore, relative to traditional enzymatic colorimetric methods, the total procedure of the proposed method was simple, rapid, and usually did not require skilled laboratory personnel for equipment handling and analysis.

Comparison between the proposed ECL method and other biosensors

As mentioned above, other lactate biosensors using various detection methods have been well developed for a series of applications (Table 1). As seen from Table 1, LOx and LDH are commonly used enzymes in the design of lactate biosensors because of their simple enzymatic reaction and relatively easy sensor design configuration. Recently, paper-based

colorimetric biosensors using these two enzymes have been reported for lactate determination [8, 9]. They produce visual results, and are usually stable, inexpensive, portable, and simple to perform. However, these colorimetric biosensors still have some limitations such as inhomogeneity of colour distribution, poor sensitivity, and as well being influenced by the local light conditions and the background noise of the sample. Fluorescence biosensors rely on the measurement of the luminescence of a substrate on excitation by an appropriate light source. Therefore, they often need a relatively complex and expensive optical detection system [7]. Similarly, spectrophotometry can also be used to detect lactate [6, 7]. However, this technique has some limitations such as longer time period for sample analysis, and involvement of tedious processes and higher cost [15]. Compared with the above-mentioned methods, electrochemical lactate biosensors have been relatively widely developed in recent years [10-19]. These biosensors have some advantages such as rapidness, cost-effectiveness, being amenable to miniaturization, and having compatible instrumental sensitivity and the ability to operate in turbid media. However, a poor coupling of biochemical recognition materials and electrochemical transducers can affect selectivity, sensitivity, dynamic range and stability of electrochemical biosensors [1]. Detection based on chemical luminescence [20-25] can combine sensitivity and simplicity, making it very suitable for simple and portable analytical devices. Nevertheless, CL detection has rarely been used in disposable sensors, because of the need to immobilize all the chemistries required and the time control to trigger the sensing reactions upon contact with the sample [27]. ECL is a very convenient technique that combines the advantages of CL and electrochemical methods. Importantly, ECL combines the sensitivity of light-emitting reactions with easy control of the reaction.

Unfortunately, ECL has rarely been used for lactate determination in a disposable and portable format. Capitán-Vallvey and co-workers have described a disposable ECL biosensor for detection of lactate in saliva [27]. In their work, all the required reagents are immobilized by a membrane placed on the working electrode of the commercially available screen-printed electrochemical cell, and therefore the biosensor preparation procedure is relatively complicated. Moreover, ECL emission is measured using an expensive and bulky PMT detector interfaced by a photo-counting unit, and thus it may be difficult to perform the ECL assays in a portable format. Recently, Su and co-workers have reported an ECL biosensor array with a wheel-like pattern for glucose, lactate and choline [26]. Although multiple optical signals from micro-cells can be captured by a CCD camera for multiplex analysis, the fabrication of the array chip involves expensive apparatuses and complex processes. Different from these two reported lactate biosensors [26, 27], an inexpensive and portable ECL system in the presented work has been developed for non-invasive detection of lactate in saliva, where the low-cost and disposable cloth-based devices can be readily fabricated by two facile screen-printing processes. It can be seen from Table 1 that the detection sensitivity and dynamic range obtained using the proposed ECL method are comparable to or better than those achieved in some other biosensors that often require an expensive and complicated detection system or sensor preparation.

Future research directions

Based on its detection sensitivity, the proposed cloth-based biosensor should have wide potential applications in the future. Taking into account normal lactate levels of 0.5-2 mM in

blood, 1.9-2.2 mM in interstitial fluid, and 1-5 mM in tear fluid [56], the presented biosensor can be used for determination of lactate in other body fluids, such as measurement of lactate in human serum (Fig. S5). Similar to other reported cases in Table 1, the proposed method can also be applied for measurement of lactate in some food samples. In addition, other analytes can be detected by applying different enzyme modifications onto the cloth-based devices. For example, the proposed biosensor can be applied for detection of glucose in human urine (Fig. S6). Notably, the measurement of glucose is an important analysis in many biochemical and medical applications, and glucose biosensors account for about 85% of the entire biosensor market [57].

Although the presented cloth-based biosensor has some abovementioned advantages, it also has some limitations, including inconvenient signal acquirement using multistep procedures, the use of relatively expensive potentiostat, the detection limit of up to μM ranges, and singleplex analysis of biological samples. Fortunately, these limitations can be overcome in further developments or improvements. For example, it may be possible to apply a smart APP software to automate the smartphone-based imaging and data analysis. Moreover, it is feasible to generate and control ECL using a smartphone coupled with a certain integrated circuit [31]. Ideally, the proposed ECL device should be integrated with cloth-based electronic elements to construct a fully integrated ECL device. In addition to these, the inherent advantages of cloth-based microfluidics and smartphone-based imaging can allow the arrayed cloth-based devices to be developed for multiplex analysis. In future, the electrode modification strategy using nanoparticles or other materials [1, 10, 11, 15, 17, 26] can be explored to improve the sensitivity. In short, the potential limitations can be

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overcome, and the proposed ECL method may find new applications in chemical and biomedical fields.

Conclusions

In this work, a cloth-based ECL biosensor with smartphone-based imaging has been proposed for the first time. The developed ECL system is low-cost, portable, and may become a potential candidate for fast, accurate and affordable point-of-care diagnostics. In the presented case, the luminol/H_2O_2-based ECL reaction system has exhibited a desirable detection of lactate. After several important factors are optimized, the sensitive lactate measurement has been obtained. The detection limit of lactate can be as low as to 0.035 mM, with acceptable dynamic range, reproducibility, stability and selectivity. Especially, the obtained detection sensitivity and dynamic range are comparable or superior to those in some other biosensors. Moreover, the proposed method has been applied for simple and rapid detection of lactate in saliva, without the need of complicated sample pretreatment procedure. Therefore, looking to the future, the developed ECL method may find broad applications such as health diagnosis, food detection and environmental monitoring.

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Table 1. Comparison of the presented biosensor and other reported in the literature.

Method	Enzyme	LOD (mM)	Range (mM)	Detector	Application	Ref.
Colorimetry	LOx	0.1	0.6-10	Smartphone	Saliva	[8]
Colorimetry	LDH	0.5	0.5-8	Scanner	NA	[9]
Fluorimetry	LDH	0.11	0.55-1.65	FIA system with a diode array	Wine	[7]
Spectrophotometry	LDH	0.56	1.12-11.2	FIA system with a spectrophotometer	Wine	[7]
Spectrophotometry	LOx	0.66	2.23-22.3	SIA system with a spectrophotometer	Wine	[6]
Chronoamperometric	LOx	0.01	0.025-0.25	Potentiostat	Saliva	[10]
Chronoamperometric	LOx	0.013	0.1-1	Electrochemical workstation	Saliva	[11]
Chronoamperometric	LOx	0.04	NA-0.2	Potentiostat	Wine and beer	[17]
Chronoamperometric	LOx	0.05	0.1-2.5	Potentiostat	Saliva and blood	[18]
Chronoamperometric	LDH	0.05	0.2-2	Potentiostat	NA	[15]
Chronoamperometric	LDH	0.05	0.075-1	Potentiostat	Wine	[19]
Chronoamperometric	LOx	0.05	0.1-1	Potentiostat	Saliva	[13]
Chronoamperometric	LOx	0.3	0.1-25	Potentiostat	Saliva	[12]
pH-based ElecFET	LOx	~0.05	1-6	ChemFET-meter and DC supply	NA	[14]

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Voltammetric	NA	1.54	11.9-188	Potentiostat	NA	[16]
CL	LOx	0.0092	0.05-4	PMT	Yoghurt	[22]
CL	LOx	0.017	0.1-1.5	FIA system with a spectrophotometer	Yoghurt	[24]
CL	LOx	0.04	NA-10	FIA system with a PMT	Reconstituted whey	[25]
CL	LOx	0.5	0.5-5	FIA system with a photodiode	Serum	[23]
CL	LOx	0.5	0.5-10	Smartphone	Saliva	[20]
CL	LOx	1	1-50	FIA system with a photodiode	Food	[21]
ECL	LOx	0.005	0.01-0.5	PMT	Saliva	[27]
ECL	LOx	0.04	0.04-2	CCD camera	NA	[26]
ECL	LOx	0.035	0.05-2.5	Smartphone	Saliva	This work

Note: LOD-limit of detection; LOx-lactate oxidase; LDH-lactate dehydrogenase; NA-not available; FIA-flow injection analysis; SIA-sequential injection analysis; ElecFET-electrochemical field effect transistor; CL-chemiluminescence; PMT-photomultiplier tube; ECL-electrochemiluminescence; and CCD-charge-coupled device.

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Figure Captions

Fig. 1. Schematic representation of the proposed portable ECL system. It mainly includes: (1) smartphone, (2) instrument container, (3) specified cables, (4) potentiostat, (5) wireless transmission of ECL signal, (6) cloth-based device which is fixed on a support, and (7) PC.

Fig. 2. Characterization of the ECL cloth-based devices. (A) Front (I) and back (II) photographs of a typical cloth-based ECL device. (B-D) SEM images of the cloth-based device. Panels (B), (C) and (D) show the structures of the pure cloth (in the hydrophilic chamber), wax-screen-printed cloth (in the hydrophobic zone), and carbon ink-screen-printed electrodes. (E) Cyclic voltammograms of 5.0 mM potassium ferricyanide in 0.5 M KCl solution using the proposed cloth-based devices at scan rates of 5, 10, 20, 30 and 40 mV/s. The relationship between the peak current and the square root of the scan rate is shown in the insert, where the fitted linear regression equations were $Y = 5.01\ X - 3.88$ ($R^2 = 0.9977$, cathodic) and $Y = -5.07\ X + 3.70$ ($R^2 = 0.9913$, anodic). The error bar represents the standard deviation from five independent measurements.

Fig. 3. The dependence of ECL intensities on the applied potential (A), pH of substrate solution (B), luminol concentration (C), and volume of assay solution (D). In panel (A), Applied potential: 0, 0.3, 0.6, 0.7, 0.8, 0.9, 1.0 and 1.2 V; pH of substrate solution: 10.0; Luminol concentration: 2.5 mM; and Volume of assay solution: 20 μL. In panel (B), Applied potential: 0.9 V; pH of substrate solution: 8.0, 9.0, 9.5, 10.0, 10.5, 11.0 and 12.0; Luminol concentration: 2.5 mM; and Volume of assay solution: 20 μL. In panel (C), Applied potential: 0.9 V; pH of substrate solution: 10.0; Luminol concentration: 0.5, 1.0, 1.5, 2.0 and 2.5 mM; and Volume of assay solution: 20 μL. In panel (D), Applied potential: 0.9 V; pH of substrate

solution: 10.0; Luminol concentration: 2.0 mM; and Volume of assay solution: 15, 20, 25, 30, 35 and 40 μL. In panels (A)-(D), the concentration of lactate was 1.0 mM. The error bar represents the standard deviation from five independent measurements.

Fig. 4. Relationship between the ECL response and the lactate concentration. Assay conditions: Applied potential: 0.9 V; pH of substrate solution: 10.0; Luminol concentration: 2.0 mM; Volume of assay solution: 35 μL; and Lactate concentration: 0, 0.05, 0.25, 0.5, 0.75, 1.0, 1.5, 2.0 and 2.5 mM. The error bar represents the standard deviation from five independent measurements.

Fig. 5. Storage stability of the presented ECL devices. Assay conditions: Applied potential: 0.9 V; pH of substrate solution: 10.0; Luminol concentration: 2.0 mM; Volume of assay solution: 35 μL; and Lactate concentration: 1.0 mM. The error bar represents the standard deviation from five independent measurements.

Fig. 6. Evaluation of selectivity for lactate determination on the presented ECL devices. Assay conditions: Applied potential: 0.9 V; pH of substrate solution: 10.0; Luminol concentration: 2.0 mM; Volume of assay solution: 35 μL; and Lactate concentration: 1.0 mM. The error bar represents the standard deviation from five independent measurements.

Fig. 7. The measurement of lactate in saliva during fasting and after a meal using the proposed method and the spectrophotometry. The error bar represents the standard deviation from five independent measurements.

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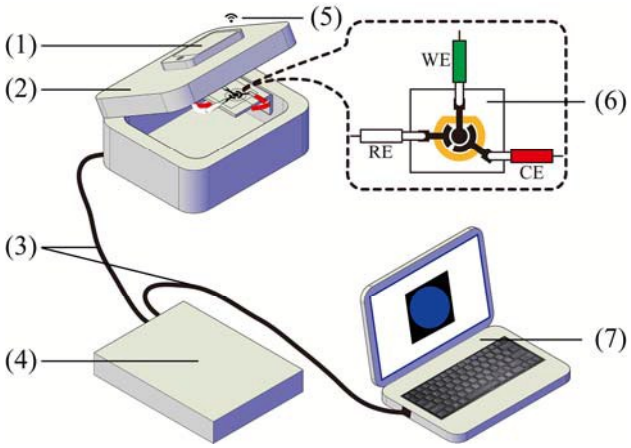

Fig. 1

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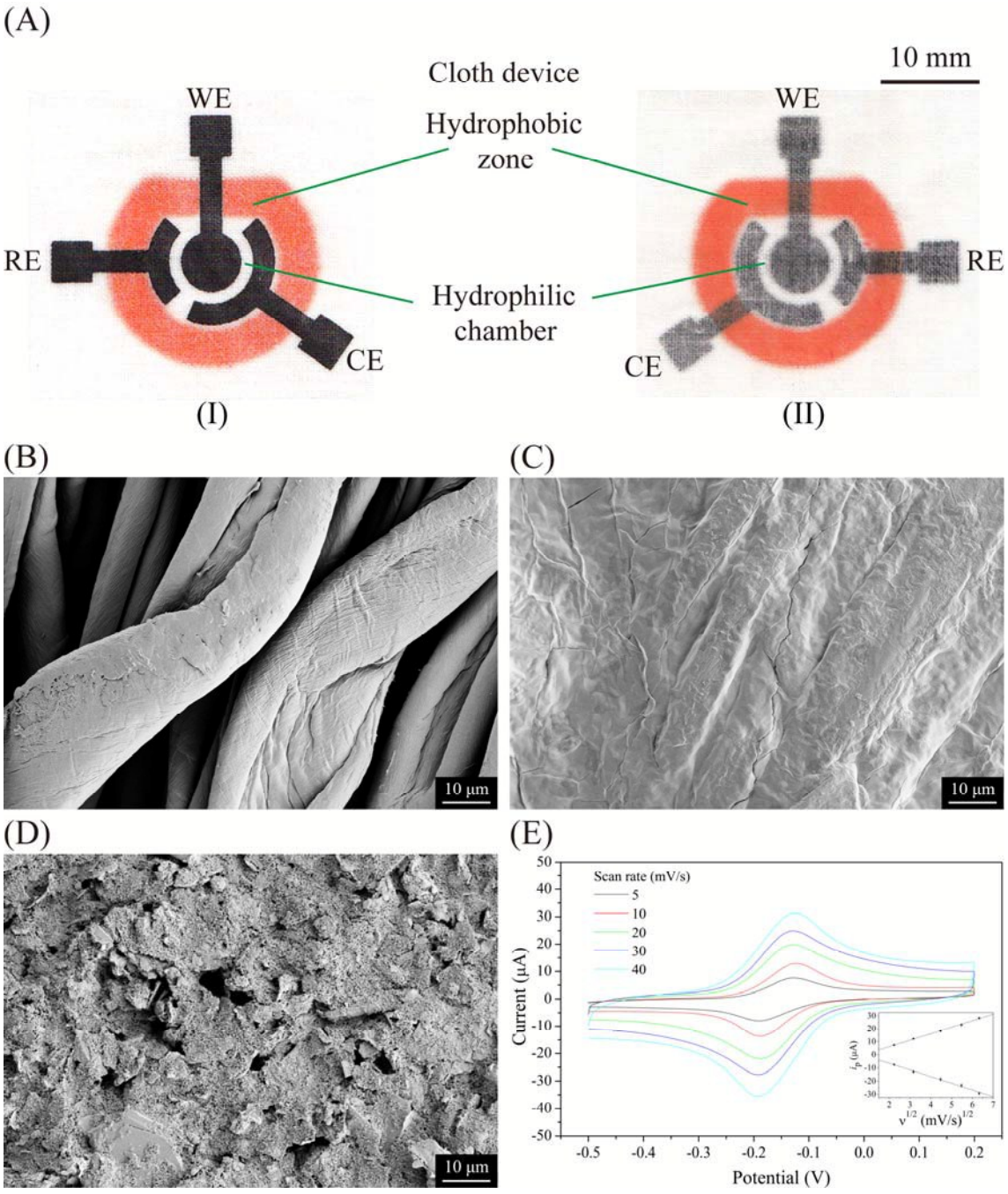

Fig. 2

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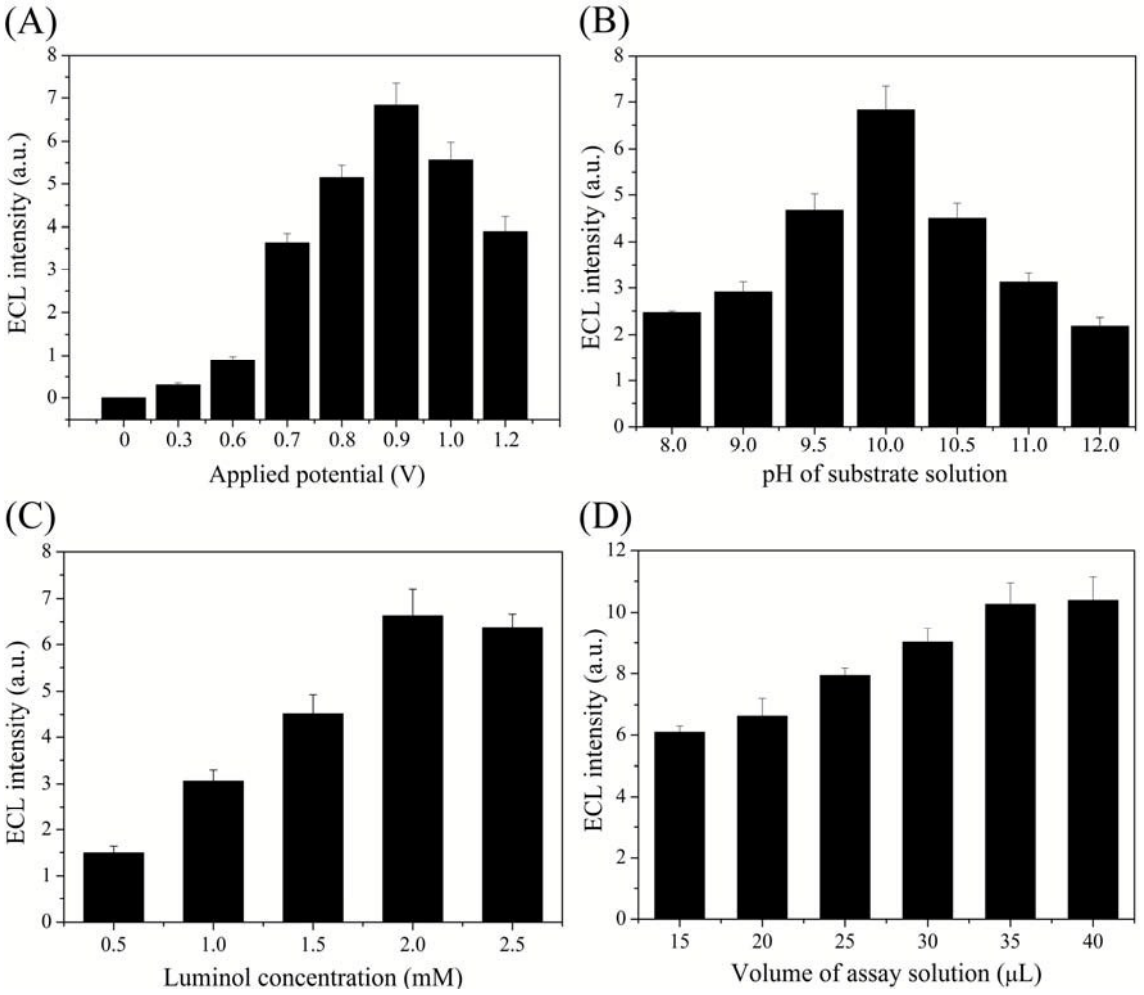

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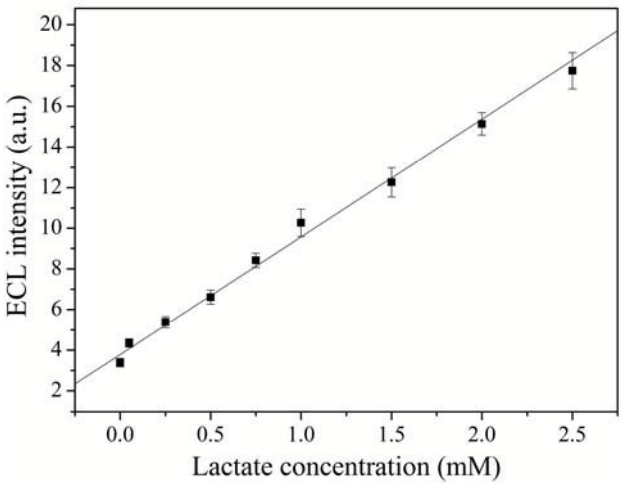

Fig. 4

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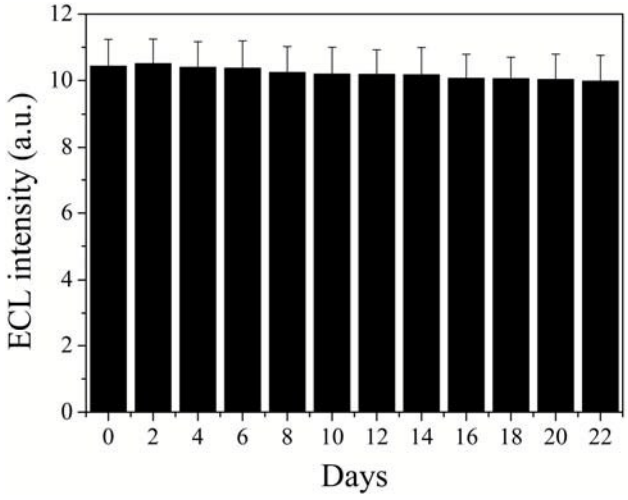

Fig. 5

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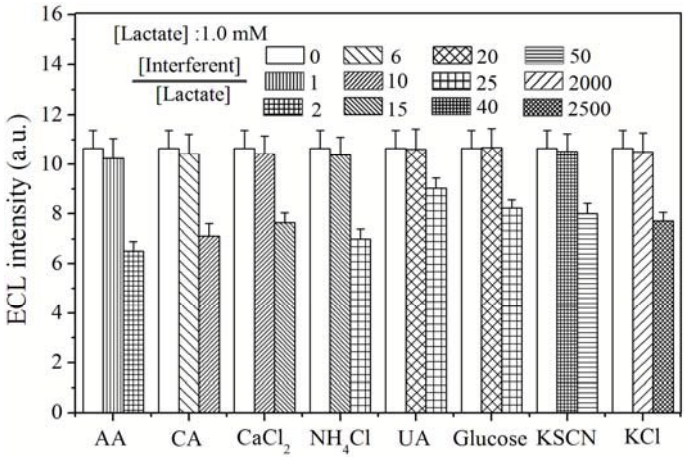

Fig. 6

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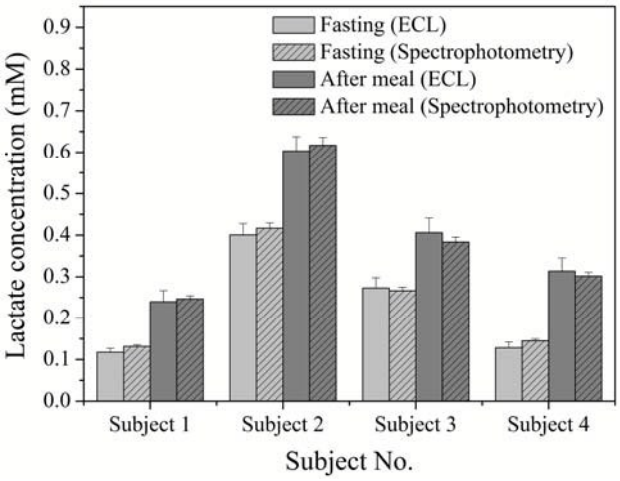

Fig. 7