**Showcasing research from the Bioinspired Engineering and Biomechanics Center (BEBC), Xi'an Jiaotong University, Xi'an, P. R. China.**

Direct writing electrodes using a ball pen for paper-based point-of-care testing

A simple strategy for directly writing electrodes on paper using a pressure-assisted ball pen is reported and applied to fabricate paper-based electrochemical devices (PEDs), which are successfully employed in point-of-care detections of glucose and melamine in sample solutions.
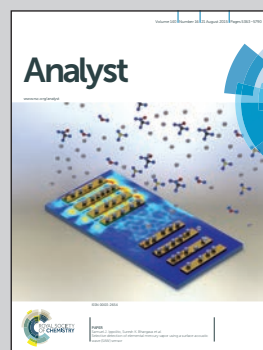
ROYAL SOCIETY
OF **CHEMISTRY**

www.rsc.org/analyst

Direct writing electrodes using a ball pen for paper-based point-of-care testing†

Zedong Li,[a,b] Fei Li,*[b,c] Jie Hu,[a,b] Wei Hong Wee,[a,b,d] Yu Long Han,[a,b] Belinda Pingguan-Murphy,[d] Tian Jian Lu[b] and Feng Xu*[a,b]

The integration of paper with an electrochemical device has attracted growing attention for point-of-care testing, where it is of great importance to fabricate electrodes on paper in a low-cost, easy and versatile way. In this work, we report a simple strategy for directly writing electrodes on paper using a pressure-assisted ball pen to form a paper-based electrochemical device (PED). This method is demonstrated to be capable of fabricating electrodes on paper with good electrical conductivity and electrochemical performance, holding great potential to be employed in point-of-care applications, such as in human health diagnostics and food safety detection. As examples, the PEDs fabricated using the developed method are applied for detection of glucose in artificial urine and melamine in sample solutions. Furthermore, our developed strategy is also extended to fabricate PEDs with multi-electrode arrays and write electrodes on non-planar surfaces (*e.g.*, paper cup, human skin), indicating the potential application of our method in other fields, such as fabricating biosensors, paper electronics *etc*.

Introduction

Due to the advantages of portability, ease of use, short turnaround time, trace sample consumption and low cost,[1] point-of-care testing (POCT) has recently attracted increasing attention in rapid diagnostics and monitoring for human health,[2,3] food safety and environmental pollution,[4] especially in resource-limited settings.[5,6] The core working principle of POCT is to convert the information of target analytes to more easily detectable signals, such as optical and electrochemical signals, and then record and analyze. Among various detection methods, electrochemical detection offers the advantages of low cost, high sensitivity, good selectivity, rapid readout and only needing minimal instrumentation.[7] Compared to the optical detection methods, it holds the special advantage of being applicable in a turbid environment or samples with suspended solids, which are common states of samples in POCT.[8] Therefore, much effort has been made on developing various electrochemical detection devices to meet the application demand of POCT in different fields.[9–11]

A typical electrochemical detection device is composed of two parts: electrode and substrate. Although significant efforts have been put on developing electrodes with various compositions (*e.g.*, carbon and metal based materials) and shapes (*e.g.*, disk, ring, band), little attention has been paid to the substrate materials.[12,13] However, taking the hand-held blood glucose meter as an example, the practical application of POCT demands the exploration of the electrochemical detection device which is low cost, consumable and disposable. Paper, a kind of ubiquitous, low-cost, disposable and recyclable material, has been extensively used as a substrate for fabricating test strips, such as pH test paper and urinalysis test pads.[13] Recently, owing to the power-free fluid transport *via* capillary action and the ability to store reagents within the fiber network, paper has been applied as a substrate material for microfluidic devices.[14] Besides, the properties of high electrical resistance and high flexibility also make paper a promising substrate for electrochemical devices.[15,16] Therefore, electrodes have been recently integrated on paper to fabricate

[a] The Key Laboratory of Biomedical Information Engineering of Ministry of Education, School of Life Science and Technology, Xi'an Jiaotong University, Xi'an 710049, P.R. China. E-mail: feili@mail.xjtu.edu.cn, fengxu@mail.xjtu.edu.cn

[b] Bioinspired Engineering and Biomechanics Center (BEBC), Xi'an Jiaotong University, Xi'an 710049, P.R. China

[c] Department of Chemistry, School of Science, Xi'an Jiaotong University, Xi'an 710049, P.R. China

[d] Department of Biomedical Engineering, Faculty of Engineering, University of Malaya, 50603 Kuala Lumpur, Malaysia

†Electronic supplementary information (ESI) available: Photographs of the designed PMMA masks (Fig. S1), photographs and resistances of five carbon electrode lines written by five different individuals using the proposed pressure-assisted ball pen device (Fig. S2), photographs of electrode patterns written on a Whatman BA95 paper, a Millipore HF180 paper and human skin, and the recorded CV curves of FcMeOH redox reaction using a PED on a Whatman BA95 paper (Fig. S3), photograph of the combination of the fabricated pressure-assisted ball pen device and a commercial plotter (Fig. S4), CV curves of FcMeOH redox reaction using PEDs with Ag QRE or Ag/AgCl RE (Fig. S5), and different sizes of carbon CEs (Fig. S6). See DOI: 10.1039/c5an00620a

paper-based electrochemical devices (PEDs) for detecting various health related markers (*e.g.*, glucose, lactate, and uric acid), proving that PEDs hold great potential for POCT.[17] For instance, paper-based electrochemiluminescence and electrochemical devices have been developed to detect tumor markers (*e.g.*, carbohydrate antigen 199) and sulfonamide antibiotics (*e.g.*, sulfapyridine).[18,19] To further achieve the practical application of PEDs in POCT, it is of great importance to fabricate electrodes on paper in a low-cost, easy, and versatile way.

The quality of the electrodes has significant effects on the performance of PEDs. Several methods have been used to fabricate electrodes (*e.g.*, carbon and gold electrodes) on paper, including sputtering,[20] sintering,[21] inkjet printing,[22,23] laser etching[24] and screen printing.[8,17,25–29] However, all these methods need special instruments, limiting their accessibility for POCT applications. More recently, direct writing with commercial pencil leads[18,30,31] and doped pencil leads[32,33] has been proposed for fabricating PEDs. However, the exfoliation of pencil lead on paper is difficult to be controlled, which may affect the stability and performance of the prepared PEDs.[34] In addition to pencil, the ball pen as another writing technique has also shown great ability to write conductive materials (*e.g.*, silver ink and Cu-metal ink) on papers,[35,36] since ball pens are compatible with various conductive inks and well suited to mark various patterns on papers. But the application of the ball pen writing method for fabricating PEDs has not yet been explored. Besides, different conductive inks have different viscosities, which has a direct effect on their conductivity during writing. However, for the direct writing technique with the ball pen, there is a strict requirement on the ink viscosity (~1–10 Pa s) for the ink to smoothly flow through the roller ball. Therefore, it needs an additional step of special pretreatment of conductive inks (*e.g.*, dilution) to tune their viscosity,[35] which is time-consuming and has high costs limiting their accessibility for POCT applications. Therefore, there is an unmet need to improve the ball pen technique for writing electrodes on paper with broad robustness.

In this work, we developed a direct writing method based on a self-designed hand-held pressure-assisted ball pen for PED fabrication, where conductive inks could flow through the roller ball smoothly without the need for any ink pretreatment. The conductivity, flexibility, surface morphology and electrochemical performance of the fabricated PEDs were characterized. The fabricated PEDs were successfully applied to detect glucose in artificial urine for human health and melamine for food safety, indicating the potential of this ball pen writing technique for making PEDs for POCT applications. Compared with the traditional methods of fabrication of PEDs, our writing method holds the advantages of being simple, low-cost, portable and easy to use and has the capability of writing electrode patterns of any shape. In addition, the applications of our developed strategy for fabricating PEDs with multi-electrode arrays and writing electrodes on non-planar surfaces and also human skin were also demonstrated.

Experimental

Chemicals and materials

Carbon ink (Electrodage 423ss) and silver (Ag) ink (C1002) were purchased from Acheson (USA) and Acheson (Korea), respectively. Syringe (5 mL), ball pen (ball size of 1.0 mm), A4 text papers (70 mg per page), paper cup, coin cell and light-emitting diode (LED) were all purchased from local stores. Polymethylmethacrylate (PMMA) boards were obtained from Legend Technology Ltd (HK, China). Ferrocene methanol (FcMeOH), sodium chloride, disodium hydrogen phosphate, sodium phosphate monobasic, D-(+)-glucose, glucose oxidase, potassium ferricyanide ($K_3Fe(CN)_6$), melamine and uric acid were all of analytical grade and obtained from Sigma-Aldrich. Artificial urine solutions were prepared following the protocol in the literature.[37] All aqueous solutions used in this work were prepared from Milli-Q reagent water (Millipore Corp., resistivity of 18.2 MΩ cm at 25 °C).

Design and fabrication of pressure-assisted ball pen device

The pressure-assisted ball pen device is composed of two parts (Fig. 1a and e): a ball pen and a pressure-assisted device, in which the ball pen was connected to the tip of the pressure-assisted device. Before reloading with carbon or silver ink, the ball pen was firstly pre-emptied by washing using water, then wiping using tissue to clean the residual oil on the inner wall
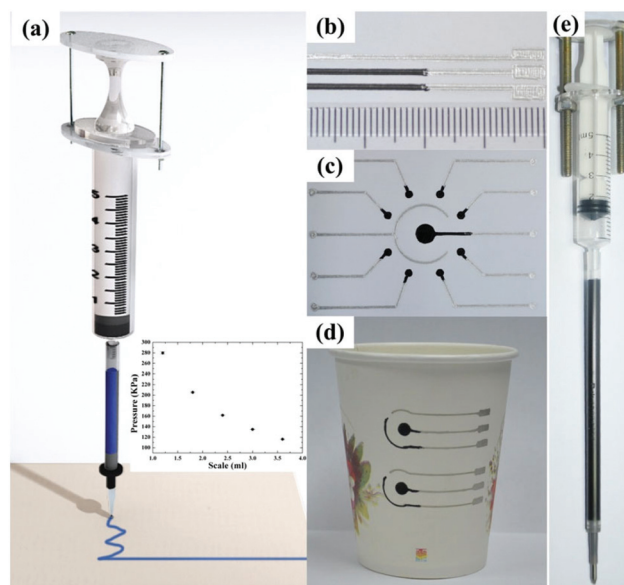

Fig. 1 (a) Schematic of direct writing electrodes on paper using a pressure-assisted ball pen. The inset represents the relationship between the pressure inside the syringe measured by a pressure meter with a syringe scale. (b–d) Photographs of the fabricated PEDs: (b) a PED on an A4 paper with a three-electrode system of carbon WE, Ag QRE and carbon CE; (c) a PED on an A4 paper with an eight-electrode array of eight carbon WEs and a communal Ag QRE and a communal carbon CE; (d) a PED on a paper cup with two three-electrode systems similar to (a) except with different electrode shapes; (e) photograph of the fabricated pressure-assisted ball pen.

of the pen reservoir, and washing again using water. The pressure-assisted device was an assembly of a 5 mL syringe and two PMMA boards, which were designed with Corel Draw 12 software and cut by a laser cutter (Versa LASER VLS3.50), with two screws and two nuts immobilized on the plunger of the syringe.

Design and fabrication of PEDs

The PEDs fabricated on A4 text papers with a standard three-electrode system (*i.e.*, carbon electrodes as the working electrode (WE) and the counter electrode (CE), silver electrode as the quasi-reference electrode (QRE)) were hand-written by the pressure-assisted ball pen device. A4 papers were used as received. Firstly, a PMMA mask, designed with Corel Draw 12 software and fabricated by the laser cutter for accurate control of the size and shape of the written electrode patterns, was placed on the paper substrate. The Ag conductive line and Ag quasi-reference electrode on paper were prepared by writing with Ag inks, and the paper was placed into an oven at about 120 °C for 30 min to cure the Ag inks. Successively, carbon inks were written on paper as the working electrode of the PEDs, and the paper was placed into an oven once again at 70 °C for 30 min to cure the carbon inks. A similar process of using PMMA masks (Fig. S1a and b†) has also been applied to fabricate an eight-electrode array on an A4 text paper and a three-electrode system on a paper cup. Writing electrodes on a Whatman BA95 paper and a Millipore HF180 paper using our device were helped with a ruler. Owing to the high porosity property, the Whatman BA95 paper and the Millipore HF180 paper were treated with wax to form hydrophobic barriers after writing.

To detect the glucose in artificial urine using the fabricated PEDs combined with a commercial glucometer (Sanrupid glucometer, Safe Accu, China), the PEDs were specially designed referring to the electrode material and the geometry of commercial test strips of the glucometer. The fabrication process of the PEDs was the same as described above but the electrode material was changed to only carbon, as used in the commercial test strip. According to the reported method,[38] a filter paper sheet (Whatman #1) of 5 mm width and 10 mm length was used to cover the written electrode surfaces. The reagents of glucose oxidase and $K_3Fe(CN)_6$ were added to the filter paper over the written electrode surfaces, *i.e.*, 8 µL of 600 mM $K_3Fe(CN)_6$ solution was dropped on the filter sheet, allowed to dry at room temperature and then treated with 500 U mL^{-1} glucose oxidase.

For preparing the PEDs for the electrochemical detection of melamine in PBS solution, the three-electrode system with a carbon working electrode was written on an A4 paper. Before electrochemical detection of melamine, the prepared PEDs were equilibrated in 0.1 M PBS (pH = 8) by applying a potential between −1.0 and 1.0 V and cycling for 20 segments, and then they were anodized by applying a potential of 2.0 V for 200 s.[39]

Characterization of conductivity and surface morphology of PEDs

The resistances and surface morphology of the written carbon and Ag electrodes on PEDs were measured by a multimeter (UT58A, UNI-T, China) and imaged by a field emission scanning electron microscope (JSM-6700F), respectively.

Electrochemical measurements

Cyclic voltammetry (CV) and differential pulse voltammetry (DPV) measurements were performed by using a CHI852D electrochemical workstation (Chenhua Instrument Ltd, China). For the electrochemical characterization of the prepared PEDs, cyclic voltammetry experiments were carried out in an aqueous solution with 1.0 mM FcMeOH and 0.1 M NaCl, and the potential was scanned from −100 mV to 400 mV (*vs.* Ag QRE) with various scan rates (10 mV s^{-1}, 20 mV s^{-1}, 50 mV s^{-1}, 75 mV s^{-1} and 100 mV s^{-1}, respectively).

The electrochemical measurements of glucose concentration in artificial urine were performed using a commercial glucometer combined with the fabricated PEDs as the test strips. When the target glucose solutions were introduced on the filter paper sheets of the PEDs, the concentration of glucose could be rapidly displayed on the glucometer screen due to the capillary action of the porous filter paper carrying the reagents to the working electrode of the PEDs.

For the detection of melamine in PBS solution using PEDs, the differential pulse voltammetry was applied to measure the melamine concentration in the solution with uric acid as the indicator. According to the reported protocol,[39] DPV measurements were performed in the sample solution with a pulse amplitude of 50 mV, a pulse width of 60 ms, and a step height of 4 mV in the potential range from −100 mV to 600 mV (*vs.* Ag QRE). Before each DPV experiment, a preconditioning step at a potential of 2.0 V was applied at the PEDs for 200 s.

Results and discussion

Proof of concept of the pressure-assisted ball pen device

In the fabricated pressure-assisted ball pen device, the syringe serves as a pressure supplier by moving its plunger to compress air in the chamber, where the pressure can be simply controlled according to the ideal gas equation of state ($P \propto 1/V$). That is, by adjusting the syringe scale (also volume (V) in this case), the pressure inside the syringe (P) can be monitored and loaded to push the conductive inks with different viscosities out from the ball pen to the paper substrate surface during writing. As shown in the inset of Fig. 1a, by moving the plunger of the syringe to different scales (1.2 mL, 1.8 mL, 2.4 mL, 3.0 mL and 3.6 mL), the pressure measured by a pressure meter changes accordingly (116.2 kPa, 134.9 kPa, 161.7 kPa, 205.4 kPa and 279.7 kPa, respectively), which approximately agrees with the relationship described in the equation of $P \propto 1/V$. It proves our concept of combining a pressure system with a ball pen to form a pressure-assisted ball pen device, so that the conductive inks with different viscosities can be written on paper.

Versatile writing of electrodes on papers using the pressure-assisted ball pen device

Using the above developed pressure-assisted ball pen device, the conductive ink inside the pen reservoir can be smoothly written on paper under a certain pressure, and the pressure of

the syringe was maintained in a relatively stable range until the writing ended. Taking a pressure of 252.5 kPa and carbon as a conductive ink as an example, the fabricated pressure-assisted ball pen device could write approximately 120 electrodes until all the conductive ink in the pen reservoir was used up. Moreover, through comparing the patterns and resistances of five carbon electrode lines written by five different individuals using our fabricated pressure-assisted ball pen device (Fig. S2†), the good reproducibility of our device in writing electrodes on paper is also proved.

A typical PED fabricated by our direct writing technique using the pressure-assisted ball pen device is shown in Fig. 1b. In the figure, the top, middle and bottom lines are the Ag quasi-reference electrode, the carbon working electrode and the carbon counter electrode, respectively. The average sizes of the three electrode patterns are: *ca.* 2.0 cm length and *ca.* 0.91 mm width for the carbon WE and carbon CE, *ca.* 2.0 cm length and *ca.* 0.87 mm width for the Ag QRE. The distances among the electrodes are *ca.* 1.33 mm. For better connecting the electrodes to the electrochemical workstation cables during electrochemical measurements, the conductive Ag lines and Ag "caps" were written under the carbon WE/CE and the ends of the three electrodes, respectively. The conductivity, flexibility, surface morphology and electrochemical behavior of the fabricated PEDs were measured and are described next. The one shown in Fig. 1b was used as a representative PED in the following experiments, except for specially mentioned.

Besides fabricating a simple three-electrode system on an A4 paper, the recent demand of simultaneous determination of various targets in complex samples in the POCT field also requires the fabrication of sensing arrays with high throughput detection function, such as PEDs with multi-electrode compositions. Herein, to investigate the versatility of our method in writing electrode array, a composite eight-electrode array system was also written on paper with our pressure-assisted ball pen. As shown in Fig. 1c, the fabricated composite electrode system consists of eight carbon working electrodes (the surrounding black electrodes), a communal silver quasi-reference electrode (the center electrode with a half-circle shape) and a communal carbon counter electrode (the center black electrode). This illustrates that this hand-held pressure-assisted ball pen is able to fabricate a complex multi-electrode system with multi-component materials. Moreover, papers with high porosity have been widely applied as PED substrate materials.[13] Besides A4 paper, we also tried writing electrodes on two other kinds of papers with high porosity, *i.e.*, Whatman BA95 paper and Millipore HF180 paper. The obtained PEDs and the CV results (Fig. S3a, b and d†) prove the feasibility of our proposed method to write electrodes on other paper substrates. In addition, patterning electrodes on non-planar or curved surfaces, such as human skin, is of great significance for fabricating wearable or flexible electronics for personal healthcare. Therefore, to further demonstrate the ability of fabricating electrodes on a non-planar or curved surface using our method, we patterned a composite with two three-electrode systems on a paper cup surface (Fig. 1d) and also on human

skin (Fig. S3c†). In Fig. 1d, the center carbon WE is surrounded by the Ag QRE and carbon CE. Besides, through further combining our device with a commercial plotter controlled by a computer (Fig. S4†), the large production of PEDs using our method could be realized for future applications, which is comparable to the traditional screen-printing technique. Furthermore, hand writing with a pen is suitable and comfortable to fabricate a prototype for concept proofing before mass production and endows the end users the capability to design and manufacture "on-demand" sensors for "on-site" applications. In contrast to the stamp method,[40] our writing method is simple, low-cost, portable and easy to use without the need for any mould and can write an electrode pattern with any shape and on both planar and non-planar surfaces. All the materials are commercially available in local stores, making the fabrication of PEDs quite easy. These advantages provide our method unique simplicity, flexibility and robustness. Moreover, it is also versatile for conductive inks with different viscosities. For example, by loading with appropriate inks such as silver based conductive inks, our device holds great potential in fabricating paper electronics other than only electrochemical devices.[41] With a pressure-assisted device, the ink can flow through the roller ball pen fluently without the need for ink pretreatment. In addition, the device is also cost-effective (~1 RMB). All these would also have a high impact on paper electronics written by the pen based techniques.

Characterization of conductivity, flexibility and surface morphology of the fabricated PEDs

The electrode fabricated through direct writing on paper may raise concerns about its conductivity and mechanical flexibility due to the properties of the paper substrate and surface conditions.[35] The conductivity and the mechanical flexibility of the carbon and Ag electrodes written on paper by our method were checked through using the electrodes as circuits to connect a LED to a coin cell (Fig. 2a and b). When putting the carbon or Ag electrode line into contact with the coin cell, the closed loop circuit was formed and the LED was then turned on (Fig. 2c and d), which proves the good conductivity of the fabricated Ag and carbon electrodes on paper in this case. As shown in Fig. 2e and f, after folding the paper and reconnecting the carbon or Ag electrode line to the loop, the LED remained lit, indicating that the folding of the paper substrate does not have a significant effect on the electrode conductivity and the fabricated PED has good mechanical flexibility. This also demonstrates a potential application of our method in fabricating flexible paper electronics in the future. In addition, it is noted that the light intensities of LEDs shown in Fig. 2d and 2f are weaker than those in Fig. 2c and 2e, respectively. The main reason for this is that the resistance of the carbon electrode is higher than that of the Ag electrode, as discussed next.

Besides conductivity, the surface morphology of electrodes also affects its electrical behavior. To provide the surface morphology information of the electrodes, the microstructures of
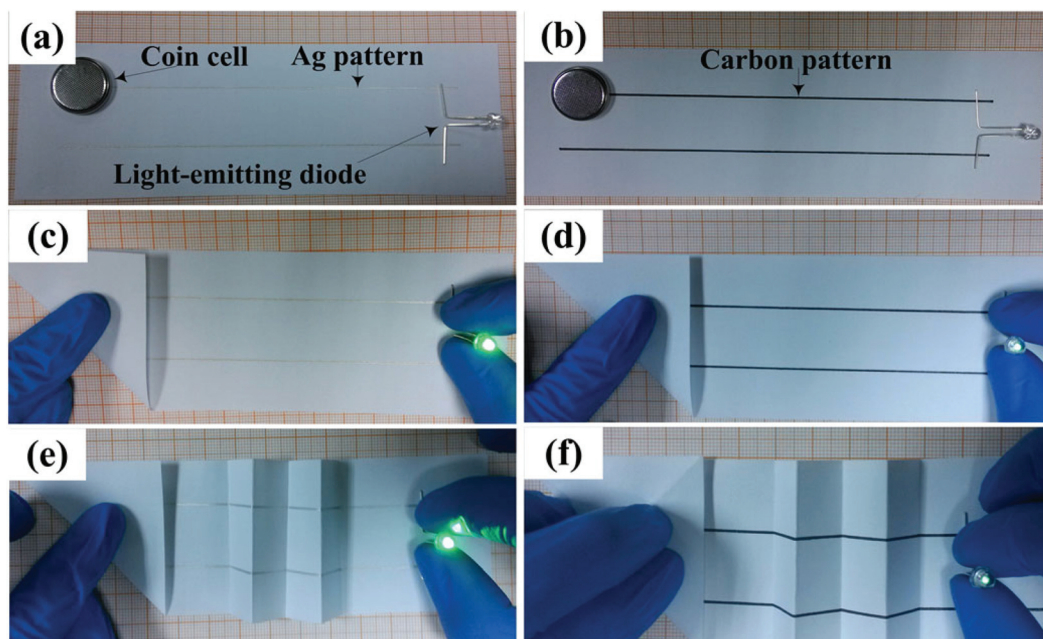
Fig. 2 Characterization of the conductivity of the written Ag and carbon electrodes as an interconnection circuit before and after folding A4 papers. Photographs of (a, b) the assemblies of a closed loop circuit with a coin cell and a LED using the written (a) Ag and (b) carbon electrodes on A4 papers; the loop circle formed (c, d) before and (e, f) after folding paper by linking the coin cell with the written (c, e) Ag and (d, f) carbon electrodes.

the written Ag and carbon electrodes on paper were imagined using SEM (Fig. 3a and b). In the figure, the surfaces of the prepared electrodes are little rough but still uniform. It can be observed that the thin flat layers are formed in the middle part of the written line, while the thick layers are formed on both boundary areas of the written line, which can be ascribed to the work principle of the ball pen writing process. To further check if such a microstructure influences the electrical performance of the electrodes, the conductivities of the electrodes were determined by measuring the resistance of the carbon and Ag electrodes with varied electrode lengths using a multimeter (Fig. 3c and 3d). When the electrode lengths vary from 2.0 cm to 12.0 cm, the resistance values of both Ag and carbon electrodes increase accordingly and exhibit a linear relationship as expected, indicating a relatively uniform writing process of carbon and Ag inks on paper using our device. Moreover, the average resistance value of carbon electrodes is calculated to be *ca.* 2.26 kΩ cm^{-1}, which is nearly 1000 times than that of Ag electrodes (*ca.* 2.29 Ω cm^{-1}) in this case. This can explain the difference of the LED light intensities for the carbon and Ag electrodes observed above. The possible effect of the high resistance of the carbon working electrode on the electrochemical performance of the fabricated PED is considered and discussed later.

Electrochemical characterization of the fabricated PEDs

The electrochemical behavior of the fabricated PEDs was checked with cyclic voltammetry in the aqueous solution containing 1 mM FcMeOH as the redox mediator. The obtained

cyclic voltammograms at various scan rates are shown in Fig. 4. From the figure, the shape of the CVs shows a typical redox reaction behavior and the ratio of the anodic and cathodic peak currents (I_{pa}/I_{pc}) is close to unity at each scan rate, which indicates that the FcMeOH redox reaction at the PEDs is electrochemically reversible (*i.e.*, a Nernstian electrochemical reaction behavior). The potential separation between the anodic and cathodic peaks ($\Delta E_p = E_{pa} - E_{pc}$) varies from 97 mV to 200 mV with the scan rate increasing from 10 mV s^{-1} to 100 mV s^{-1}, which are greater than the predicted potential separation value for a reversible one-electron redox process (~60 mV). The reason for the large peak splitting could be related to the uncompensated resistance present in the PEDs from the slightly large distance between the carbon WE and the Ag QRE in our design (*ca.* 1.33 mm) and the high resistance of the carbon working electrodes (2.26 kΩ cm^{-1}). But from the plots illustrated in the inset of Fig. 4, the anodic (I_{pa}) and cathodic peak currents (I_{pc}) seem linearly proportional to the square root of the scan rate ($v^{1/2}$), which fits the Randles–Sevcik equation and indicates that the mass transfer of the electroactive species (FcMeOH in this case) is a diffusion-controlled process. Similar to the redox reaction occurring at a traditional electrode surface, the redox reaction rate of FcMeOH is governed by its diffusion to the carbon WE surface. The absolute values of the slopes of the forward and reverse sweeps (0.7142 for the forward sweep, 0.6988 for the backward sweep) are nearly identical as normally found at traditional electrode materials, proving that the electrochemical performance of our fabricated PEDs meets the demand for the following electrochemical measurements.
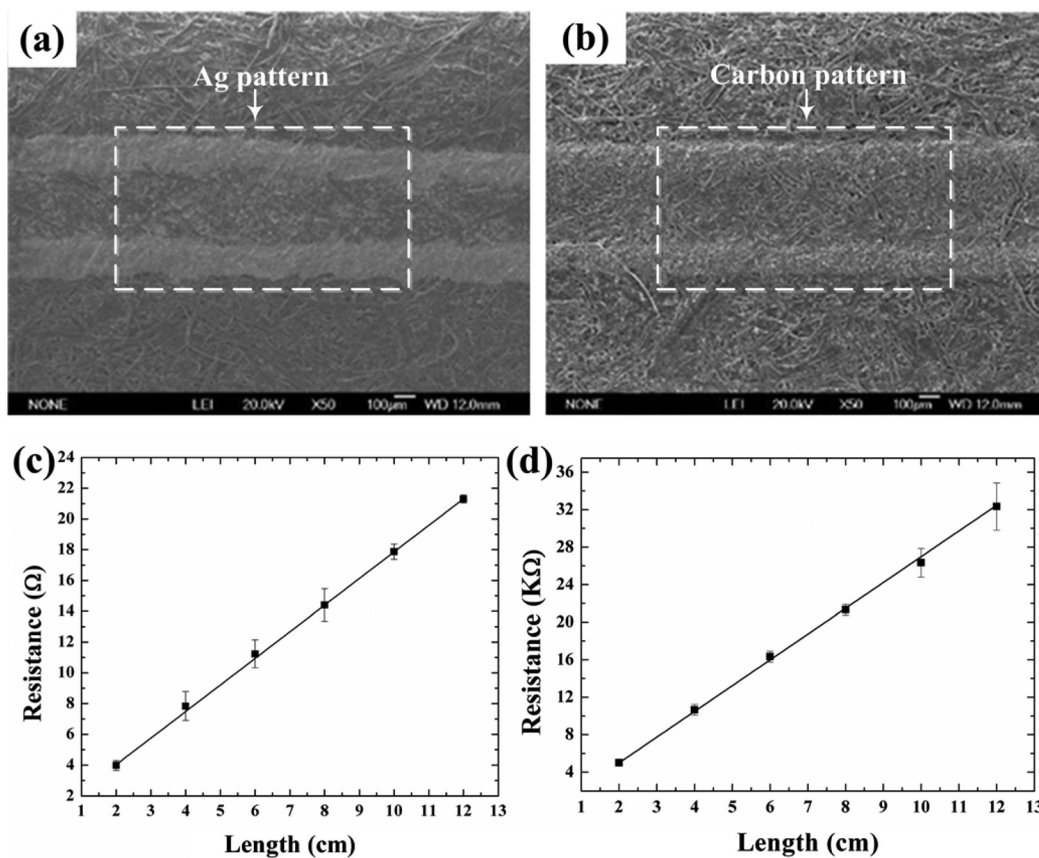
Fig. 3 Characterization of the surface morphology and resistance values of the written (a, c) Ag and (b, d) carbon electrodes on A4 papers. SEM images of the (a) Ag and (b) carbon electrodes on A4 papers. Measured resistance values of the (c) Ag and (d) carbon electrodes on A4 papers over the electrode lengths ranging from *ca.* 2.00 cm to *ca.* 12.00 cm (*n* = 5).
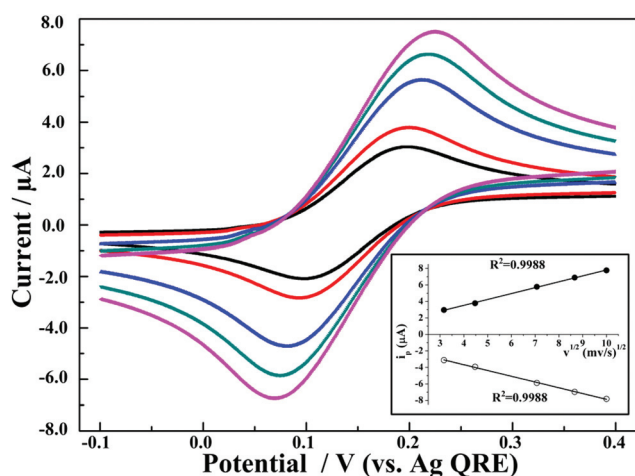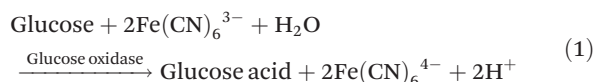

Fig. 4 Cyclic voltammograms recorded by a fabricated PED in the aqueous solution containing 1.0 mM FcMeOH and 0.1 M NaCl at various scan rates (10 mV s^{-1} (black curve), 20 mV s^{-1} (red curve), 50 mV s^{-1} (blue curve), 75 mV s^{-1} (green curve) and 100 mV s^{-1} (pink curve), respectively). The inset represents the plots of the anodic (●) and cathodic (○) peak currents (I_p) *vs.* the square root of the scan rate ($v^{1/2}$). The solid lines represent a linear fit to (●) with the regression equation: $y = 0.7142x + 0.6621$, $R^2 = 0.9988$, and a linear fit to (○) with the regression equation: $y = -0.6988x - 0.8794$, $R^2 = 0.9988$.

Moreover, after running the same experiment for over 100 cycles, the obtained CV curves (figure not shown) do not present obvious changes, indicating the electrochemical stability of our fabricated PEDs. But since the reference electrode of our PEDs is the Ag quasi-reference electrode, rather than the typical Ag/AgCl reference electrode,[42] we also electrodeposited AgCl on the Ag electrode to form the Ag/AgCl RE through the electrolysis method (inset of Fig. S5†), and tried the same CV measurement in the FcMeOH solution using two PEDs with Ag QRE and Ag/AgCl RE, respectively. From the obtained CV results (Fig. S5†), except for the shift of CV curves about 43.5 mV, the shape and ΔE_p of two CVs are similar, which means that the Ag QRE does not influence the electrochemical performance of PEDs and thus could be used as the reference electrode of PEDs for the following electrochemical measurements. Furthermore, in our designed PED, the size of the carbon counter electrode is about the same as that of the working electrode, while the counter electrode is normally bigger than the working electrode in the traditional electrochemical system. To check the possible size effect of the counter electrode on the PED electrochemical performance, we also fabricated PEDs with a carbon CE with larger size (inset of Fig. S6†). Through comparing the two recorded CVs of

FcMeOH redox reaction using two PEDs with two carbon CEs with areas of 21.92 mm^2 and 36.49 mm^2, respectively (Fig. S6†), we can conclude that our PEDs with using carbon CE of the same size as the working electrode also present good electrochemical behavior.

Electrochemical detection of glucose in artificial urine using the fabricated PEDs combined with a glucometer

Since the detection of glucose in human physiological fluids (*e.g.* blood, urine) is of great significance for diabetes mellitus diagnosis, to prove the feasibility and provide the potential application of the PEDs fabricated by our method in practical POCT, we firstly selected the detection of glucose using our PEDs as an example of human health diagnosis. The sample solution we used was the artificial urine because urine can be acquired easily and noninvasively. Considering the blood-glucose concentrations of both people not afflicted by diabetes (in the 4.0–8.0 mM range) and people with diabetes (in the 2.0–30.0 mM range),[43] the artificial urine solutions containing glucose with concentrations of 2.0, 5.0, 10.0, 15.0 and 20.0 mM were used as the sample solutions in this case. The detection of glucose concentration in the sample solution is normally performed by electrochemical measurement based on the following redox reaction (eqn (1)),

$$\text{Glucose} + 2\text{Fe(CN)}_6{}^{3-} + \text{H}_2\text{O}$$
$$\xrightarrow{\text{Glucose oxidase}} \text{Glucose acid} + 2\text{Fe(CN)}_6{}^{4-} + 2\text{H}^+ \qquad (1)$$

where the concentration of the reactant, glucose, is related to the amount of the product, $\text{Fe(CN)}_6{}^{4-}$. Through measuring the oxidation current of the produced $\text{Fe(CN)}_6{}^{4-}$ based on eqn (2),

$$\text{Fe(CN)}_6{}^{4-} \xrightarrow{\text{Oxidizing electrode}} \text{Fe(CN)}_6{}^{3-} + \text{e}^- \qquad (2)$$
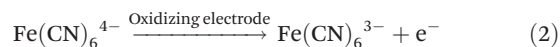
the amount of $\text{Fe(CN)}_6{}^{4-}$, then the glucose concentration, can be obtained.

In this experiment, we firstly designed and prepared the PEDs (Fig. 5a(i)) covered with filter papers (Fig. 5a(ii)) as the test strips for the glucometer. The reason for covering a filter sheet on the electrode surfaces of the PEDs is explained as follows.[34] Considering the difference between the electrode sizes of the commercial test strips and our fabricated PEDs, a filter sheet with a special size of 5 mm width and 10 mm length was designed and used to cover the electrode surfaces to adjust the reaction area of the PEDs, so that the detection range using our PEDs could be in the readout range of the glucometer. Moreover, because paper is a porous substrate and can transfer the sample to the electrode surface rapidly, the reagents of $\text{K}_3\text{Fe(CN)}_6$ and glucose oxidase were stored on the filter paper covering on the electrode surfaces as an alternative method for electrode modification. When inserting the fabricated PEDs into the sampling injection port of the glucometer, a read out was displayed on the glucometer screen (Fig. 5a(iii)), proving the feasibility of combining our PEDs with the commercial glucometer. The fabricated PEDs were then used as the test strips of the glucometer to measure the concentrations of glucose in the artificial urine solutions and the obtained results are illustrated in Fig. 5b. According to the work principle of the glucometer, when the oxidation potential of $\text{Fe(CN)}_6{}^{4-}$ is applied to the electrode, the obtained current is linearly proportional to the concentration of $\text{Fe(CN)}_6{}^{4-}$. Thus, the concentration of glucose in the sample can be obtained by the read out of the glucometer. In Fig. 5b, it is observed that the read outs displayed on the glucometer using our PEDs increase linearly with the concentration of glucose in the range of 2.0–20.0 mM in the sample solutions, and the slope of the fitting plot is calculated to be 1.2299 ($R^2 = 0.9982$). This result agrees well with the one recorded by the commer-
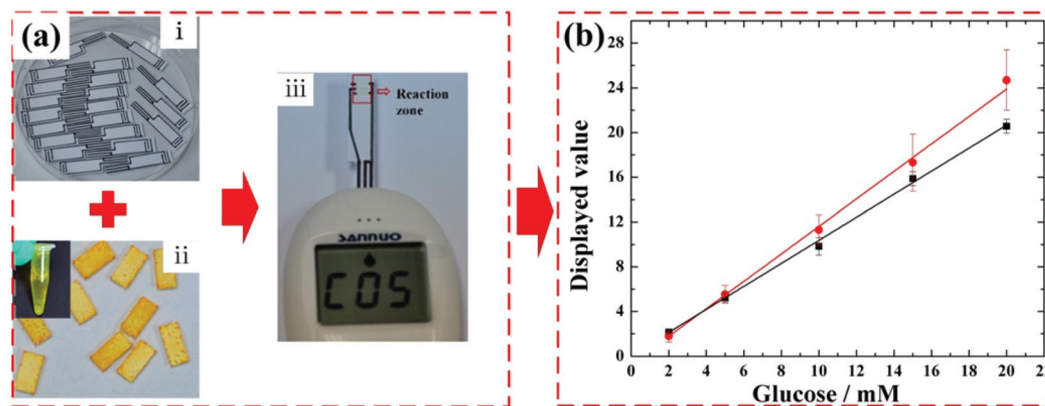

Fig. 5 Electrochemical detection of glucose in artificial urine samples using fabricated PEDs as test strips combined with a commercial glucometer. (a) Photographs of (i) the fabricated PEDs specially designed as test strips for the glucometer, (ii) the filter papers after adding the solution with 600 mM $\text{K}_3\text{Fe(CN)}_6$ and 500 U mL^{-1} glucose oxidase on their surfaces, (iii) a read out displayed on the glucometer screen after inserting the fabricated PEDs to the sampling injection point of the glucometer. (b) The linear relationships between the displayed values of the glucometer using (●) the fabricated PEDs and (■) the commercial test strips with the glucose concentrations of 2.0, 5.0, 10.0, 15.0, 20.0 mM in the artificial urine samples ($n = 3$). The red plot represents a linear fit to (●) with the regression equation: $y = 1.2299x - 0.6847$, $R^2 = 0.9982$. The black line represents a linear fit to (■) with the regression equation: $y = 1.0310x + 0.0590$, $R^2 = 0.9992$.

cial glucose test strips, which produces a similar linear plot with a slope of 1.0310 (R^2 = 0.9992). The above results prove that our method provides an *in vitro* detection of glucose concentration in artificial urine. Even though the detection limit of our fabricated PED (2.0 mM) and the linear detection range (2.0–20.0 mM) are not as good as the commercial test strips with a detection limit of 1.1 mM and a detection range of 1.1 mM to 33.3 mM, they are enough for diagnosing people with diabetes since the minimum glucose level of people with diabetes is about 2.0 mM. A wider linear range of glucose concentrations could be achieved by optimizing the geometry and the surface area of the working electrode of PEDs, although the precision of the device might suffer. Furthermore, by modifying the filter paper with appropriate chemicals, our PEDs could also be used to measure the concentration of analytes other than glucose, such as cholesterol and lactate in human plasma.

Electrochemical detection of melamine using the fabricated PEDs

The detection of melamine has attracted increasing attention since illegal addition of melamine to dairy products may cause kidney failure and even death.[44] To further demonstrate the feasibility of our PEDs in food safety analysis, the written PEDs were employed to detect melamine as an example. The sample solutions are PBS solutions (pH = 8) with a serial concentration of melamine (from 0 to 100.0 μM) with 1.0 mM uric acid as the indicator.[39] Differential pulse voltammetry was applied to detect the melamine in sample solutions with the fabricated PEDs. In the obtained differential pulse voltammograms shown in Fig. 6a, it is observed that the peak currents corresponding to the currents of uric acid oxidation decrease on increasing the concentration of melamine. This is because of the competition between melamine and uric acid for the adsorption on the carbon WE surface. At the beginning of the experiment without adding melamine, uric acid dominated the adsorption step on the electrode. When melamine was introduced, melamine replaced uric acid to adsorb on the electrode surface leading to the reduction of the amount of uric acid on the electrode surface. Therefore, as the amount of melamine continues to increase, the values of DPV peak currents of uric acid oxidation decrease. The concentration of melamine in sample solutions was determined by analyzing the reduction of the peak currents of uric acid. The obtained plot in Fig. 6b shows a linear relationship between the concentration of added melamine (from 0 to 40.0 μM) and the values of the reduced peak currents (from 0 to 10.22 μA), and the detection limit of melamine is found to be 1.0 μM in this case. According to the safe ingestion limit of melamine in an infant formula of 20 μM (2.5 ppm) in USA and EU, and 8 μM (1 ppm) in China,[44] our detection limit is much lower than the safe ingestion limit and could be satisfied for real sample detection. A wider linear range of melamine concentrations could also be achieved by optimizing the electrode dimension of our PEDs. The above results demonstrate that the PEDs fabricated by our method could be used as POCT devices to determine
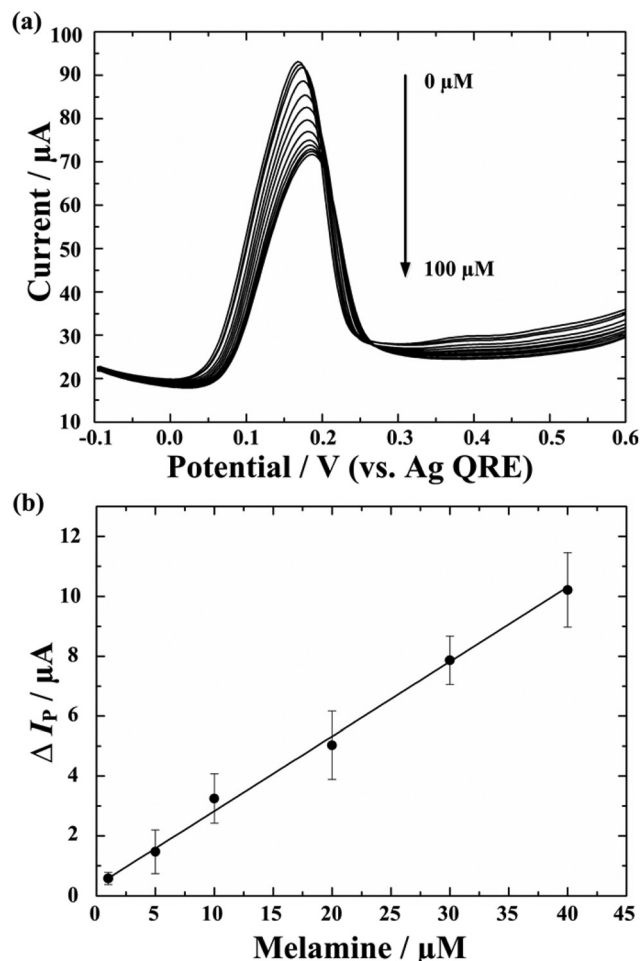

Fig. 6 Electrochemical detection of melamine in PBS solutions using fabricated PEDs. (a) Differential pulse voltammetric responses for the uric acid oxidation on increasing the concentrations of melamine (from top to bottom, 0, 1.0, 5.0, 10.0, 20.0, 30.0, 40.0, 50.0, 60.0, 70.0, 80.0, 90.0, 100.0 μM, respectively) in 0.1 M PBS (pH 8) with 1.0 mM uric acid. (b) The linear relationship between the reduced anodic peak currents and the melamine concentrations with a regression coefficient of 0.9969 (*n* = 3).

the melamine concentration in samples. The writing electrode technique by our pressure-assisted ball pen device can also be explored to prepare PEDs for the quantitative analysis of other illegal additives in the food safety field.

Conclusions

In summary, we have developed a direct writing method based on the ball pen combined with a pressure assisted accessory for fabricating electrodes on paper to make PEDs in a simple, low-cost way, and without needing sophisticated facilities and skilled individuals. The prepared PEDs were successfully employed to quantitatively analyze the concentrations of glucose in artificial urine and melamine in sample solutions. Our results indicate the potential applications of the fabricated

PEDs for POCT of human health and food safety, especially in resource-limited settings. Moreover, our method could also be used to fabricate PEDs with multi-electrode arrays and to write electrodes on a non-planar paper surface and also on human skin. The characteristic of no ink pretreatment of our method makes it compatible with other particle-based inks, indicating its potential for application in other fields such as paper electronics. However, due to the manual procedures and the principle of writing process of the ball pen, the surface roughness of the electrodes fabricated by direct writing is slightly higher than that of conventional printing methods, such as the screen printing technique. This could be improved by optimizing the pressure applied on the ink.

Acknowledgements

References

1 R. Sista, Z. Hua, P. Thwar, A. Sudarsan, V. Srinivasan, A. Eckhardt, M. Pollack and V. Pamula, *Lab Chip*, 2008, **8**, 2091–2104.

2 D. C. H. Burgess, J. Wasserman and C. A. Dahl, *Nature*, 2006, **444**, 1–2.

3 P. Yager, G. J. Domingo and J. Gerdes, *Annu. Rev. Biomed. Eng.*, 2008, **10**, 107–144.

4 M. Tudorache and C. Bala, *Anal. Bioanal. Chem.*, 2007, **388**, 565–578.

5 V. Gubala, L. F. Harris, A. J. Ricco, M. X. Tan and D. E. Williams, *Anal. Chem.*, 2012, **84**, 487.

6 R. Peeling and D. Mabey, *Clin. Microbiol. Infect.*, 2010, **16**, 1062–1069.

7 J. J. Xu, N. Bao, X. H. Xia, Y. Peng and H. Y. Chen, *Anal. Chem.*, 2004, **76**, 6902–6907.

8 Z. Nie, F. Deiss, X. Liu, O. Akbulut and G. M. Whitesides, *Lab Chip*, 2010, **10**, 3163–3169.

9 L. Ge, P. Wang, S. Ge, N. Li, J. Yu, M. Yan and J. Huang, *Anal. Chem.*, 2013, **85**, 3961–3970.

10 A. Robinson, S. Harroun, J. Bergman and C. Brosseau, *Anal. Chem.*, 2012, **84**, 1760–1764.

11 M. Li, Y. T. Li, D. W. Li and Y. T. Long, *Anal. Chim. Acta*, 2012, **734**, 31–44.

12 B. Liu, D. Du, X. Hua, X. Y. Yu and Y. Lin, *Electroanalysis*, 2014, **26**, 1214–1223.

13 E. J. Maxwell, A. D. Mazzeo and G. M. Whitesides, *MRS Bull.*, 2013, **38**, 309–314.

14 D. M. Cate, J. A. Adkins, J. Mettakoonpitak and C. S. Henry, *Anal. Chem.*, 2015, **87**, 19–41.

15 D. Tobjörk and R. Österbacka, *Adv. Mater.*, 2011, **23**, 1935–1961.

16 Y. Zhang, L. Ge, M. Li, M. Yan, S. Ge, J. Yu, X. Song and B. Cao, *Chem. Commun.*, 2014, **50**, 1417–1419.

17 W. Dungchai, O. Chailapakul and C. S. Henry, *Anal. Chem.*, 2009, **81**, 5821–5826.

18 H. Yang, Q. Kong, S. Wang, J. Xu, Z. Bian, X. Zheng, C. Ma, S. Ge and J. Yu, *Biosens. Bioelectron.*, 2014, **61**, 21–27.

19 F. Conzuelo, M. Gamella, S. Campuzano, D. G. Pinacho, A. Julio Reviejo, M. Pilar Marco and J. M. Pingarrón, *Biosens. Bioelectron.*, 2012, **36**, 81–88.

20 L. Y. Shiroma, M. Santhiago, A. L. Gobbi and L. T. Kubota, *Anal. Chim. Acta*, 2012, **725**, 44–50.

21 D. D. Liana, B. Raguse, L. Wieczorek, G. R. Baxter, K. Chuah, J. J. Gooding and E. Chow, *RSC Adv.*, 2013, **3**, 8683–8691.

22 C. Hu, X. Bai, Y. Wang, W. Jin, X. Zhang and S. Hu, *Anal. Chem.*, 2012, **84**, 3745–3750.

23 A. Määttänen, U. Vanamo, P. Ihalainen, P. Pulkkinen, H. Tenhu, J. Bobacka and J. Peltonen, *Sens. Actuators, B*, 2013, **177**, 153–162.

24 M. Santhiago, J. B. Wydallis, L. T. Kubota and C. S. Henry, *Anal. Chem.*, 2013, **85**, 5233–5239.

25 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.

26 D. Zang, L. Ge, M. Yan, X. Song and J. Yu, *Chem. Commun.*, 2012, **48**, 4683–4685.

27 J. P. Metters, S. M. Houssein, D. K. Kampouris and C. E. Banks, *Anal. Methods*, 2013, **5**, 103–110.

28 C. Renault, M. J. Anderson and R. M. Crooks, *J. Am. Chem. Soc.*, 2014, **136**, 4616–4623.

29 L. Ge, S. Wang, J. Yu, N. Li, S. Ge and M. Yan, *Adv. Funct. Mater.*, 2013, **23**, 3115–3123.

30 N. Dossi, R. Toniolo, A. Pizzariello, F. Impellizzieri, E. Piccin and G. Bontempelli, *Electrophoresis*, 2013, **34**, 2085–2091.

31 N. Dossi, R. Toniolo, E. Piccin, S. Susmel, A. Pizzariello and G. Bontempelli, *Electroanalysis*, 2013, **25**, 2515–2522.

32 N. Dossi, R. Toniolo, F. Impellizzieri and G. Bontempelli, *J. Electroanal. Chem.*, 2014, **722**, 90–94.

33 N. Dossi, R. Toniolo, F. Terzi, F. Impellizzieri and G. Bontempelli, *Electrochim. Acta*, 2014, **146**, 518–524.

34 N. Kurra and G. Kulkarni, *Lab Chip*, 2013, **13**, 2866–2873.

35 A. Russo, B. Y. Ahn, J. J. Adams, E. B. Duoss, J. T. Bernhard and J. A. Lewis, *Adv. Mater.*, 2011, **23**, 3426–3430.

36 W. Li and M. Chen, *Appl. Surf. Sci.*, 2014, **290**, 240–245.

37 T. Brooks and C. Keevil, *Lett. Appl. Microbiol.*, 1997, **24**, 203–206.

38 S. N. Tan, L. Ge and W. Wang, *Anal. Chem.*, 2010, **82**, 8844–8847.

39 C. W. Liao, Y. R. Chen, J. L. Chang and J. M. Zen, *Electroanalysis*, 2011, **23**, 573–576.

40 J. R. Windmiller, A. J. Bandodkar, S. Parkhomovsky and J. Wang, *Analyst*, 2012, **137**, 1570–1575.

41 Y. L. Tai and Z. G. Yang, *J. Mater. Chem.*, 2011, **21**, 5938–5943.

42 E. T. S. G. da Silva, S. Miserere, L. T. Kubota and A. Merkoçi, *Anal. Chem.*, 2014, **86**, 10531–10534.

43 A. Heller and B. Feldman, *Chem. Rev.*, 2008, **108**, 2482–2505.

44 C. W. Liao, Y. R. Chen, J. L. Chang and J. M. Zen, *J. Agric. Food Chem.*, 2011, **59**, 9782–9787.