

Portable 3D printed Electrochemiluminescence Platform with Pencil Graphite Electrodes for Point of Care multiplexed analysis with Smartphone based Read-out

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Abstract:

Herein, a portable 3D-printed miniaturized bipolar electrode-electrochemiluminescence (BPE-ECL) platform is presented. The platform has a smartphone enabled read-out method for ECL signal analysis, 9 V Hi-watt battery for power supply and graphitized mesoporous carbon/multiwalled carbon nanotube (GMC/MWCNT) nanomaterial modified pencil graphite electrodes (PGE) as bipolar and driving electrodes. The nanomaterial modified pencil graphite bipolar electrode (PG-BPE) showed great electro-catalytic activity towards luminol-H₂O₂, luminol-O₂ ECL reaction in neutral medium. The sensitized luminol-O₂, luminol-H₂O₂ reactions were successfully applied for sensing of H₂O₂, O₂ and CO₂. With optimized parameters determination of H₂O₂, O₂ and CO₂ can be in the linear range of 0.08 μM -5000 μM, 0.3 - 9 mg/L, 0.6 - 9 mg/L with detection limit of 0.069 μM, 0.15 mg/L, 0.45 mg/L. As a prototype application, quantitative detection of glucose has been carried out with modified PGE anchored with GOX (GMC/MWCNT@GOX). The prepared electrode was analysed for physico-chemical and microscopic characterizations. The modified PGE showed an excellent ability to detect glucose in a linear range of 1 μM – 10 mM with a detection limit of 0.31 μM. Finally, the platform was subjected to real sample analysis of H₂O₂ in clinical H₂O₂, cosmetic bleach,

O₂ and CO₂ in lake water and tap water, and glucose in human blood serum samples. The results indicated that the proposed platform offered excellent reliability, accuracy and amenable to be used for multiple point-of-care biochemical analysis.

Keywords: 3D-printed miniaturized platform; bipolar electrodes; Electrochemiluminescence; pencil graphite; smartphone diagnostics

1. Introduction:

Development of chemiluminescence (CL)sensors is an active research area due to their proven utility to detect metal ions, antioxidants and various biochemical [1]. Amongst different CL methods, the electrochemiluminescence (ECL) detection gained more attention owing to features like absence of background signal, excellent controllability over the position or time of light emission, and possibility to enhance ECL intensity with nano-material [2]. Therefore, ECL is a highly sensitive analytical method for a wide range of applications. It involves the process of light emission at the electrode surface due to exergonic electron transfer reaction [3].

Typically, an ECL assay was accomplished by harnessing two or three electrode system [4], [5]. However, a few ECL assays are implemented using bipolar electrode (BPE) either in open or closed system. A BPE is an electronic conductor that gets exposed to an applied electric field without any direct contact with the electric circuit. A uniform electric field is applied across the solution by providing voltage to the two driving electrodes (DE). As a result, the potential difference is generated between the BPE and the electrolyte. The redox reaction is driven at the two poles of BPE due to anodic or cathodic over potentials. Such platform has advantages such as no need of direct contact, inexpensive, integration with portable system and possibility of high throughput analysis [6].

Arora et al. developed the first BPE-ECL based miniaturized sensor with U-shaped platinum film as a BPE [7]. Subsequently, several research groups have developed BPE-ECL based miniaturized devices [4], [5]. The devices developed by Crook and Wang group consists of poly(dimethylsiloxane) (PDMS) based microchannel irreversibly bonded to a glass slide fabricated using standard lithography procedure with indium tin oxide (ITO) [8] or gold as a BPE [9]. There are a couple of reports wherein the BPE was placed inside the capillary tube to perform BPE-ECL.

For integrating electrode in PDMS, miniaturized devices, techniques like electrode insertion, usage of screen printed electrode (SPE) and a single step fabricated 3D printed device with electrode have been adopted to gain accuracy [10], [11], [12], [13], [14]. However, while performing biological studies, experimental replication from the multiple samples were usually required. Hence, device-to-device or electrode reproducibility became a major concern. In recent few decades, efforts have been made to create hybrid, reusable devices using standard lithography process. Nevertheless, these cells require expensive device fabrication process and electrode materials. Moreover, expensive peripheral device, such as CCD camera, Photomultiplier Tube (PMT) or ECL analyser for data collection, bulky DC power supply to provide the potential and external micro pumps for injection and extraction of solution, are generally required. Therefore, an alternative and novel BPE-ECL method to overcome these limitations is necessary.

Usually, ITO, gold, platinum and carbon based electrodes have been the conventional electrodes used in ECL sensing. In comparison to these traditional electrodes such as glassy carbon electrode (GCE) [15], [16], pencil graphite electrodes (PGE), gives an advantage of high electrochemical reactivity, mechanical rigidity, reproducibility and ease of modification that are often necessary to develop disposable electrochemical sensors [17]. Considering these advantages, PGE are often used in analytical application and can be used as BPE.

Luminol is an inexpensive and widely-reported classic reagent that can be used for both CL and ECL reactions, different mechanisms have been proposed for ECL reaction of luminol. The luminol ECL reaction happens in the presence of hydrogen peroxide (H_2O_2) or also in dissolve oxygen but higher ECL intensity is obtained with H_2O_2 . Recently, various efforts are made to sensitize and use luminol ECL reaction for detection of biological compounds. The luminol ECL reaction can be sensitize by the production of reactive oxygen species when specific potential is applied. The electrogenerated reactive species present at electrode surface oxidise luminol, and the product of luminol generates the excited state 3-aminophthalate anion which emit light.

The electrode modified with functionalized carbon nanomaterial is another way to produce and enhance ECL intensity. The sensitized luminol reaction can be used for detection of superoxide dismutase which is based on the quenching effect of luminol O_2 ECL system [18]. The sensitized ECL reaction make a probability of sensing dissolve O_2 and CO_2 in food, environmental and biomedical industry. A reliable, rapid, cost-effective, portable gas sensor is still an issue. Determination of dissolved oxygen (O_2) and carbon dioxide (CO_2) in aqueous solution is of great importance many efforts have been made to detect dissolve oxygen using CL, electrochemical and optical sensor, but few papers are reported using ECL technique [19].

In the present work, a portable 3Dprinted miniaturized platform (PG-BPE-ECL), integrated with 9V Hi-watt battery and a smartphone for ECL signal analysis is proposed. The key features of PG-BPE-ECL are: (i) reusable (sample can be injected directly using a micro-pipette) (ii) the PGE is readily available and inexpensive (iii) requires low driving potential with minimum energy consumption (a 9V battery can be used for 20 hours), (iv) usage of smartphone as a read out. Henceforth, the platform is appropriate for portable biochemical analysis at a point-of-care level. For method validation and authenticate the practicability, the fabricated PG-BPE-ECL was used for determining H_2O_2 . In addition, the applicability of the

proposed platform was explored for O₂, CO₂ and glucose sensing with modified and optimized PGE.

2. Experimental Section

2.1 Chemicals, Materials and Apparatus

The details of chemical, materials, instrumentation and electrode preparation used in this work are available in Supplementary Information (S1)

2.2 3D Printed Device Design and Fabrication

Detailed 3D printed miniaturized device design (**Scheme. IA**) and fabrication procedure are provided in supplementary information (S2). 3D printed device CAD model (**Scheme. IC-D**) with length as 25 mm, width as 10 mm and height as 8 mm with total volume of the reagent reservoir as 320 $\mu\text{L} \pm 10 \mu\text{L}$. The open reservoir for BPE of dimension 9 mm \times 4 mm \times 3 mm was in the middle in contact with the DE for the fluid to flow and interact with all the three electrodes. A holder for the driving electrode (DE) was fabricated with dimensions 4 mm \times 4 mm \times 6 mm. The BPE and the DE were kept equidistant (~ 3 mm) for achieving the potential difference across electrode. The developed platform was fully reusable, after each experiment, the platform was cleaned using water gun and was dried in oven at 40°C for 30 sec.

2.3 Design and Assembly of Portable ECL Platform

Scheme. II A shows the overall design of portable ECL platform comprising a dark-box, miniaturized ECL sub-system (2.5 cm \times 1 cm \times 0.8 cm), 9V hi-watt battery (operating current 500 mA), electronic circuit module and a smartphone. The 3D-printed miniaturized device (**Scheme. II B-C**) and dark-box (**Scheme. II D- E**) was prepared using a black acrylic sheet (4 mm) with dimensions 17 cm \times 8 cm \times 10 cm. The voltage was regulated, maintained and displayed using an electronic circuit module LM2596. From the potentiometer, the output

voltage was regulated for the electrodes, and with adequate driving potential, the ECL signal was captured using a smartphone. The smartphone camera was placed on top of the miniaturized ECL platform at a distance of 10 cm to ensure that the captured image is clearly focussed. The ECL images were captured clearly without any mechanical movement of the platform or smartphone, and were wirelessly transmitted to desktop for rapid analysis. The overall cost of the components of the portable ECL platform was less than \$8 (detailed cost estimate is given in **Table S3**), excluding the cost of the smartphone.

2.4 Data acquisition and Analysis

The captured images were recorded in JPG format and were analysed using ImageJ software (openware free imaging software). An automated image processing macro program was created in ImageJ for batch image analysis. As a result, the developed program measured the relative light intensity after converting them, into 8-bit mode. This was followed by image inversion to acquire a white background for light area, with different shades of gray area to capture the ECL intensity leading to the calibration of optical density. The gray spot with 1700 pixels area on the BPE anode was analysed for each image and mean gray values were obtained. The calculated relative light intensities value was in relative light units (RLU). With this automated program, the images with maximum ECL intensity were easily obtained. The attained ECL data was used and calibration graph were plotted.

2.5 Preparation of nanomaterial modified PG-BPE

GMC decorated MWCNT-COOH composite were prepared by adding 1:1 ratio of both in ethanol and then ultra-sonicated in water bath for 15 mins. The prepared suspension of GMC and MWCNT was drop casted on the anode part of the BPE and was dried in oven at 90°C for 10 mins. Control experiments after each modification were performed (Supplementary Fig. S4) to study the role of each layer. It was discovered that the ECL intensity increased with each

modification. The prepared composite was used for H₂O₂, CO₂, O₂ sensing. For glucose sensing, GOX enzyme was anchored to the modified PGE by immersing in the GOX enzyme solution of 5 U mL⁻¹ for 30 minutes followed by air drying for 30 minutes.

3. Results and Discussion:

The PG-BPE-ECL assays based on the luminol/H₂O₂ reaction system were performed to explore the applicability of the system. The luminol/ H₂O₂ reaction mechanism was proposed by Marquette and Blum [20] (supplementary information S5). The detailed principle is given in the supplementary information S6 and is shown in **Scheme. 1B**. Smartphone was used as read-out method for ECL signal, avoiding CCD camera and PMT module. Evidently, smartphone was used as an effective readout method for colorimetric detection in miniaturized devices but seldom used for ECL signal analysis [21]. The smartphone was placed on blackbox and its camera was aligned with the PG-BPE-ECL platform with focus kept on BPE anode. For clear capture of ECL image, it was observed that the distance should be ~10 cm (for less than 10 cm, images were blurred whereas for more than 10 cm it was difficult to capture).

3.1 Characterization of nanocomposite modified Electrode

Modified electrode was characterized by physico-chemical and microscopic characterization. **Fig. 1** shows the SEM characterization of GOX modified and unmodified systems. **Fig. 1A** shows dark sphere like structure describing the attachment of GOx on PGE, **Fig. 1B** shows PGE modified with MWCNT+GMC where agglomerated spheres and tube type structures indicates the basic structure of MWCNT+GMC composite. **Fig. 1C** reveals that GOX has covered the entire layer of composite (GMC/MWCNT).

Fig. 1D-F depicts the physico-chemical characterizations by Raman, UV-Vis, and FT-IR spectroscopy of PGE/MWCNT + GMC and PGE/MWCNT+GMC@GOx modified electrodes. Raman Spectroscopic response (**Fig. 1D**) of PGE/MWCNT+GMC with (a) and

without (b) GOx showed D and G band at 1350.6 cm^{-1} , 1583.77 cm^{-1} and 1336 cm^{-1} , 1594 cm^{-1} respectively. The G-band, corresponding to the graphitic nature (Sp^2 carbons), showed a significant decrement while the disordered graphitic structure (Sp^3 carbons), due to oxygen functional groups, showed an increment post modification with GOX [22]. The I_D/I_G ratio for GOx unmodified system was 0.71 whereas, GOx modified system gave value of 1.17, which is due to π - π interaction between the GOx enzyme and graphitic units of GMC+MWCNT[23]. UV-Vis response (**Fig. 1E**) of the fabricated electrode before and after modification with GOx showed almost similar absorption peak only with a slight bathochromic shift 312 nm to 312.5 nm indicating the insensitivity if this technique for identification of GOx immobilization. The FT-IR characterization (**Fig. 1F**) showed peaks at 3146 cm^{-1} , 1640 cm^{-1} , 1540 cm^{-1} and 1110 cm^{-1} corresponding to O-H (stretch), C=N (stretch), N-O (stretch) and C-O (stretch) respectively for GOx alone. On the other hand, the composite of MWCNT+GMC showed 3129 cm^{-1} (O-H stretch), 1401 cm^{-1} (O-H bend) and 1121 cm^{-1} (C-O stretch) due to the carboxylic groups on MWCNT. Upon modification of the carbon matrix with GOX, there was a substantial increase in (O-H) stretching at 3140 cm^{-1} (plausibly due to OH groups of GOx). Similarly, peaks at 1642 cm^{-1} (C=N), 1537 cm^{-1} (N-O) and 1113 cm^{-1} (C-O) were also significantly observed indicating immobilization of GOX on MWCNT+GMC surface.

3.2 Effect of length of PGE on driving potential

Investigations were carried out on the effect of PG-BPE length utilized with respect to the driving potential keeping DE length constant as 12 mm. The reservoir was first filled with 320 μL mixture of 10 mM luminol and 1 mM H_2O_2 . As the ECL intensity is related to BPE length, the study was done on 2 mm diameter PGE with length ranging from 1 mm – 7 mm. As shown in **Fig.2A & B**, the intensity on BPE is directly proportional to the length, with the maximum obtained at 7 mm. Hence, 7 mm length was chosen to achieve the strongest ECL intensity (**Fig.**

2A & B). With optimized driving potential, longer BPE goes high partial potential, which leads to lower ECL initial potential and strong ECL intensity. It was witnessed that the ECL intensity increases with the increase in driving potential from 3 V to 7 V, which can be due to the increase in EC oxidation rate of luminol to form 3-aminophthalate. With the driving voltage less than 3 V, no ECL signal was obtained, as the potential is not sufficient to oxidise the luminol. Whereas, with more than 7 V potential, the ECL signal decreased plausibly due to background reaction like oxidation of H₂O releasing oxygen. The optimized length was 7 mm as greater than this reduced the intensity.

3.3 Effect of Luminol Concentration

Herein, luminol works as a luminescence reagent, hence, its concentration plays a key role in the ECL response. As shown in **Fig. 3 A & B**, the ECL intensity grows with the increase in luminol concentration ranging from 1 mM to 7.5 mM. This is possibly due to the luminolendoperoxide formation as the concentration increases [24]. Nonetheless, the ECL intensity decreases with more than 7.5mM luminol, credibly due to the self-quenching effect [25].

3.4.1 ECL behaviour of luminol-H₂O₂ system

Under optimum conditions, PG-BPE-ECL platform was used for quantification of H₂O₂. Based on charge balance mechanism, the ECL intensity increased with gradual increase in H₂O₂ concentration over the range from 5 μM to 5000 μM as shown in **Fig.4 A & B**. It was observed that the ECL intensity was directly proportional to H₂O₂ concentration ranging from 0.08 μM to 100 μM (inset in **Fig.4B**) whereby, the limit of detection (LOD) was estimated to be 0.069 μM. The measured LOD was lower than that obtained using carbon ink SPE in paper based BPE-ECL device (46.6 μM), cloth based BPE-ECL (24 μM) [26] using CCD as an imaging system and using commercial chemiluminescence imaging system with PDMS-ITO glass

microchip (60 μM) [27]. The proposed method gives wide linear range, with better sensitivity and LOD. The LOD was calculated using the given formula where σ is standard deviation,

$$LOD = \frac{3\sigma}{SLOPE}$$

3.4.2 ECL behaviour of luminol-O₂ System

In luminol – O₂ ECL system, the dissolve oxygen is considered to be endogenous co- reactant that generates O₂ on the working electrode reacting with luminol anion that emits light. For determining the efficiency of the luminol – O₂ process, the experiments were carried out with GMC modified PG-BPE ina working electrolyte of luminol in neutral and alkaline aqueous medium (0.5 M phosphate buffer solution). It was found that in neutral aqueous solution, the ECL signals were greatly enhanced as compared to that of alkaline medium. This may be attributed to proton as well as hydroxide ion as a quencher in ECL reaction [28]. The ECL measurements were carried out under saturated O₂, N₂ and in air electrolyte solution. It was observed that under saturated O₂, strong ECL signal were obtained and in N₂ hardly any ECL signal were observed. When the air free solution was prone to air, ECL signals were observed and its intensity increase respect to prone time, with the result that ECL signal are dependent on O₂. It seems that at higher concentration of dissolved O₂ give higher ECL intensity as it might be able to enhance O₂ adsorption.

Under the optimal condition, luminol - O₂ system, it was observed that ECL intensity is dependent on the concentration of dissolve oxygen. As shown in **Fig.5 A & B**, the ECL intensity increases with increase in oxygen concentration. The ECL intensity was observed to be linear in the range of 0.3 - 9 mg/L with a detection limit of 0.15 mg/L. The measured LOD was lower than obtained using fluorescence probe sensor of 0.35 mg/L [29] and optical fibre sensor method giving LOD of 0.98 mg/L [30].

3.4.3 ECL behaviour of luminol-CO₂ system

In CL, it has been reported that CO₂ greatly increase the luminol CL in the presences and absence of added oxidant. But no studies are reported in ECL for CO₂ sensing with and without added oxidant. For verifying the feasibility of CO₂ gas sensing, the experiments were carried out in presence and absence of added oxidant (H₂O₂). It was observed that in the absence of added oxidant no ECL signal was obtained. The plausible principle of CO₂ ECL sensing can be CO₂ reacts with H₂O₂ to form peroxy carbonate, that decomposes to highly reactive radical anion which are capable of oxidizing luminol [31], [32]. The concentration of sensing solution was optimized as luminol (7.5 mM) and H₂O₂ (100 μM).

As expected, the ECL signal increases with an increase in CO₂ concentration with the optimized PG-BPE in the linear range of 0.60 – 9 mg/L. Corresponding calibration plot (**Fig.6B**) gives a slope value of 0.666 ± 0.0295 and $R^2 = 0.994$ with an appreciable detection limit of 0.45 mg/L.

3.4.4 ECL behaviour of Luminol-glucose system

Glucose sensing was studied by anchoring Glucose oxidase enzyme (GOx) on the PGE electrode surface. The developed platform should have the potential for glucose assay, as H₂O₂ detection is usually the basis for glucose biosensing. For this, the PGE was chemically modified with MWCNT+GMC composite and GOx enzyme and has been shown in **scheme. 1C**.

Further, the optimal pH, drying time, concentration of enzyme was also optimized based on the intensities obtained (Supplementary S7).

3.4.5 Glucose detection by the proposed sensor

As a model system, glucose sensing in PBS was performed in the range 1 μM to 10000 μM. As shown in **Fig.7 A & B**, the glucose concentration increases linearly from 1 μM to 30 μM (inset in **Fig.7B**), whereby the LOD was estimated to be 0.30 μM. The obtained results

show that the PG-BPE-ECL platform has the ability to detect glucose and can provide low LOD in comparison to the previously known procedures. Even though the direct comparison at present stage is difficult as different detection methodologies have been used elsewhere, but it is significant that the present detection limit is much lower than that obtained using colorimetric, electrochemical and electrochemiluminescence methods as tabulated in **Table S8** (supplementary information S8).

Besides, the stability, reproducibility and selectivity of the proposed ECL biosensor was also tested (supplementary information S9). It was found that the fabricated electrode is highly stable upto 2 days with less than 2% error. Also, upon testing with various biochemical, the electrode showed high selectivity (supplementary information S9).

3.5 Real sample analysis

In order to find the selectivity of the prepared electrode in real sample, sensing of the analytes like clinical H_2O_2 and cosmetic bleach, lake water, tap water and human serum were tested for H_2O_2 , O_2 , CO_2 and glucose. Real Sample preparations are mentioned in supplementary information S10. **Table 1 and 2** shows the real sample analysis recovery values indicating the applicability of the system. Here the recovery was above 100% may be due the calculated concentration of the reaction contain more impurities which may result in greater concentration value than it actually would be if the real sample used was pure. This is considered as positive error [33-34].

4. Conclusion:

In the present work, a portable ECL platform is presented integrated with PG-BPE-ECL, 3D printed miniaturized device and a battery with smartphone imaging. With luminol/ H_2O_2 as the base for ECL reaction, the developed platform gave an excellent O_2 , CO_2 and glucose detection. The developed ECL system gives high throughput and sensitive biochemical analysis that can be easily performed at a POC level with corresponding limit of detection of H_2O_2 , O_2 , CO_2 and

glucose as 0.069 μM , 0.15 mg/L, 0.45 mg/L and 0.31 μM respectively. In future, the developed portable ECL system may find board application in environmental monitoring, bimolecular interaction and clinical diagnostics.

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Conflict of Interest

The authors have no conflict of interest

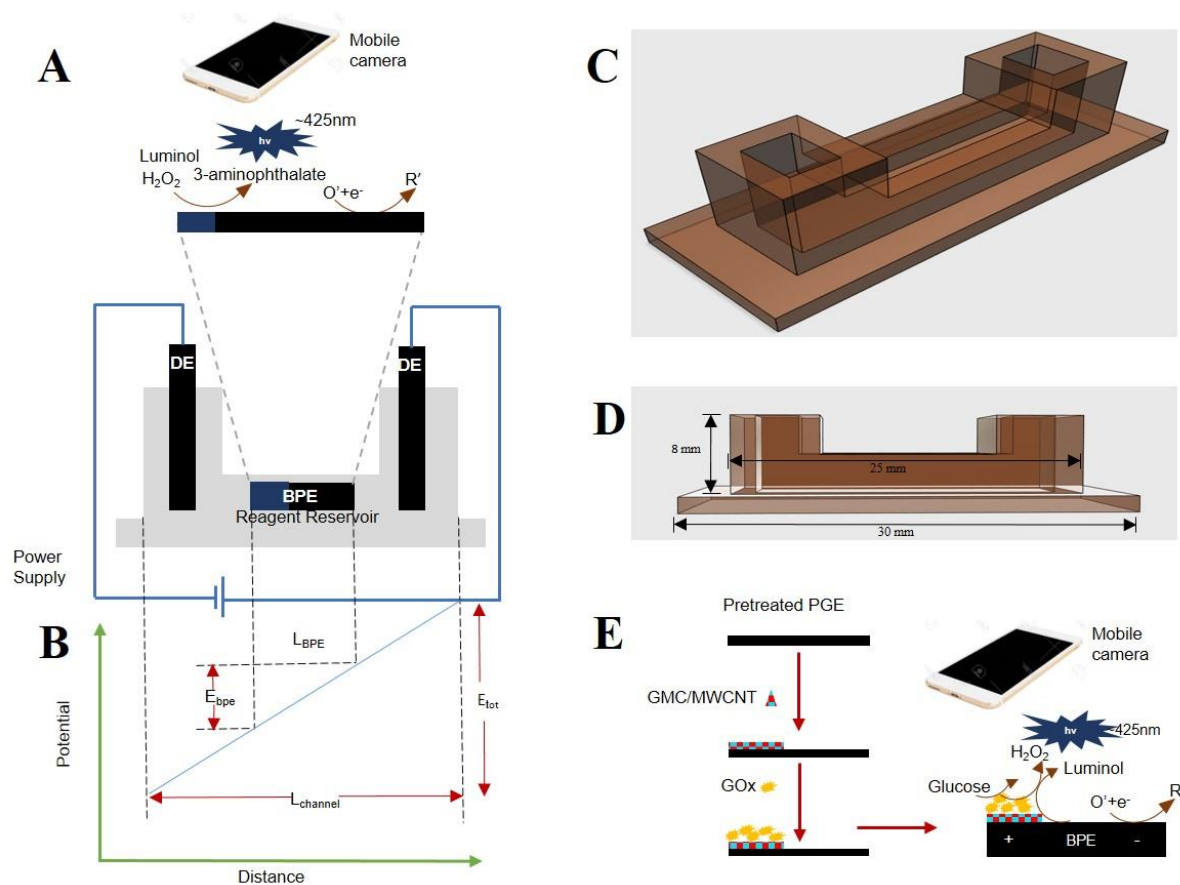
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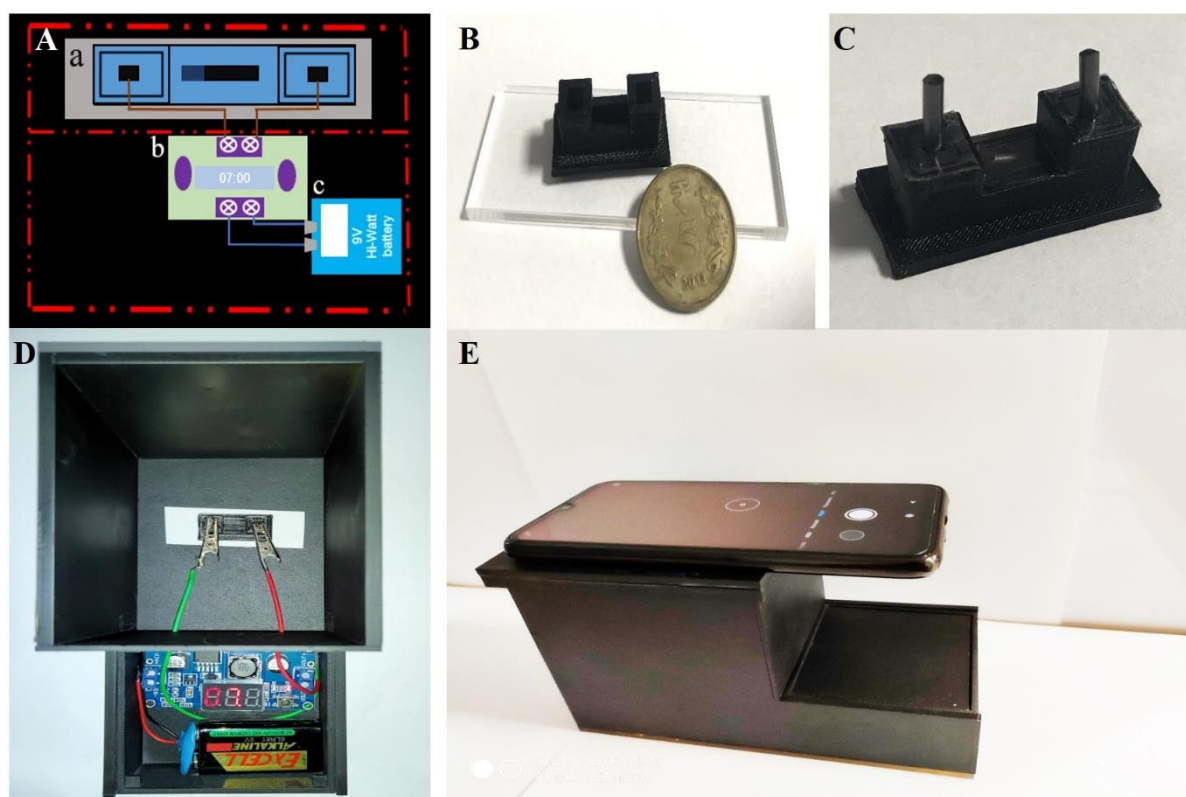
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Scheme I



Scheme I. Schematic illustration of the proposed design and sensing principle (A) Proposed 3D printed miniaturized design (BPE: bipolar electrode; DE: driving electrode). (B) Principle of Open-BPE-ECL for biochemical analysis. (C-D) Side and front view of CAD model of proposed design. (E) PG-BPE modification process and luminol based sensing for glucose detection

Scheme II



Scheme. II Schematic of the proposed platform (A) Illustration of the proposed platform (top-view) (a) PG-BPE-ECL 3D printed platform (b) electronic circuit module (c) 9V battery. (B) Picture of 3D printed miniaturized channel and (C) bonded with acrylic sheet for optical window containing with PG-BPE and DE. (D-E) Dark box open and closed view

Figure 1

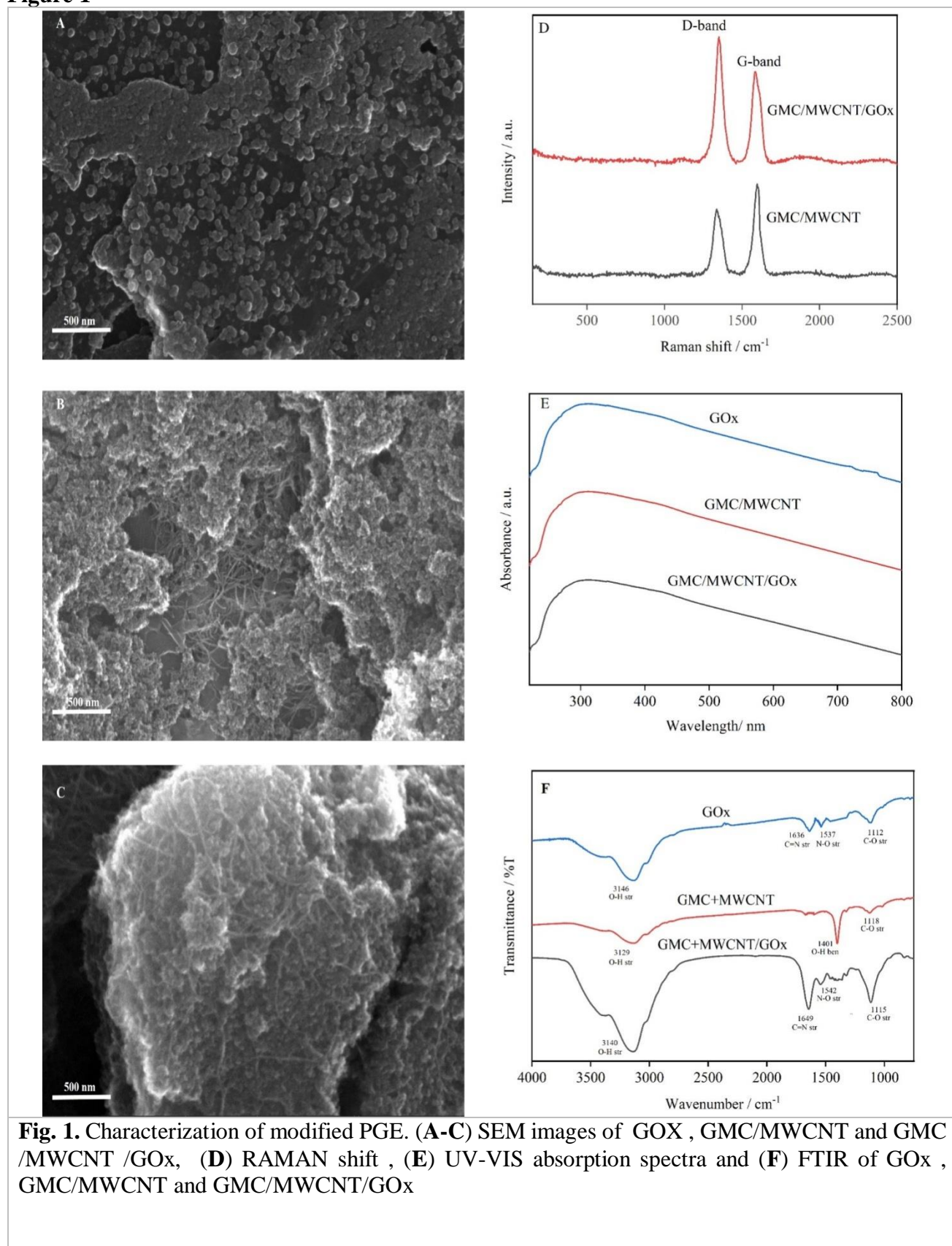


Figure 2

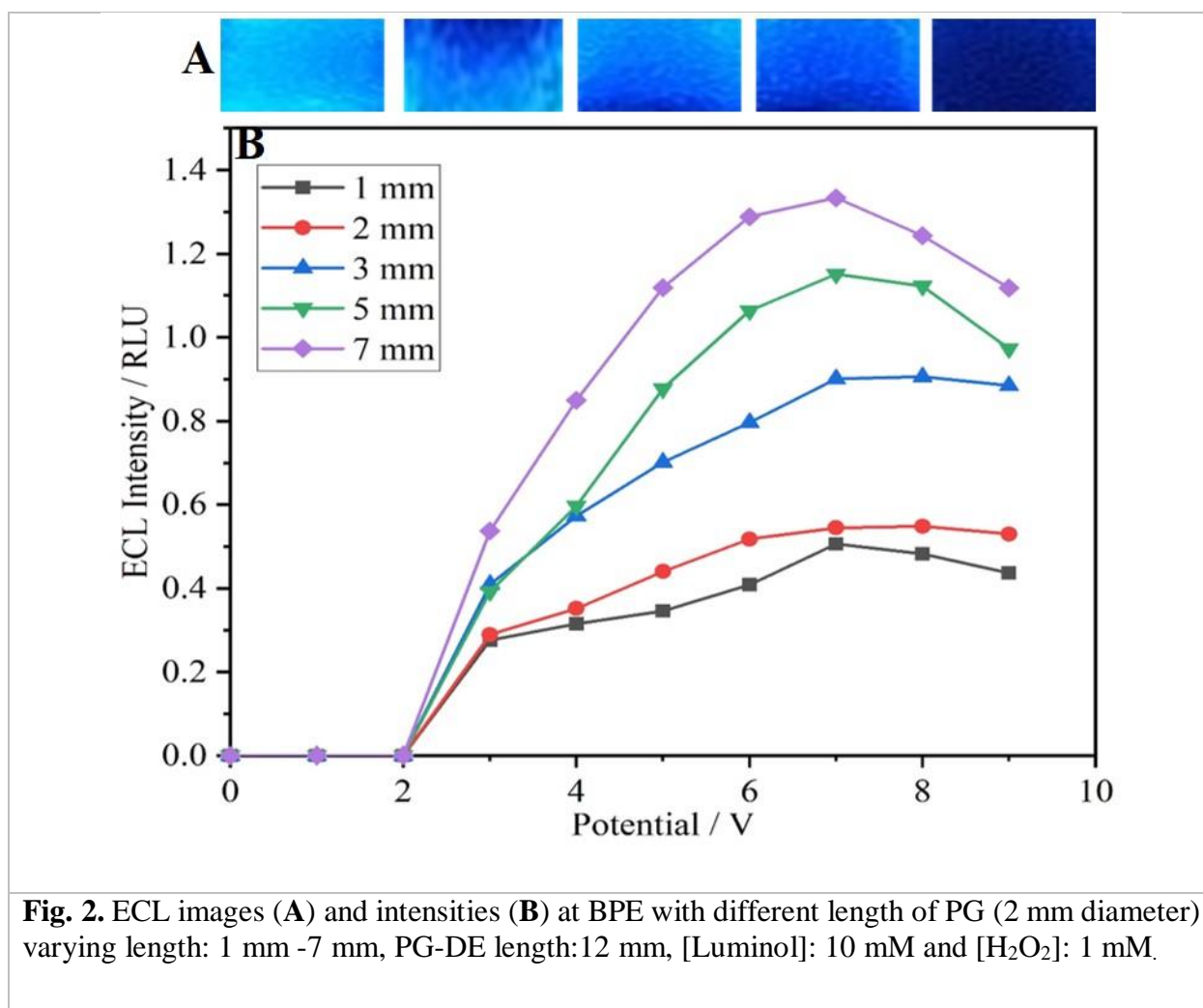


Fig. 2. ECL images (A) and intensities (B) at BPE with different length of PG (2 mm diameter) varying length: 1 mm -7 mm, PG-DE length:12 mm, [Luminol]: 10 mM and [H₂O₂]: 1 mM.

Figure 3

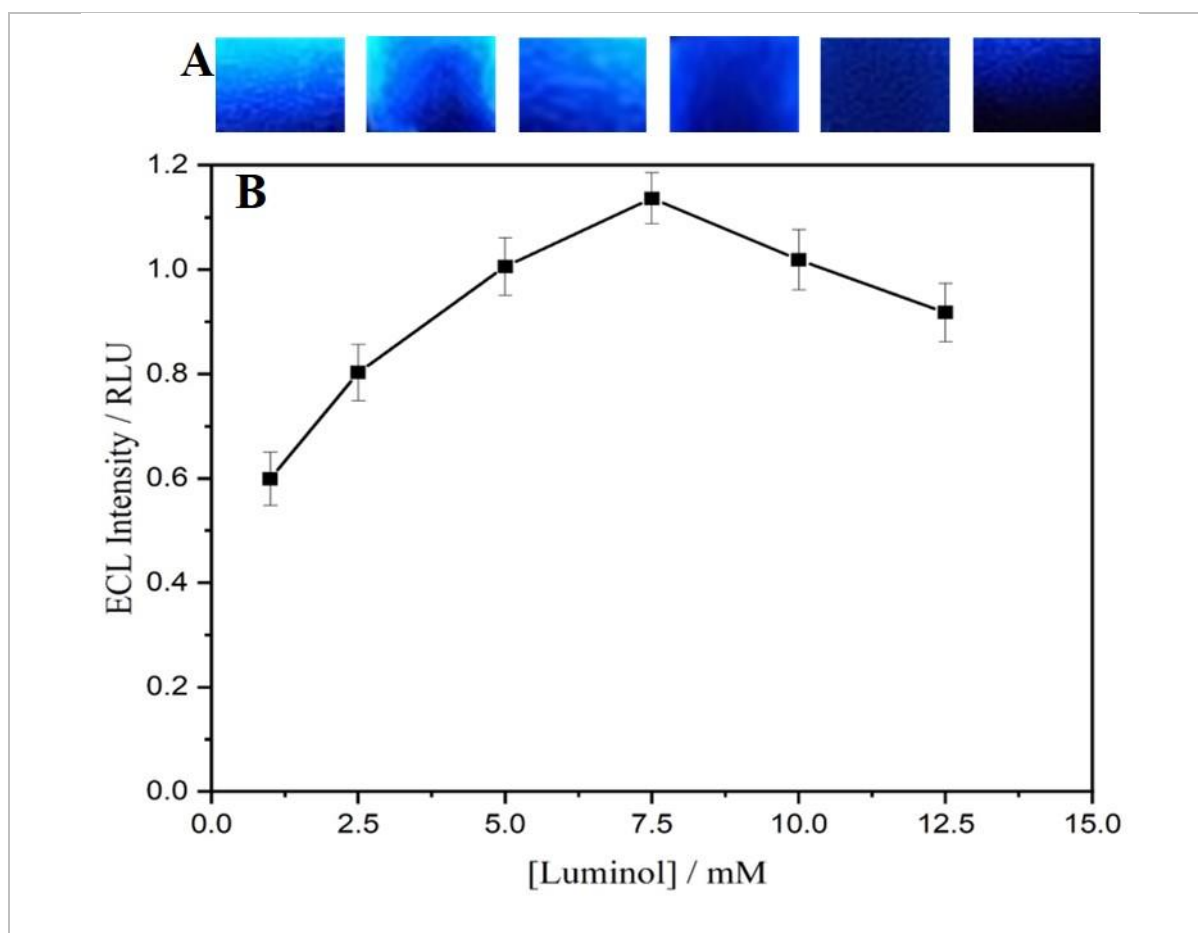


Fig. 3. ECL images (A) and intensities (B) Effect of [Luminol] on PG-BPE. Experimental conditions: [Luminol]:1, 2.5, 5, 7.5, 10 and 12.5 mM, [H₂O₂]:1mM, PG-BPE: 7 mm, PG-DE: 12 mm, driving voltage: 7V. The error bar represent the standard deviation from five independent experiment.

Figure 4

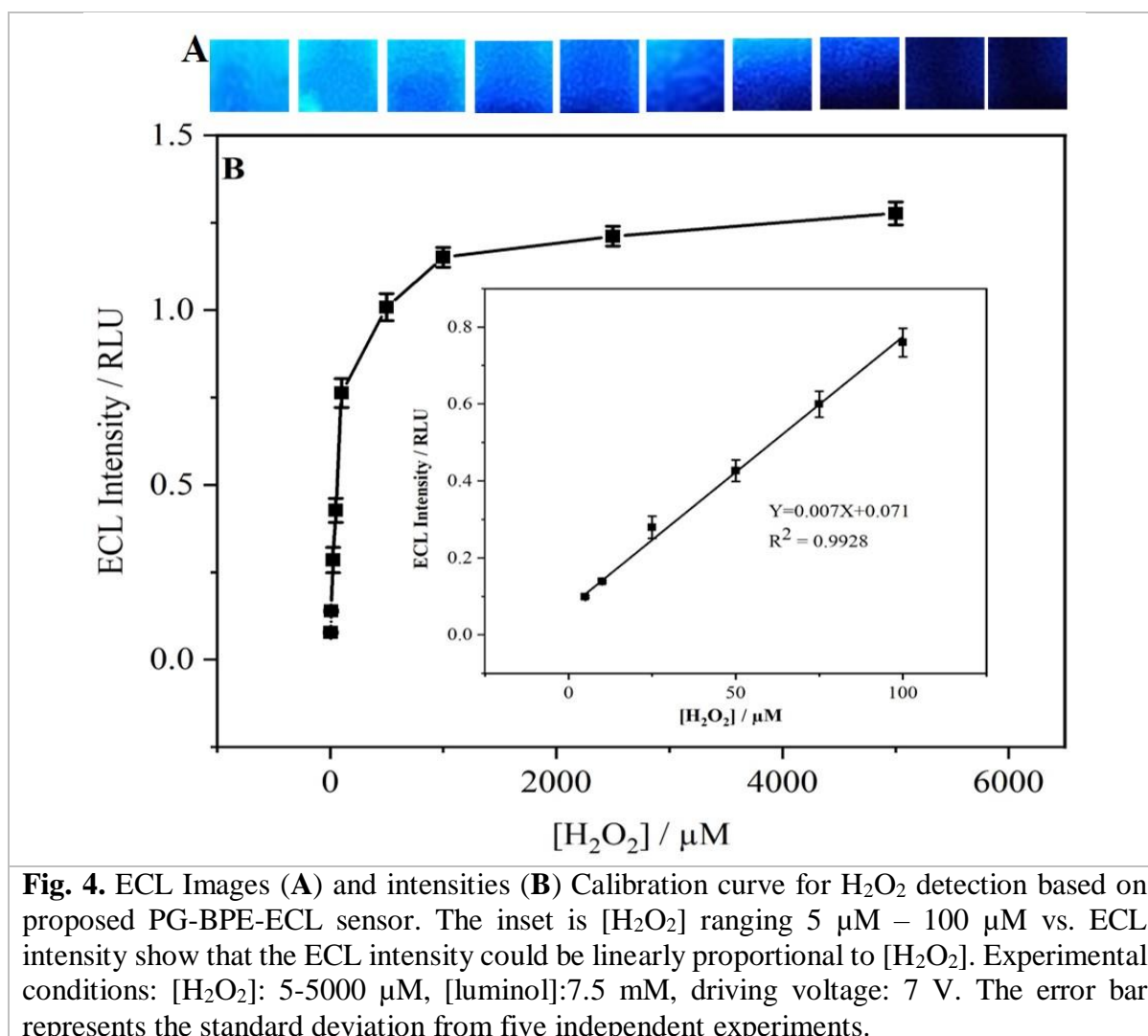


Fig. 4. ECL Images (A) and intensities (B) Calibration curve for H_2O_2 detection based on proposed PG-BPE-ECL sensor. The inset is $[H_2O_2]$ ranging $5 \mu M - 100 \mu M$ vs. ECL intensity show that the ECL intensity could be linearly proportional to $[H_2O_2]$. Experimental conditions: $[H_2O_2]$: $5-5000 \mu M$, $[luminol]$: $7.5 mM$, driving voltage: $7 V$. The error bar represents the standard deviation from five independent experiments.

Figure 5

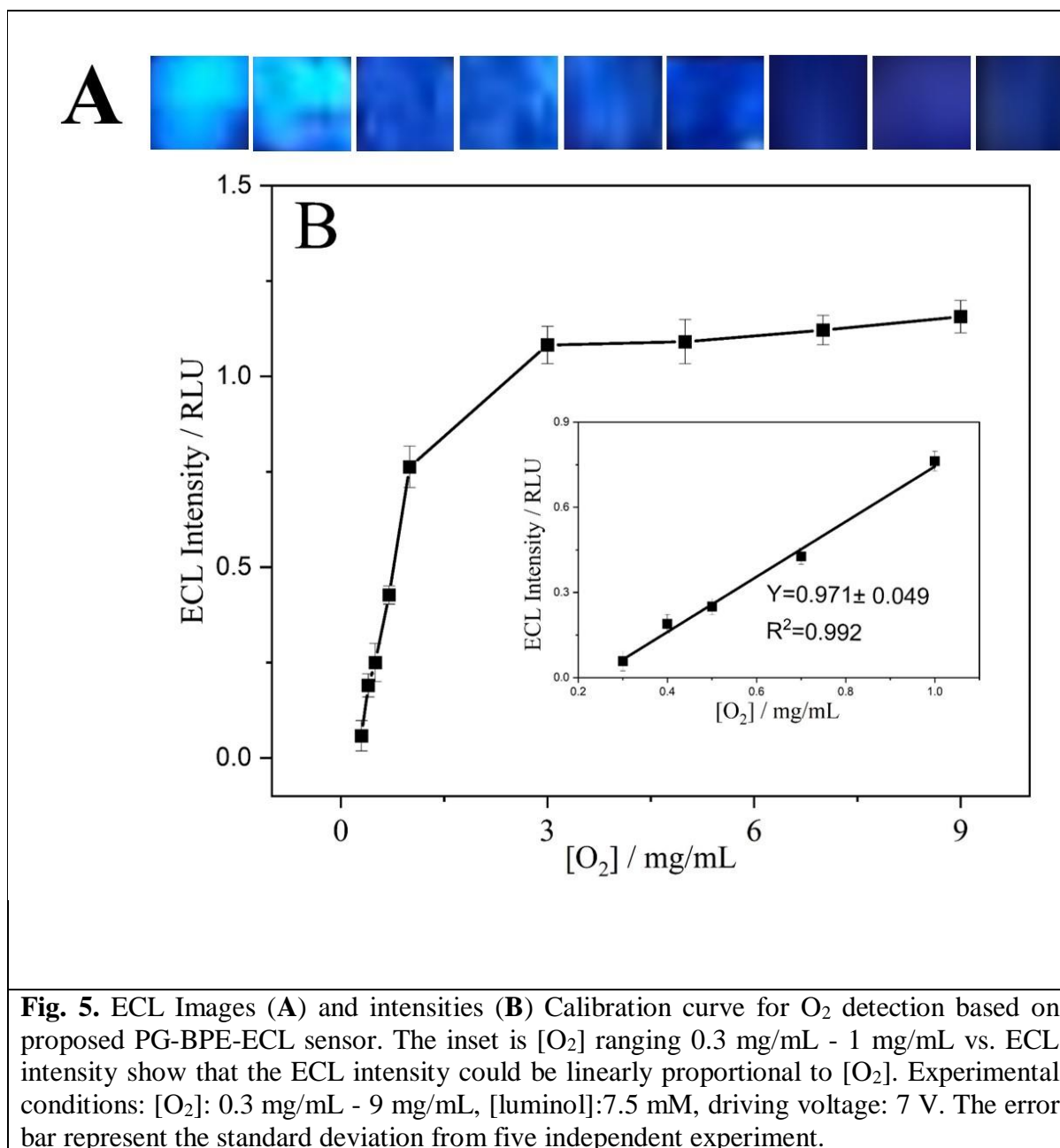


Fig. 5. ECL Images (A) and intensities (B) Calibration curve for O₂ detection based on proposed PG-BPE-ECL sensor. The inset is [O₂] ranging 0.3 mg/mL - 1 mg/mL vs. ECL intensity show that the ECL intensity could be linearly proportional to [O₂]. Experimental conditions: [O₂]: 0.3 mg/mL - 9 mg/mL, [luminol]:7.5 mM, driving voltage: 7 V. The error bar represent the standard deviation from five independent experiment.

Figure 6

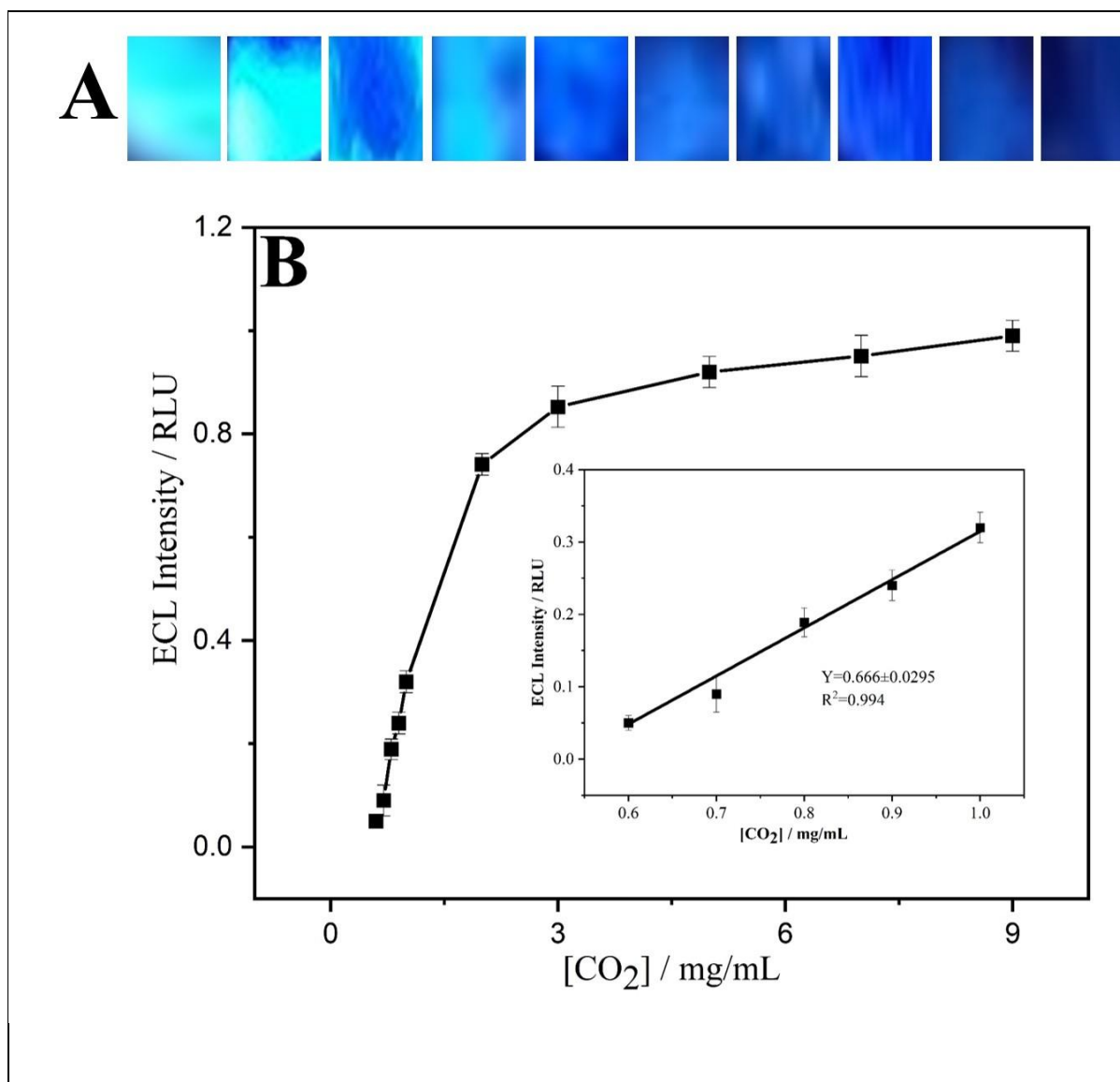


Fig. 6. ECL Images (A) and intensities (B) Calibration curve for CO₂ detection based on proposed PG-BPE-ECL sensor. The inset is [CO₂] ranging 0.6 mg/mL-1 mg/mL vs. ECL intensity show that the ECL intensity could be linearly proportional to [O₂]. Experimental conditions: [CO₂]: 0.6 mg/mL - 9 mg/mL, [lumino]:7.5 mM, [H₂O₂]: 1 mM, driving voltage: 7 V. The error bar represent the standard deviation from five independent experiment.

Figure 7

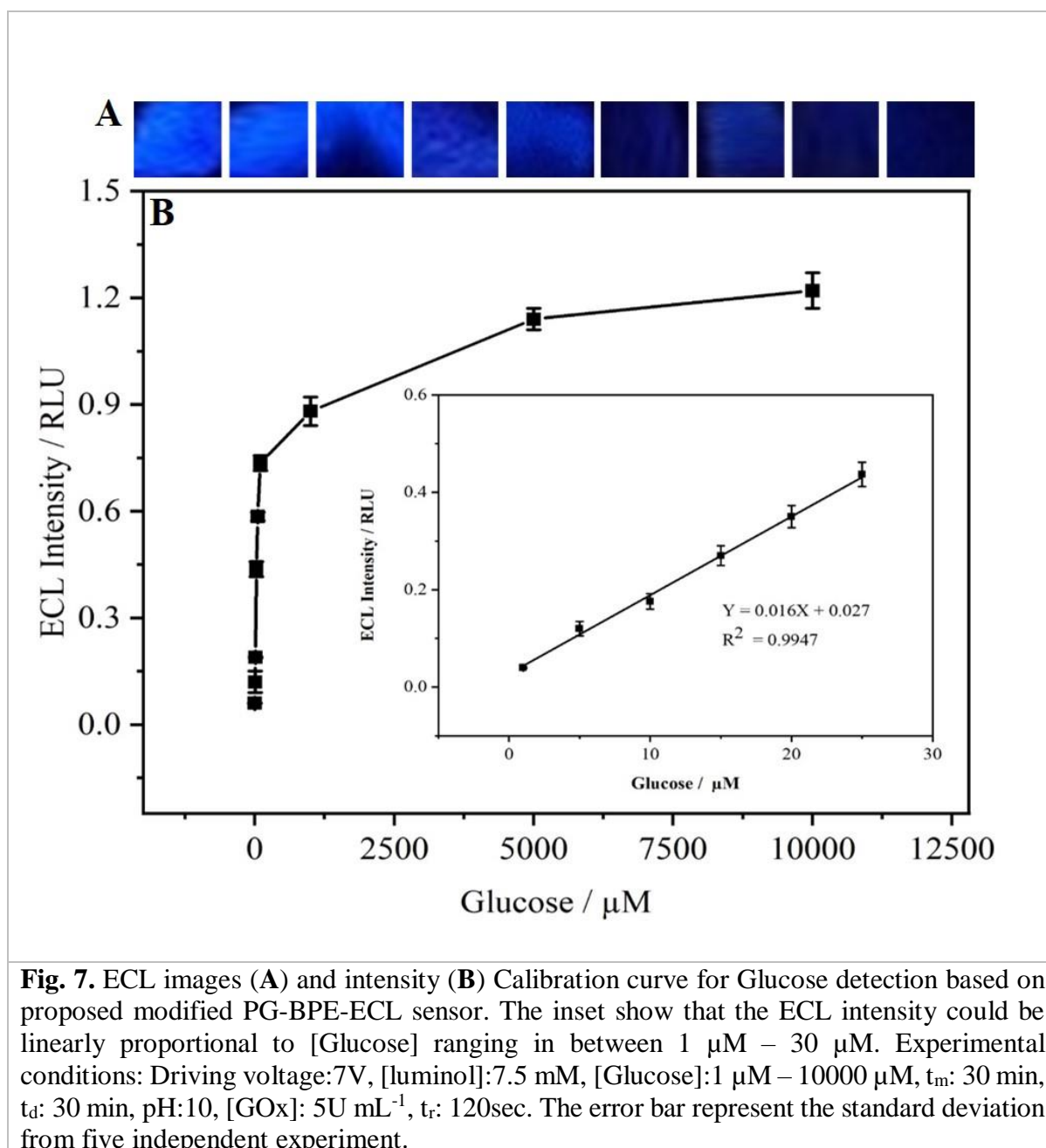


Fig. 7. ECL images (A) and intensity (B) Calibration curve for Glucose detection based on proposed modified PG-BPE-ECL sensor. The inset show that the ECL intensity could be linearly proportional to [Glucose] ranging in between 1 μM – 30 μM . Experimental conditions: Driving voltage:7V, [luminol]:7.5 mM, [Glucose]:1 μM – 10000 μM , t_m : 30 min, t_d : 30 min, pH:10, [GOx]: 5U mL⁻¹, t_r : 120sec. The error bar represent the standard deviation from five independent experiment.

Table 1: Real Sample Analysis

Found In sample	H₂O₂ added (μm)	Found In sample (μm)	Recovery %
Clinical H₂O₂ (3%)			
7.94	20	29.05	103.99
	40	51.11	106.61
	60	70.722	104.09
FEM Bleach (4%) H₂O₂			
9.055	20	29.05	105.16
	40	51.11	100.78
	60	70.722	100.40
Found In sample (mg/ml)	O₂ added (mg/ml)	Found In sample (mg/ml)	Recovery %
O₂ in Tap Water			
1	1	1.98	99.01
	1.5	2.6	104.32
	2	3.02	100.67
O₂ in Lake Water			
4	1	4.23	105.75
	1.5	4.64	102.22
	2	5.	101.8
Found In sample (mg/ml)	CO₂ added (mg/ml)	Found In sample (mg/ml)	Recovery %
CO₂ in Tap Water			
0.8	1	1.83	101.66
	1.5	2.51	108.69
	2	2.76	98.57
CO₂ in Lake Water			
3	1	4.17	104.25
	1.5	4.64	103.11
	2	5.13	102.66

Table 2. Real Sample Analysis in Human Serum

Clinical method (μM) (500 times diluted)	This method (μM)	Added (μM)	Found (μM)	Recovery%
16.98	17.25	5	22.16	99.59551
		10	28.16	103.3394
		15	32.42	100.5271