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Paper-based graphene oxide biosensor coupled with smartphone for the quantification of glucose in oral fluid

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Abstract

Rapid, disposable, point-of-care (POC) oral fluid testing has gained considerable attention in recent years as saliva contains biomarker and components of the serum proteome that offer important information on both oral and systemic disease. Microfluidic paper-based analytical devices (μPADs) coupled with smartphone reflectance sensing systems have long been considered to be an effective POC tool for the diagnostics of biomarkers in oral fluid. However, the existing portable systems are limited by the poor color distribution in the detection area as well as not being universally applicable. Therefore, using the properties of nanomaterials to our advantage, we present a simple, universally applicable approach that features graphene oxide (GO) coated μPADs coupled with smartphone-based colorimetric detection for the direct quantification of glucose. An integrated portable system is used to implement the approach. Owing to the enhanced reagents absorptivity, reactive efficiency and homogeneity of color distribution from the deposition of GO, the glucose assay performance was improved. Also, by using a self-developed app, the glucose concentrations in physiological range can be automatically quantified. Finally, the approach is universally applicable as the modification of μPADs with GO can be achieved without the use of any linker, binder or retention aid, which avoids possible enzyme cross contamination. The system was first calibrated by standard glucose buffer solutions, and the limit of detection as well as the linear dynamic range were found to be 0.02 mM and 0~1 mM, respectively, which are appropriate for analyzing glucose concentrations in a clinically relevant range. Finally, the system was used for quantifying glucose concentrations in artificial saliva and the results obtained using our portable system showed reasonable agreement with the actual use concentrations. Thus, the utility of the system in sensitively quantifying glucose concentrations in a portable, and repeatable manner is demonstrated.

Keywords Graphene oxide · Paper microfluidics · Smartphone-based analytics · Oral fluid sensing

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1 Introduction

In recent years, microfluidic paper−based analytical devices (μPADs) have emerged as a new class of disposable microfluidic systems that possess the capability to be used for point-of-care (POC) applications (Yao and Zhang 2016; Hegener et al. 2017; Horst et al. 2018). Compared with other glass or polydimethylsiloxane (PDMS)-based microfluidic devices, μPADs have been proven to be advantageous in the following areas: (1) low-cost and recyclable, (2) highly biocompatible, (3) power-free for liquids transportation, (4) scalable (i.e. printing and roll-to-roll manufacturing friendly) (Evans et al. 2014). Also, thanks to the exponentially increasing in smartphone internal memory capacity, processing speed, and the enhanced performance of complementary metal-oxide semiconductor (CMOS)-based smartphone cameras, when μPADs is paired with smartphones (termed MS^2), they are able to provide relatively sophisticated tests at

locations lacking laboratory facilities, such as rural areas, homes, border points (Hart et al. 2011). To date, POC detection of biological specimen including proteins (Yetisen et al. 2014), nucleic acid (Priye et al. 2016), cells (Kim et al. 2017), bacterial (Park et al. 2013) and virus (Magro et al. 2017) have been demonstrated using the MS^2 approach. Of these, most of the tests utilizes blood sample. However, oral fluid-based tests have been gaining substantial interests as it has proven to be a suitable alternative for a number of analytes.

Oral fluid (saliva) contains biomarker and components of the serum proteome that offer important information on both oral and systemic disease (Hart et al. 2011). It is simple to collect, easy to store. Also, the non-invasive collection method of oral fluid sampling reduces the anxiety and discomfort of patients, thereby increasing their willingness to participate screening events at a much higher rate (Oblath et al. 2013). At last, saliva is considered as a constantly regenerating fluid, which offers a better snapshot of the patients' current heath state (Jokerst et al. 2009). Bearing the advantages, various applications including monitoring growth factors (Murdock et al. 2013), detecting steroids (Ogirala et al. 2017), infectious diseases (Zarei 2018), were proposed for MS^2 systems, exploiting specific enzymatic reactions, gene probe and immunoassays with different analytical formats. Among all the developed systems, colorimetric assays-based approaches are particularly attractive due to their ease-of-use, lack of complicated external equipment and ability to provide semiquantitative information (Roda et al. 2016). Therefore, in the field of oral fluid POC diagnostics, the advantages of simplicity, sensitivity, cost-effectiveness and the ability to provide quantitative color intensity measurements using imaging processing algorithm are key reasons that colorimetric detection coupled with MS2 systems have tremendous potential to be an effective POC diagnostic tool.

However, the most noticeable drawback associated with paper-based bioassays relying on reflectance measurements is the heterogeneity (i.e. poor quality) of the color distribution in the detection area. This is mainly caused by unrestricted flow of the solutions containing enzymes and reagents towards the edge of detection areas in the paper surface area upon sample addition (Calabria et al. 2017). In addition, most analytes are presented in saliva at a much lower concentration than in blood. For example, the saliva glucose concentration of a healthy person is between 1/10 and 1/50 of his/her blood glucose level (Kumar et al. 2014). As a result, existing MS^2 based oral fluid diagnostic tools need improvements to increase signal detectability, assay accuracy and applicability, thus enabling the cost-effective quantification of analytes presented at sub-micromolar levels, as required for convenient oral fluid POC tests.

Several strategies have been used to address the analytical performance limitations of the colorimetric MS^2 detection system, most of them used different bioconjugation reactions to immobilize the enzymes via carbonyl, aldehyde, or amino groups formed on the cellulose substrate (Cate et al. 2015; Parolo and Merkoci 2013; Xu et al. 2016). Most recently, Calabria et al., used a "functional wafer-like" multi-layered coating of Poly (allyl amine hydrochloride/poly(sodium 4-styrene sulfonate) (PAH/PSS) to separately immobilize analyte-specific oxidases, thereby alleviating the color heterogeneity issue (Calabria et al. 2017). Although each of these strategies has its own advantages, they are not universally applicable, require multiple reagents and functionalization steps to execute, which defeat the purpose of a cost-efficient, simple POC testing.

Nanomaterials can potential be the solution to the problems because of their high specific surface area and unique electrochemical properties. Out of the available materials, graphene oxide (GO), a functionalized form of graphene with epoxy and hydroxyl groups on its basal plane and carboxyl groups at its edge (Boukhvalov and Katsnelson 2008) is particularly of interest due to its cost-effectiveness and adaptiveness to paper-based biosensing. Song, Yujun, et al. demonstrated carboxyl-modified graphene oxide has a peroxidase-like activity that can catalyze the reaction of peroxidase substrate TMB in the presence of H_2O_2 to produce a color change (Song et al. 2010). In addition, existing studies have showed that GO improves the homogeneity of color distribution in colorimetric assays by increasing the linkage of aromatic molecules through π-π stacking or van der Walls forces and absorbing biomolecules via electrostatic interactions or hydrogen bonding (Figueredo et al. 2016). Therefore, using the properties of GO to our advantage, we present a simple, universally applicable approach that features graphene oxide (GO) coated μPADs coupled with smartphone-based colorimetric detection for the direct quantification of glucose in saliva. An integrated portable system is used to implement the approach. Owing to the increased specific surface area and the homogeneity of color distribution from the coating of GO, both the sensitivity and accuracy of the system are improved. Also, the approach was universally applicable as the modification of cellulose fibers with GO can be achieved without the use of any linker, binder or retention aid, which avoids possible enzyme cross contamination and can potentially be used for a vast variety of other bioanalytical applications. In addition, by using a self-developed app, the glucose concentrations in physiological range can be colorimetrically quantified via cellphone photography and automatic image analysis. Finally, the μPADs are fabricated batch-to-batch, using a cost-efficient photolithography process. Up to 48 chips can be fabricated on a filter paper (210 × 210 mm) within 10 mins with a total cost of $1.92, well suited for disposable use. The system was first calibrated by standard glucose buffer solutions, and the limit of detection as well as the linear dynamic range were found to be 0.02 mM and 0~1 mM, respectively, which are appropriate for analyzing glucose

concentrations in a clinically relevant range (Kumar et al. 2014). Finally, the system was used for quantifying glucose concentrations in artificial saliva and the results obtained using our portable system showed reasonable agreement with the actual use concentrations. Thus, the utility of the system in sensitively quantifying glucose concentrations in a portable, and repeatable manner is demonstrated.

2 Material and methods

2.1 Materials

Peroxidase (HRP) (type VI-A from horseradish, 950–2000 U/mg protein), 3,3′,5,5′-tetramethylbenzidine (TMB), dimethyl sulfoxide (DMSO), D-glucose and glucose oxidase (GOx) (Type II, ≥15,000 U/g protein) were purchased from Sigma Aldrich (St. Louis, MO). Whatman® qualitative filter paper (Grade 3) was purchased from GE healthcare Life Sciences (Pittsburgh, PA, USA). Graphene oxide dispersion (5 mg/mL) were purchased from XFNANO Materials (Suzhou, Jiangsu, China). Phosphate buffered saline (0.1 mol L^{-1} PBS, pH 7.5) was employed as a buffer. Artificial oral fluid at pH 6.8 was prepared based on ISO 10271 standard by dissolving 0.78 mg mL^{-1} Na_2HPO_4, 0.795 mg mL^{-1} anhydrous $CaCl_2$, 0.4 mg mL^{-1} KCl, 0.4 mg mL^{-1} NaCl, 0.005 mg mL^{-1} Na_2S and 1 mg mL^{-1} urea in deionized water.

2.2 Chip Design and fabrication

There are two types of paper-based microfluidic designs in general, the reservoir array-based design and the lateral-flow design. In this paper, both design strategies were incorporated to our portable system to verify the effectiveness of our approach in a universal setting. Specifically, a 3 by 11 circular detection reservoir (DR) array each with a diameter of 6.0 mm was used for the calibration of the portable system. Also, a 1 by 6 lateral-flow assay array each with a circular detection area (3.0 mm in diameter) and a hydrophilic channel inlet (1.5, 20.0 mm in width, length, respectively) was used for the quantification of glucose concentrations in saliva (Fig. 1). The μPADs fabrication process was described elsewhere in detail (Jia et al. 2017). Briefly (Fig. 1a), a photoresist mixture that included stearyl acrylate (monomer for UV polymerization), 2,2-dimenthoxy-2-phenylacetophenone (photoinitiator for initializing radical polymerization), and 1,10-Bis (acryloyloxy) decane (solvent and crosslinker for the cured co-polymer) were placed in a glass tank (300 × 300 × 50 mm). Next, A A4-sized Whatman® filter paper was submerged in the tank for photoresist coating and subsequently transferred to a UV curing machine for pattern exposure. Finally, the exposed paper was developed in acetone and allowed to dry at room temperature (Fig. 1b). For our μPADs fabrication, photolithography was used instead of wax printing as the former method enabled more precise fabrication of various μPADs sizes.

2.3 Paper functionalization and assay procedure

The fabricated μPADs were then functionalized by the following steps. A 100 μg mL^{-1} GO solution was first prepared from the 5 mg mL^{-1} stock suspension in DI water. It was vigorously sonicated for 2 h prior to use to improve the GO dispersion. A 15 mM TMB solution in DMSO was obtained by adding 3.6 mg of TMB to 1 mL of DMSO and shaking for 30 min at room temperature. Enzymes solutions of GOx and HRP (120:30 U mL^{-1}) were prepared in PBS.

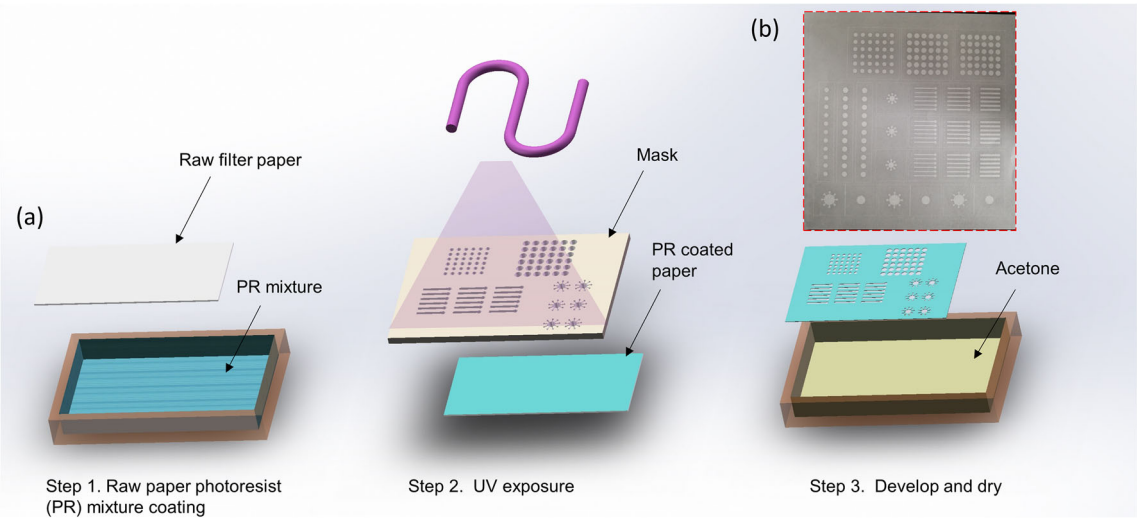

Fig. 1 Design and fabrication of the μPADs; **a** self-developed photoresist-based μPADs fabrication process flow; **b** fabricated filter paper with different μPADs designs

The sensing principle of our work is based on a cascade reaction catalyzed by GOx and HRP and is described in detail in Section I of the supplementary information (SI). The glucose colorimetric assay was performed in the following process. 1.5 μL of the dispersed GO solution was first pipetted to the μPADs detection zones (Fig. 2a), and the chip was allowed to dry. Then 1 μL of chromogenic substrate (15 mM TMB) and the mixture of enzymatic solutions (GOx and HRP) were added to the detection zones in two independent steps and the chip was dried over a plastic film at room temperature. Known concentrations of glucose solutions ranging from 0.05 to 1.5 mM were prepared by dissolving glucose in both the PBS buffer and the prepared artificial saliva solution. During the assay, a control experiment was first conducted by placing functionalized μPADs without glucose on an alignment cartridge and subsequently loaded to a black box UV analyzer (Qinke Tech, Shanghai, China) to minimize the effects of ambient light. Back camera of a smartphone (iPhone 7) with a fixed focus distance and the "always on" flash mode was used to take assay images 60 s after the chip loading (Fig. 2b). The cartridge was then removed from the UV analyzer, and 5 μL of the glucose solution with predetermined concentrations was applied to the μPADs. Similar to the control experiment, after 60 s of inserting the cartridge back to the chamber (same location), glucose assay images were acquired with identical photographic parameters. For each glucose concentration, the assay was repeated for at least 3 times.

2.4 Image analysis app

An app was developed on the iOS platform for images quantitative analysis using the developer toolset Xcode 8.0 via Swift 3.0 programming language (Fig. 2c). Since there were multiple μPADs designs involved, a MATLAB program was first compiled using a simple circular object edge tracking algorithm to locate the center and calculated the diameter of the detection regions of interest (ROI) in units of pixels. Based on the size of detection reservoirs in each design (3.0 mm and 6.0 mm in diameter), the ROI corresponded to a pixel diameter of 42 and 84 (calculated based on the resolution of an iPhone 7 camera: 4608 × 2592). Next, the locations of the ROI centers were manually inputted in the app, and we set the pixel diameter to 40 ~ 45 (80 ~ 85 for DRs with a diameter of 6 mm) to track all the available ROIs in a captured image (Fig. 2d). Once the ROIs were defined, the app extracted values of R, G, B channels of each pixel within the ROIs for both the assay images and the background images. Subsequently, a generalized "pixel value" ΔP for each pixel was calculated by the following equation (Eq. 1):

$$\Delta P = \sqrt{\left(R_{assay}-R_{bg}\right)^2 + \left(G_{assay}-G_{bg}\right)^2 + \left(B_{assay}-B_{bg}\right)^2} \quad (1)$$

where R_{assay}, G_{assay}, B_{assay} and R_{bg}, G_{bg}, B_{bg} were the extracted value of the R, G, B channels of each pixel within the ROI for the assay and background images, respectively. Finally, the app converted the calculated ΔP value to glucose concentrations based on the results of the system calibration. Notably, although the app was developed on the iOS platform, images taken from other cellphones and cameras could also be processed.

3 Results and discussion

3.1 GO enhanced colorimetric assays

First, the hypothesis presented in this paper, the addition of GO improved the overall analytical performance of our portable system was validified. Before doing so, both Raman spectroscopy and Scanning Electron Microscopy were used to characterize and verify the deposition of GO onto the paper substrate (SI-Section II, and SI Figure 2). After the

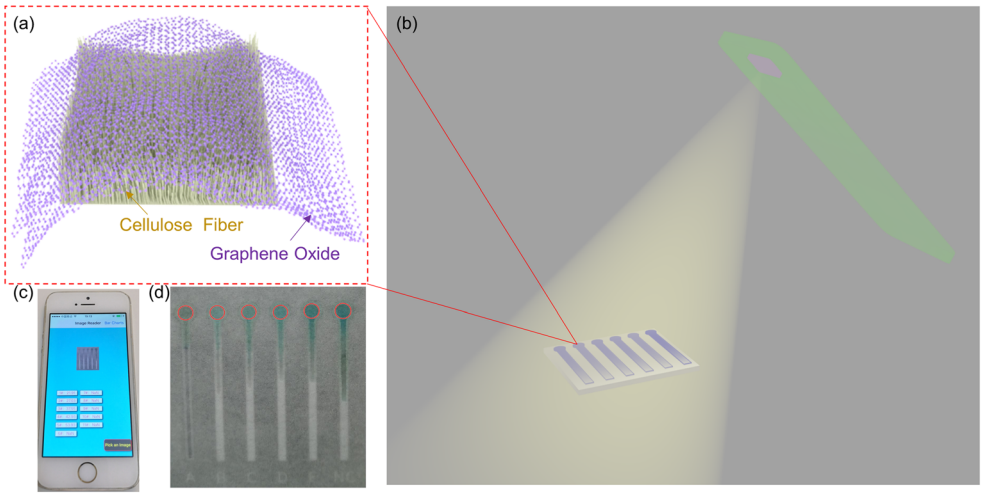

Fig. 2 Portable system operating principle: **a** deposition of the GO onto the paper substrate; **b** illustration of the smartphone imaging process; **c** IOS app interface on an iPhone 7; **d** detection regions of interest tracking using the algorithm

verification, μPADs consisted of 1 by 5 circular detection reservoirs arrays (6 mm in diameter for each DR) were cut from the fabricated paper sheets. Of these, one of the DR arrays was first deposited with 0.75 μL of 100 μg/mL dispersed GO colloidal solution and followed with the addition of 0.5 μL of TMB and enzymatic solutions in 2 independent steps. As a comparison, only 0.5 μL of TMB and enzymatic solutions were introduced to the other DR array in the same concentrations. In parallel, a constant glucose concentration of 0.5 mM was prepared by dissolving 1.8 mg of glucose in 20 mL of the PBS buffer. Colorimetric assays were then performed by pipetting 5 μL of the glucose solution to the reservoir arrays in order. The results of the assays were shown in Fig. 3a–d. For better viewing purposes, the images were taken under daylight with the same focus distance and without flash. As illustrated in the figure, for the assay without the GO coating (Fig. 3a, b), compared with control (DRs on the second row of Fig. 3c, d) the color change occurred predominately in only one half of the DRs, which yielded a sub-par color uniformity. On the other hand, the assay with the GO coating showed a much better color uniformity within the DRs, especially towards the edge of the DRs.

Next, the images were analyzed quantitively. To avoid errors of using the not-yet calibrated portable system, A MATLAB program was compiled to convert the images to grey scale, access and plot the grey intensity values in each DR. Representative 3D mesh plots for a DR in both assays were shown in Fig. 3e–f. For the DR without the GO coating, as expected, the maximum intensity area was located at the top half of the DR (near the edge) while the minimum occurred at the bottom half the DR. Comparatively, although the grey intensities obtained in the DR with GO coating showed a cone shape structure with the maximum area located in the center of the DR, the grey intensity near the edge was much more uniform. This demonstrated that the GO coating restricted the flow of enzymatic solutions on the paper surface, which improved the overall color uniformity. Additionally, ImageJ (NIH, Bethesda, MD) was used to analyze the average grey intensity and standard deviation of all DRs (Fig. 3g–h). As a result, the DRs coated with GO showed an improved average grey intensity (134 compared with 127, without subtracting the background) and a slightly smaller intensity standard deviation over the DRs without the GO coating. Since in all cases there was better color intensity and uniformity for μPADs coated with GO than in native form, our hypothesis that the addition of GO enhanced the analytical performance of the portable system was demonstrated. Notably, the average grey intensity dropped slightly for both assays 60 s after the sample introduction compared with 30 s after sample introduction, this could be explained by the oxidation of the enzymatic solutions.

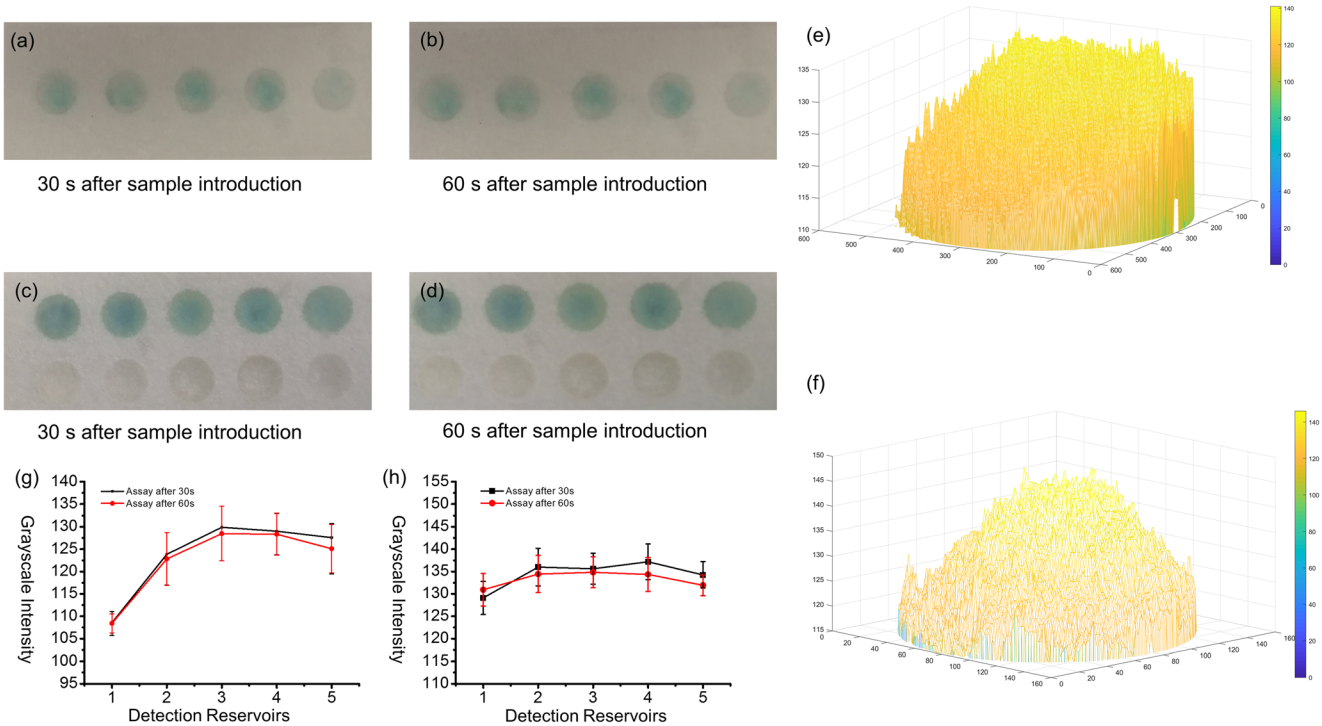

Fig. 3 Glucose assay induced color change on μPADs without GO coating (**a**) 30 s after sample introduction; (**b**) 60 s after sample introduction; Glucose assay induced color change on μPADs with GO coating (**c**) 30 s after sample introduction; (**d**) 60 s after sample introduction; 3D mesh plots of grey intensities of representative DRs; (**e**) without GO coating; (**f**) with GO coating. Determined average grey intensities and standard deviation of each DR; (**g**) without GO coating; (**h**) with GO coating

3.2 Calibration using metal salt solutions

After confirming the effectiveness of the GO coating, our portable system was calibrated by using known concentrations of glucose solutions to correlate between the generalized pixel value and the glucose concentrations. First, glucose concentrations of 0.05, 0.1, 0.2, 0.5, and 1.0 mM were prepared by dissolving the respective amount of glucose in the PBS buffer. Next, μPADs with circular DRs arrays were prepared using the same process described in the last section starting with the deposition of 0.75 μL of 100 μg/mL dispersed GO colloidal solution to each DR. Following the experimental procedure, colorimetric assays were initiated by pipetting 5 μL of the glucose solutions in predetermined concentrations to the respective DRs (2 DR per concentration) except for the last one, which was introduced with 5 μL of buffer, and used as an intra negative control (INC) (Fig. 4a). The assay was repeated for at least 3 times to calculate the standard deviation of the determined ΔP. After inputting the determined DR center location, the pixel diameter of the DRs and specifying the location of the INC to the app, it automatically calculated the generalized pixel value (ΔP) for each DR according to Eq. (1). A corresponding plot (Fig. 4b) was shown below to demonstrate a highly linear correlation between the glucose concentration and the calculated ΔP. Based on the fitting residue and the slope of the linear fit, the limit of detection (LOD) of our portable system was found to be 0.02 mM. In addition, the assay color would change from bright blue to dark orange (data not shown) when a glucose concentration of greater than 1 mM was used, therefore this linear dynamic range (LDR) of our portable system was found to be between 0 and 1 mM. Nevertheless, based on the levels of the glucose concentrations in saliva for both healthy people and diabetic patients, the LOD and LDR of our portable system were considered clinically relevant (Kumar et al. 2014).

To verify the validity of our portable system, same images were analyzed by both ImageJ and a self-compiled MATLAB program. As shown in figures below (Fig. 4c–g), the calculated ΔP values within a ROI for each concentration was presented in 2D MATLAB surface plots. As expected, the calculated ΔP also increased monotonically with respect to the increasing of glucose concentrations. In addition, as indicated by the color chart, the ΔP values within the ROI determined by the MATLAB program were found to be consistent (difference within 2% in average) with the values determined using our portable system, thus demonstrating the validity

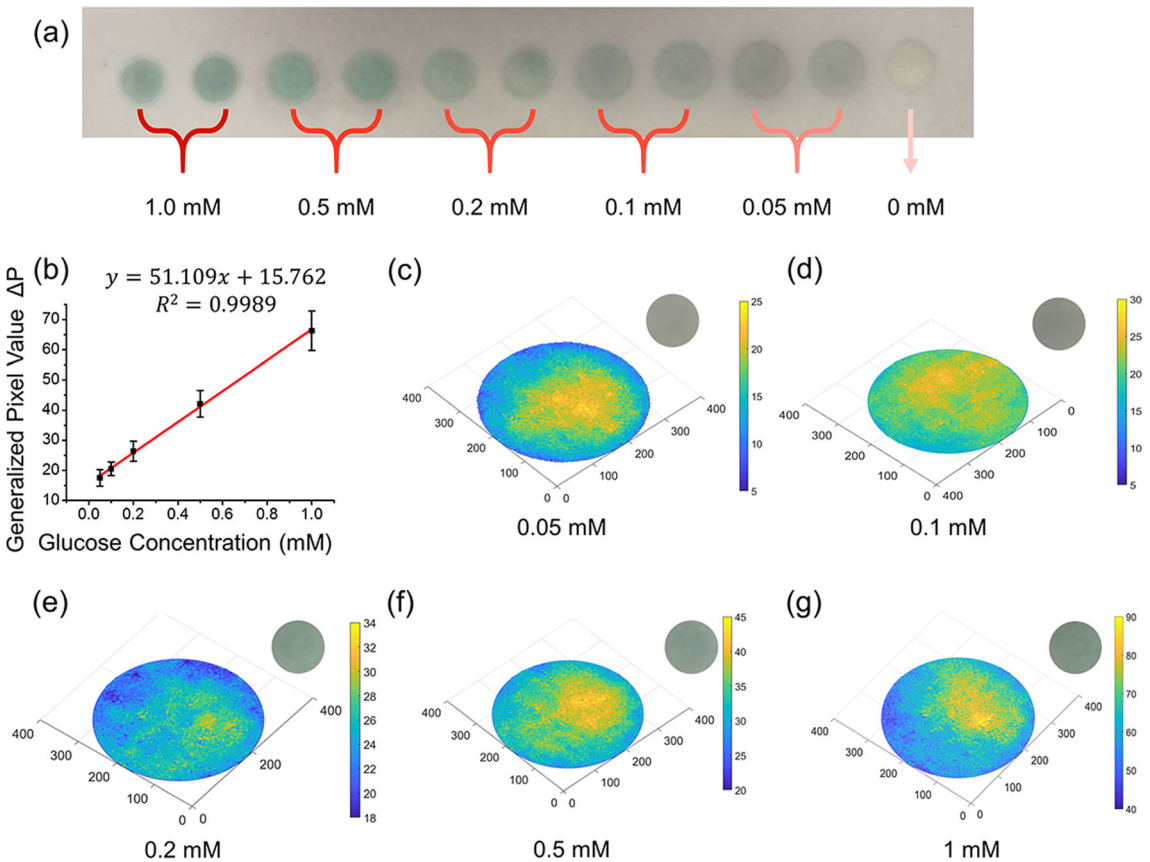

Fig. 4 Calibration of the portable system; **a** assay color change with respect to glucose concentrations; **b** calibration curve of the portable system, error bar was obtained from 3 repeated tests; determined (background subtracted) average pixel values of DRs for glucose concentration of (**c**) 0.05 mM, (**d**) 0.1 mM, (**e**) 0.2 mM, (**f**) 0.5 mM and (**g**) 1 mM

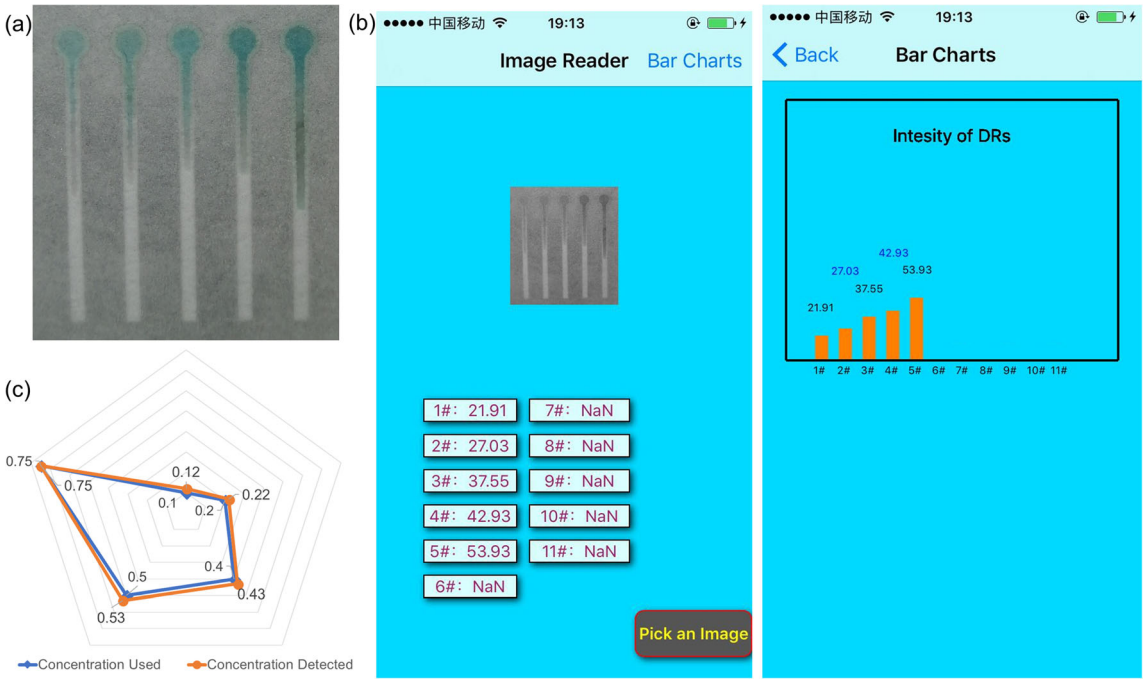

Fig. 5 Quantification of glucose concentrations in artificial saliva; **a** the results of glucose assay on a lateral-flow based μPAD design; **b** background subtracted ΔP values of DRs automatically determined by the app; **c** comparison of the glucose concentrations determined by the app with the actual concentrations used

our portable system in analytically quantifying glucose concentration changes.

3.3 Quantification of glucose concentrations

Finally, the calibrated portable system was used for the quantification of glucose concentrations in artificial saliva. Glucose concentrations with physiological significance (0.1 mM, 0.2 mM, 0.4 mM, 0.5 mM, and 0.75 mM) were prepared by dissolving the respective amount of glucose in artificial saliva. Next, the lateral-flow μPADs were prepared following the assay procedure described above starting with the introduction of 0.75 μL of the dispersed GO colloidal solution to the circular detection region of the μPADs (3 mm in diameter). Unlike the calibration experiments, a control experiment was first performed by pipetting buffer to a black chip to record the background RGB values. Then, colorimetric assays were performed one at a time by pipetting 5.0 μL of perspective glucose solution onto the sample inlet of the lateral-flow assay chips. For each concentration, the assays were repeated for 3

Table 1 Comparison of analytical performances of different paper-based sensors for the quantification of glucose

Paper-based sensors	Methods	Portability	Detection Limit (μM)	Ref
Aggregation induced emission of fluorogens	FS	No	10/ via visual observation	(Chang et al. 2018)
Zinc oxide nanowires electrode	EC	No	94	(Li et al. 2015)
Cobalt phthalocyanine/ionic liquid/graphene composite enzyme free biosensor	EC	No	0.67	(Chaiyo et al. 2018)
TMB/paper-based colorimetric biosensor	CM	No	10	(Gabriel et al. 2017)
Prussian blue-modified screen-printed electrodes	EC	No	10	(Sekar et al. 2014)
Screen printed carbon electrode	EC	No	350	(Yao et al. 2016)
Nafion-coated platinized paper electrodes	EC	No	120	(Parrilla et al. 2017)
Carbon ink for the working electrode	EC	No	120	(Amor-Gutierrez et al. 2017)
Polyethylene glycol /paper-based colorimetric biosensor	CM	No	3120	(Li et al. 2017)
Colloidal CdSe/ZnS quantum dots/ paper-based colorimetric biosensor	CM	No	90	(Duran et al. 2016)
Paper-based graphene oxide biosensor coupled with smart phone	CM	Yes	20	This work

FS Fluorescence, *EC* Electrochemical, *CM* Colorimetric

times to compute the average pixel RGB values within the ROIs. The result of the assay was presented in the figure below. Based on the visual inspection of the captured optical micrographs presented in Fig. 5a, the linear increment on the characteristic blue color of TMB in the DRs corresponded to the monotonic increase of the glucose concentrations. The captured assay figures were then analytically quantified by the portable system. As shown in Fig. 5b, the generalized pixel values determined by our portable system were consistent with the visual inspection results. The values were then converted back to glucose concentrations using the system calibration curve. The determined glucose concentrations and the actual concentration used were given in a radar plot (Fig. 5c). The difference between the actual and determined glucose concentration was mostly within 10%, with the exception of 18.7% found at the concentration of 0.1 mM. This could potentially be explained by the concentration was approaching the system LOD. Nevertheless, the results still demonstrated the practical usefulness of our portable system in rapid quantification of glucose concentrations in a convenient, cost-effective setting.

To illustrate the advantage of our portable system, Table 1 below compares the analytical performance and portability of some recent work on paper-based sensors for quantification of glucose with this work. As a result, the system described in our work is the only one capable of quantifying glucose concentrations portably. Also, since the GO μPADs do not require any form of nanomaterial functionalization, this work can be considered as the most economical option for quantifying glucose concentrations. Finally, this work reports a relatively high sensitivity comparing with other sensors listed in the table.

4 Conclusion

This paper for the first time described a simple, universally applicable approach that features GO modified μPADs coupled with smartphone-based colorimetric detection for the direct quantification of glucose in saliva. Based on the presented results, the approach demonstrated enhanced analytical performance owing to the increased specific surface area and the homogeneity of color distribution provided by GO modified μPADs. Also benefitting from automatic smartphone image analysis, rapid, quantitative characterization of glucose concentrations in oral fluid physiological range was allowed. At last, the approach was universally applicable as modify cellulose fibers with GO can be achieved without the use of any linker, binder or retention aid, which avoids possible enzyme cross contamination. The approach was implemented in a portable system that was calibrated to have a LOD of 0.02 mM and an LDR of 0~1 mM, suitable for oral fluid glucose detection. Finally, the portable system yielded satisfactory results when used for quantification of glucose concentrations in artificial saliva. Therefore, the advancement achieved by this study had potential to enable non-invasive glucose POC diagnostics and a vast variety of future bioanalytical applications with convenience, and low cost.

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References

O. Amor-Gutierrez, E.C. Rama, A. Costa-Garcia, M.T. Fernandez-Abedul, Paper-based maskless enzymatic sensor for glucose determination combining ink and wire electrodes. Biosens. Bioelectron. **93**, 40–45 (2017)

D.W. Boukhvalov, M.I. Katsnelson, Modeling of graphite oxide. J. Am. Chem. Soc. **130**, 10697–10701 (2008) 2008/08/13

D. Calabria, C. Caliceti, M. Zangheri, M. Mirasoli, P. Simoni, A. Roda, Smartphone-based enzymatic biosensor for oral fluid L-lactate detection in one minute using confined multilayer paper reflectometry. Biosens. Bioelectron. **94**, 124–130 (2017)

D.M. Cate, J.A. Adkins, J. Mettakoonpitak, C.S. Henry, Recent developments in paper-based microfluidic devices. Anal. Chem. **87**, 19–41 (2015)

S. Chaiyo, E. Mehmeti, W. Siangproh, T.L. Hoang, H.P. Nguyen, O. Chailapakul, et al., Non-enzymatic electrochemical detection of glucose with a disposable paper-based sensor using a cobalt phthalocyanine ionic liquid graphene composite. Biosens. Bioelectron. **102**, 113–120 (Apr 2018)

J.F. Chang, H.Y. Li, T. Hou, W.N. Duan, F. Li, Paper-based fluorescent sensor via aggregation induced emission fluorogen for facile and sensitive visual detection of hydrogen peroxide and glucose. Biosens. Bioelectron. **104**, 152–157 (2018)

G.M. Duran, T.E. Benavidez, A. Rios, C.D. Garcia, Quantum dot-modified paper-based assay for glucose screening. Microchim. Acta **183**, 611–616 (2016)

E. Evans, E.F.M. Gabriel, W.K.T. Coltro, C.D. Garcia, Rational selection of substrates to improve color intensity and uniformity on microfluidic paper-based analytical devices. Analyst **139**, 2127–2132 (2014)

F. Figueredo, P.T. Garcia, E. Cortón, W.K.T. Coltro, Enhanced analytical performance of paper microfluidic devices by using Fe3O4 nanoparticles, MWCNT, and Graphene oxide. ACS Appl. Mater. Interfaces **8**, 11–15 (2016) 2016/01/13

E.F.M. Gabriel, P.T. Garcia, F.M. Lopes, W.K.T. Coltro, Paper-based colorimetric biosensor for tear glucose measurements. Micromachines, 8 (2017)

R.W. Hart, M.G. Mauk, C. Liu, X. Qiu, J.A. Thompson, D. Chen, et al., Point-of-care oral-based diagnostics. Oral Dis. **17**, 745–752 (2011)

M.A. Hegener, H. Li, D. Han, A.J. Steckl, G.M. Pauletti, Point-of-care coagulation monitoring: First clinical experience using a paper-based lateral flow diagnostic device. Biomed. Microdevices **19** (2017)

A.L. Horst, J.M. Rosenbohm, N. Kolluri, J. Hardick, C.A. Gaydos, M. Cabodi, et al., A paperfluidic platform to detect Neisseria

gonorrhoeae in clinical samples. Biomed. Microdevices **20**, 1–7 (2018)

Y. Jia, H. Dong, J.P. Zheng, H. Sun, Portable detection of trace metals in airborne particulates and sediments via mu PADs and smartphone. Biomicrofluidics **11** (2017)

J.V. Jokerst, A. Raamanathan, N. Christodoulides, P.N. Floriano, A.A. Pollard, G.W. Simmons, et al., Nano-bio-chips for high performance multiplexed protein detection: Determinations of cancer biomarkers in serum and saliva using quantum dot bioconjugate labels. Biosens. Bioelectron. **24**, 3622–3629 (2009)

S.C. Kim, U.M. Jalal, S.B. Im, S. Ko, J.S. Shim, A smartphone-based optical platform for colorimetric analysis of microfluidic device. Sensors Actuators B Chem. **239**, 52–59 (Feb 2017)

S. Kumar, S. Padmashree, R. Jayalekshmi, Correlation of salivary glucose, blood glucose and oral candidal carriage in the saliva of type 2 diabetics: A case-control study. Contemp Clin Dent **5**, 312–317 (2014)

X. Li, C. Zhao, X. Liu, An electrochemical microfluidic paper-based glucose sensor integrating zinc oxide nanowires, in *2015 28th IEEE International Conference on Micro Electro Mechanical Systems (MEMS)* (2015) pp. 447–450

Z.X. Li, Y.H. Zhu, W.J. Zhang, C.H. Xu, Y.J. Pan, Y. Zhao, A low-cost and high sensitive paper-based microfluidic device for rapid detection of glucose in fruit. Food Anal. Methods **10**, 666–674 (2017)

L. Magro, B. Jacquelin, C. Escadafal, P. Garneret, A. Kwasiborski, J.C. Manuguerra, et al., Paper-based RNA detection and multiplexed analysis for Ebola virus diagnostics. Sci. Rep. **7** (2017)

R.C. Murdock, L. Shen, D.K. Griffin, N. Kelley-Loughnane, I. Papautsky, J.A. Hagen, Optimization of a paper-based ELISA for a human performance biomarker. Anal. Chem. **85**, 11634–11642 (2013)

E.A. Oblath, W.H. Henley, J.P. Alarie, J.M. Ramsey, A microfluidic chip integrating DNA extraction and real-time PCR for the detection of bacteria in saliva. Lab Chip **13**, 1325–1332 (2013)

T. Ogirala, A. Eapen, K.G. Salvante, T. Rapaport, P.A. Nepomnaschy, A.M. Parameswaran, Smartphone-based colorimetric ELISA implementation for determination of women's reproductive steroid hormone profiles. Med. Biol. Eng. Comput. **55**, 1735–1741 (2017)

T.S. Park, W.Y. Li, K.E. McCracken, J.Y. Yoon, Smartphone quantifies Salmonella from paper microfluidics. Lab Chip **13**, 4832–4840 (2013)

C. Parolo, A. Merkoci, Paper-based nanobiosensors for diagnostics. Chem. Soc. Rev. **42**, 450–457 (2013)

M. Parrilla, R. Canovas, F.J. Andrade, Paper-based enzymatic electrode with enhanced potentiometric response for monitoring glucose in biological fluids. Biosens. Bioelectron. **90**, 110–116 (2017)

A. Priye, S.S. Wong, Y.P. Bi, M. Carpio, J. Chang, M. Coen, et al., Lab-on-a-drone: Toward pinpoint deployment of smartphone-enabled nucleic acid-based diagnostics for Mobile health care. Anal. Chem. **88**, 4651–4660 (2016)

A. Roda, E. Michelini, M. Zangheri, M. Di Fusco, D. Calabria, P. Simoni, Smartphone-based biosensors: A critical review and perspectives. Trac-Trends Anal Chem **79**, 317–325 (2016)

N.C. Sekar, S.A.M. Shaegh, S.H. Ng, L. Ge, S.N. Tan, A paper-based amperometric glucose biosensor developed with Prussian blue-modified screen-printed electrodes. Sensors Actuators B Chem. **204**, 414–420 (2014)

Y.J. Song, K.G. Qu, C. Zhao, J.S. Ren, X.G. Qu, Graphene oxide: Intrinsic peroxidase catalytic activity and its application to glucose detection. Adv. Mater. **22**, 2206–2210 (May 2010)

Y. Xu, M. Liu, N. Kong, J. Liu, Lab-on-paper micro- and nano-analytical devices: Fabrication, modification, detection and emerging applications. Microchim. Acta **183**, 1521–1542 (May 01 2016)

Y. Yao, C.S. Zhang, A novel screen-printed microfluidic paper-based electrochemical device for detection of glucose and uric acid in urine. Biomed. Microdevices **18** (2016)

Y. Yao, L. Zeng, Y. Huang, The enhancement of chondrogenesis of ATDC5 cells in RGD-immobilized microcavitary alginate hydrogels. J. Biomater. Appl. **31**, 92–101 (2016)

A.K. Yetisen, J.L. Martinez-Hurtado, A. Garcia-Melendrez, F.D. Vasconcellos, C.R. Lowe, A smartphone algorithm with inter-phone repeatability for the analysis of colorimetric tests. Sensors Actuators B Chem. **196**, 156–160 (2014)

M. Zarei, Infectious pathogens meet point-of-care diagnostics. Biosens. Bioelectron. **106**, 193–203 (2018)