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ORIGINAL PAPER

Multiplexed and simultaneous biosensing in a 3D-printed portable six-well smartphone operated electrochemiluminescence standalone point-of-care platform

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Abstract

3D-printed portable devices have immense and proven potential to transform the field of electrochemiluminescence (ECL) for diverse biochemical applications. 3D printing (3DP) offers unparalleled ability to build tiny devices in a single step with high accuracy and compatibility, and integrability as per the requirement. In this study, for the first time, a six-well 3D-printed closed bipolar electrochemiluminescence (3DP-CBPE-ECL) device has been successfully fabricated and validated by performing single-step detection of various biochemicals such as glucose and choline. Luminol/H_2O_2-based enzymatic reactions were performed with optimized parameters for selective sensing of glucose and choline. The single-step detection of glucose and choline was accomplished for the linear ranges of 0.1 to 10 mM and 0.1 to 5 mM, with a limit of detections (LODs) of 24 µM and 10 µM, respectively. A smartphone was leveraged to execute multiple activities such as powering the ECL device, capturing ECL images, and calculating the ECL intensity of the obtained ECL signal. The feasibility of a six-well 3DP-CBPE-ECL device was tested by sensing glucose and choline simultaneously in a single device at three different concentrations. Furthermore, the concentration of glucose and choline was calculated in real blood serum using the conventional additive (spiking) method, demonstrating the high practicability of the fabricated ECL device and yielding promising findings. Finally, based on the obtained results and other advantages such as low-cost, fast prototyping and requirement of a minimum sample volume, the fabricated six-well 3DP-CBPE-ECL device has shown potential to be used in the field of biochemical applications.

Keywords 3D printing · Electrochemiluminescence · Closed bipolar device · Biochemical sensing · Point-of-care testing

Introduction

Three-dimensional printing (3DP) is a layer-by-layer fast prototyping or additive manufacturing technique that has piqued the interest of researchers and is widely used in diverse applications including automotive, defense industries [1, 2], point-of-care testing, biochemical applications, lab-on-chip, organ printing, industrial design, and healthcare [3, 4]. When compared to traditional fabrication methods such as photolithography, 3DP fabrication methodology provides many various crucial benefits such as low-cost, fast prototyping [5], reduced manufacturing time, and complicated designs that may be easily created [6, 7]. Further, numerous biochemical sensing techniques, such as chemiluminescence, electrochemical, and electrochemiluminescence, are compatible with the 3DP technique [8, 9]. However, selecting an optimal and adaptive detection methodology to incorporate 3DP devices for biochemical sensing is critical for such sensing techniques. Amongst them, ECL, whereby electrical energy is converted to the radiative one [10–12], is the most promising approach since it provides major advantages such as being very selective and sensitive, having a wide operating range, having minimal background noise, and many more [13–15].

ECL devices with bipolar electrodes (BPEs) have been widely employed as an effective analytical tool for sensing numerous biomolecules [16, 17]. BPEs operate as conductors as they are immersed in the channel and have no direct connections to the applied external potential. As ECL-BPE

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devices are easily fabricated, controlled, and integrated with POCT devices, they have been employed as a powerful tool in electrochemistry [16, 18]. Generally, two types of BPE systems have been reported: open BPE-ECL (OBPE-ECL) and closed BPE-ECL (CBPE-ECL) systems [19, 20]. The open BPE-based ECL approach has two major drawbacks: very low current efficiency and background noise signals produced by driving electrodes, making it impractical to use open BPE-ECL systems in practical applications [21]. On the other hand, the closed BPE-ECL approach can improve current efficiency to 100% ideally and by placing anode and cathode of BPE into two different wells (reporting and supporting wells), the background noise from driving electrodes can easily be eliminated [22–24]. Considering the advantages of the CBPE-ECL system, herein, a novel six-well 3D-printed ECL device has been realized and was validated experimentally by detecting various biomolecules like glucose and choline.

Continuous monitoring of glucose and choline is essential to work the human body correctly. Hence, in the presented work, a novel six-well 3D-printed closed bipolar electrode-based ECL device was fabricated and single-step detection of glucose and choline was performed. The primary focus was to develop a portable, rapid diagnosis ECL system to detect various biomolecules accurately. Keeping that in mind, to obtain better selectivity and sensitivity, enzymatic reactions were carried out to detect glucose and choline. All the materials and equipment necessary to fabricate the six-well 3DP-CBPE-ECL system are inexpensive and widely available. Additionally, the proposed device was fabricated using a one-step manufacturing process, which avoids following the lengthy fabrication flow and sophisticated equipment.

Experimental section

Materials and equipment

Luminol, glucose, choline, glucose oxidase (GOx), and choline oxidase (COx) were obtained from Sigma Aldrich, India. As the luminol is soluble in base, in order to make luminol stock solution, the base solution was first prepared using sodium hydroxide (NaOH). The NaOH was procured from Sisco Research Laboratories, India. The same step-by-step procedure was used to make luminol stock solution, as detailed in a previously published article by our group [25]. Stock solution of luminol (10 mM) was prepared in 50 mL (47 mL luminol + 3 mL NaOH having 0.1 M concentration) DI water. Further, different concentrations of luminol (1 to 7 mM) were prepared with standard dilution method in DI water using stock solution of luminol. Similarly, stock solutions of glucose and choline (10 mM each) were prepared in DI water, and different molar concentrations were prepared using standard dilution method. Isopropanol (IPA) and dimethylforamide (DMF) were procured from SRL, India.

To fabricate ECL devices, Creator PRO Flashforge 3D printer was effectively used. Commercially available graphene filament (1.75 mm diameter) was used and procured from Black Magic 3D, USA, to fabricate the closed BPE electrodes for the six-well ECL device. The white PLA filament (1.75 mm diameter) was purchased from Amazon, India. To capture ECL signals, the Samsung Galaxy M11-12 MP smartphone was exactly placed over a 3D-printed black box at an ideal distance. A DC-DC (2.4 to 24 V) buck-boost converter was utilized to power the ECL device, essentially eliminating the need for an external power supply.

Working principle and fabrication of six-well 3DP-CBPE-ECL

Enzymatic reactions based on luminol/H_2O_2 have been carried out to better comprehend the working principle of the fabricated ECL device. Anode and cathode of BPE electrodes were placed in different channels. When a sufficient external voltage was provided to the ECL device, a strong electric field was formed across the closed bipolar electrode (CBPE), triggering ECL processes such as oxidation and reduction on the anode and cathode of the CBPE, respectively, resulting in the emission of an ECL signal at the anode of the CBPE. The working principle of the six-well 3DP-CBPE-ECL device with anodic, cathodic, and enzymatic reactions is depicted in Fig. 1a.

A dual-extruder FDM 3D printer was efficiently used to fabricate a six-well 3DP-CBPE-ECL. Prior to the fabrication, the ECL device design was created using computer-aided design (CAD) software-fusion 360 and later, design was saved in ".stl" format. Following that, graphene and white PLA filament were used to create electrodes and channels by adjusting the temperature (220 °C). The electrodes and device dimension are shown in Fig. 1b and c. The generated file was then stored in the ".x3g" format, which is compatible with 3D printers. Finally, the completed design was sent to a dual-extruder FDM 3D printer through a graphical user interface (GUI). Image of final fabricated six-well 3DP-CBPE-ECL device with electrodes and channels is shown in Fig. 1d.

After the device fabrication, the six-well 3DP-CBPE-ECL devices were DMF treated to enhance device performance on several fronts, such as the surface of the electrode being more porous, to achieve better detection limit [26]. All six wells of the device were filled with DMF for a 10-min optimum time [27]. The device was then cleaned with 60% diluted IPA. Finally, DMF-treated devices were allowed to dry for 6 to 7 h at room temperature.

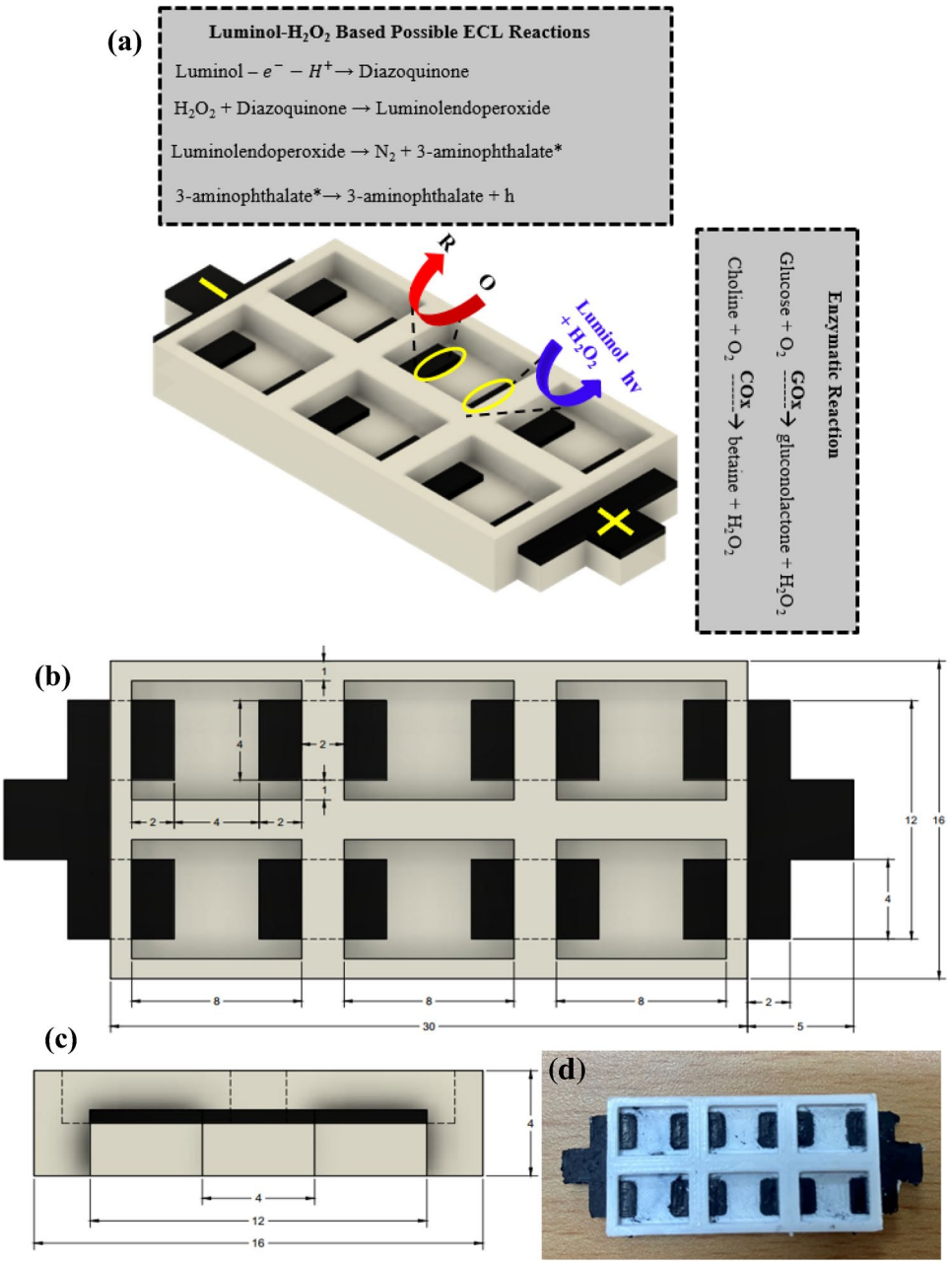

Fig. 1 **a** Working principle of six-well 3DP-CBPE-ECL device with anodic, cathodic, and enzymatic reactions, **b** and **c** top and side view for 3DP-CBPE-ECL device with dimensions, **d** final fabricated 3DP-CBPE-ECL device

Data acquisition and analysis

Herein, for the first time, smartphone was efficiently leveraged to carry out the following functions: (1) to power the ECL device through buck-boost converter which successfully makes the system portable and eliminates the usage of external bulky power supply, (2) to capture the ECL signals, and (3) to calculate the ECL intensity of captured signals which eliminates to use third-party software such as ImageJ and MATLAB. The ECL intensity calculator mobile app was developed in Java built-in Android Studio with read–write storage. The ECL intensity calculator android app in mobile is shown in Fig. 2b. The android app was designed in such way that it can calculate the intensity of real-time captured images or saved images in a gallery. The captured ECL image using an android app is shown in Fig. 2c. After capturing the ECL signal, a high intensity portion of an image was clipped and ECL intensity was calculated by pressing the "MEASURE" button shown on the display displayed in Fig. 2d.

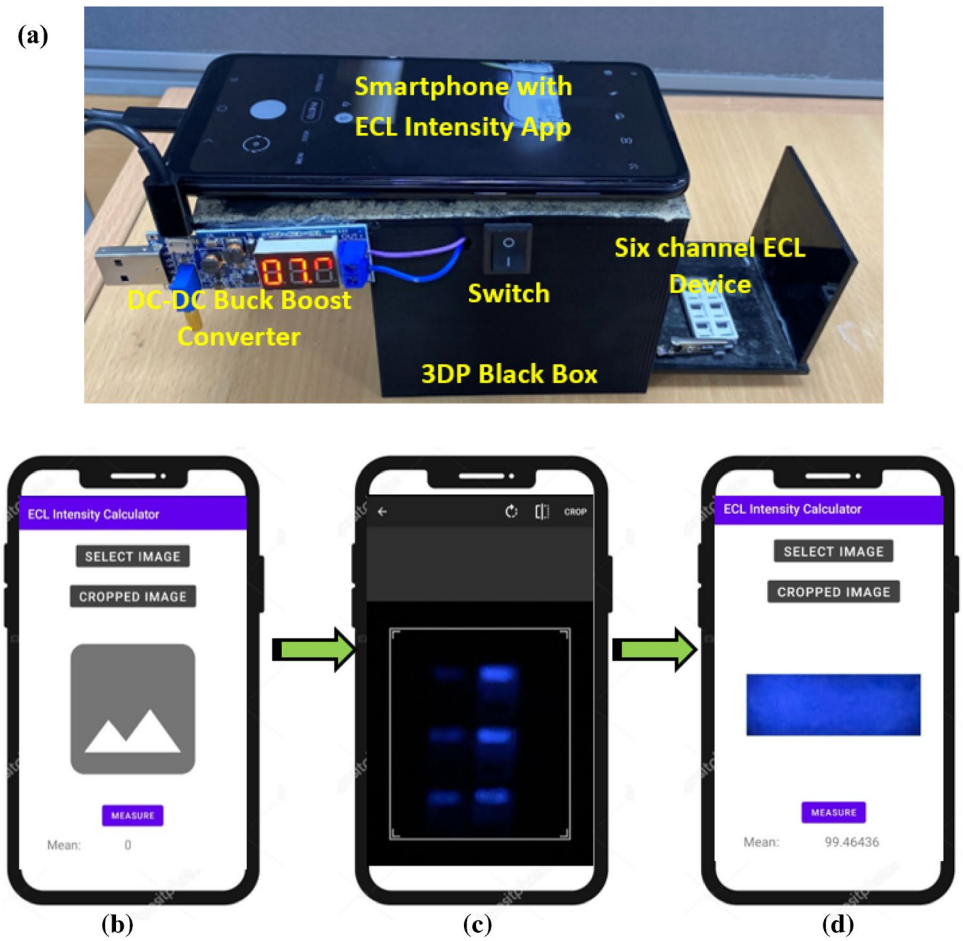

Fig. 2 Data acquisition and analysis, **a** 3D-printed black box assembly integrated with smartphone and converter, **b** display for android ECL intensity app, **c** captured real-time image, **d** selected region of interest and calculated ECL intensity

Results and discussion

Characterization

The morphological changes on the surface of graphene electrodes for the fabricated ECL device were investigated using SEM analysis before and after DMF treatment. Figure 3a and b show the SEM images of graphene filaments before and after DMF treatment [28]. With optimized parameters, the performance of six-well 3DP-CBPE-ECL device was validated by doing single-step detection of glucose and choline for six different concentrations.

Analytical performance of six-well 3DP-CBPE-ECL

Before validating the analytical performance of the ECL device, it was mandatory to optimize several parameters over which ECL signal intensity was highly dependent. Primarily, in luminol/H_2O_2-based chemistry, ECL signal strength

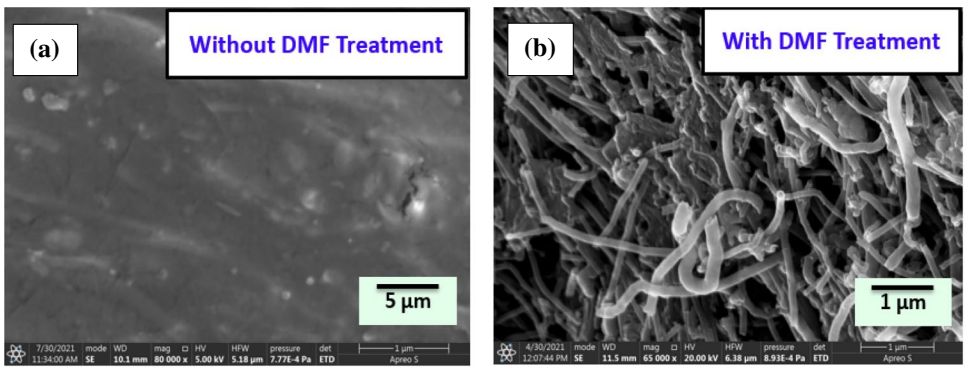

Fig. 3 SEM analysis of graphene filament, **a** SEM image of graphene filament before DMF treatment, **b** SEM image of graphene filament after DMF treatment

is dependent on parameters such as concentration of pH, luminol, and voltage. All of the optimized values in this study were obtained directly from a prior article published by our group. For validation of the six-well 3DP-CBPE-ECL, the optimal values for luminol (4 mM), pH (9), and voltage (7 V) were used. The six-well 3DP-CBPE-ECL device was validated by detecting glucose and choline in a single step. First, before the sensing of glucose and choline, the response of blank was calculated. For this, all the six wells were filled with 4-mM luminol concentration and ECL intensity was calculated. It was observed that when only luminol concentration was used, no ECL signal was obtained. Next, the glucose was detected by keeping six different concentrations in six distinct channels, as illustrated in Fig. 4a. To sense the glucose, glucose oxidase (10 mg/mL) was used and an enzymatic approach as described below was used. Experimentally, it was observed that minimum time of 3 min was required to react glucose with glucose oxidase to produce H_2O_2. Hence, luminol, glucose, and glucose oxidase were pipetted into the channel for an optimized time period of 3 min. The ECL signal was then achieved by applying an optimized value of external voltage (7 V) to the anode and cathode of the ECL device, and the corresponding ECL signal was successfully captured with android smartphone. ECL signal intensity for different six concentrations is shown in Fig. 4b. The single-step detection of glucose was accomplished for the linear range 0.1 to 10 mM with a limit of detection (LOD) of 24 µM.

Similarly, choline was detected by pipetting six different concentrations of choline with luminol and choline oxidase into the six distinct channels shown in Fig. 4c. Choline, luminol, and choline oxidase were pipetted into the channel

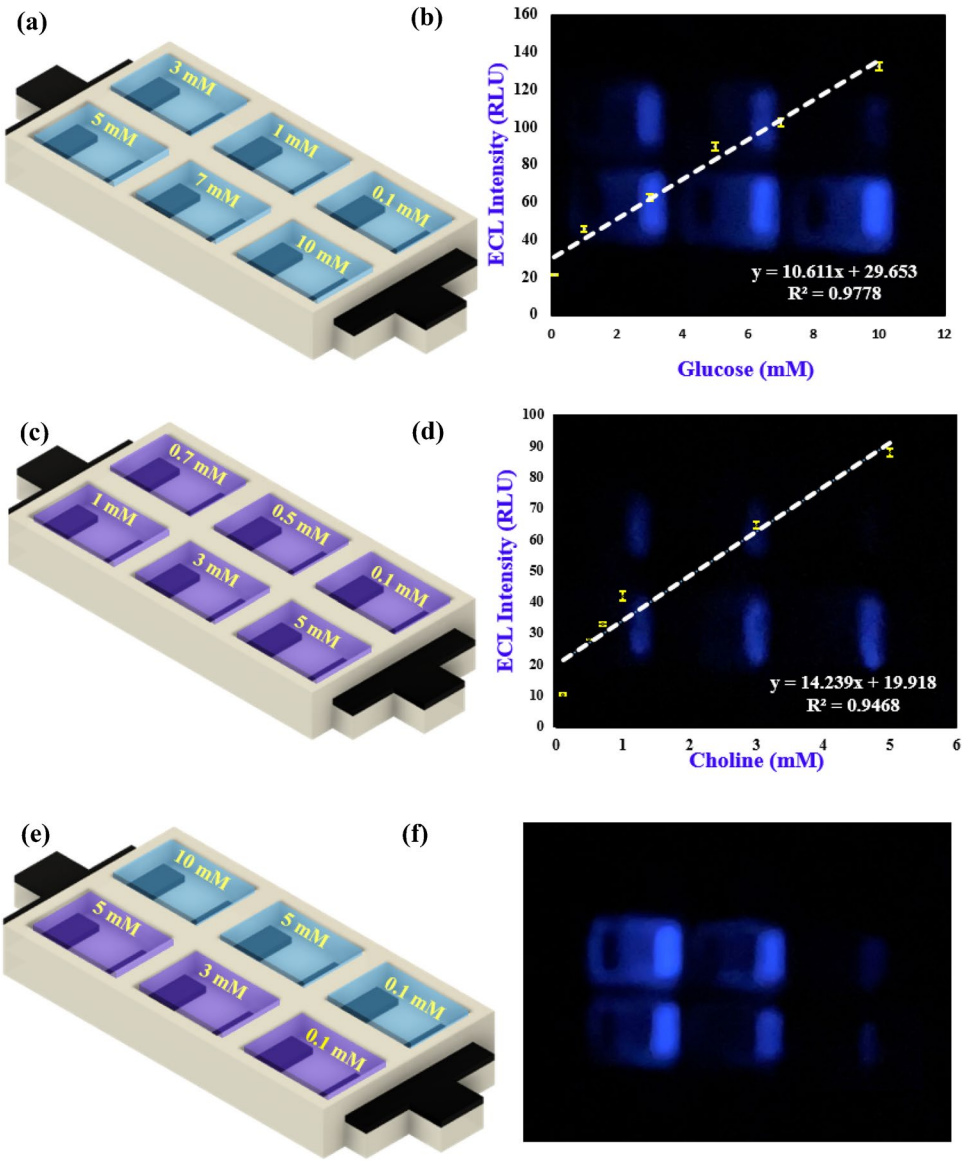

Fig. 4 **a** Schematic representation of glucose with different concentrations, **b** ECL intensity vs glucose, glucose = 0.1, 1, 3, 5, 7, and 10 mM, luminol = 4 mM, GOx = 10 mg/mL, applied voltage = 7 V, **c** schematic representation of choline with different concentrations, **d** ECL intensity vs choline, choline = 0.1, 0.5, 0.7, 1, 3, and 5 mM, luminol = 4 mM, COx = 10 mg/mL, applied voltage = 7 V, **e** simultaneous detection of glucose and choline using six channel CBPE-ECL device, **f** real-time ECL signal image corresponding to various concentrations of glucose and choline [$N = 3$]

Table 1 Comparative study for different ECL biosensing platforms to sense glucose and choline

Application	Electrode type	Device type	Detector	LOD (μM)	Real sample	Ref
Glucose	Bipolar electrode	Paper-based system	Smartphone	30	Urine	[13]
Choline	Three-electrode system	Large size	Photo multiplier tube	8.8	NA	[29]
Choline	Two-electrode system	PDMS chip	Charge-coupled device	10	NA	[30]
Choline	Three-electrode system	PDMS glass substrate-based hybrid chip	Charge-coupled device	97	NA	[31]
Choline	U-shaped bipolar electrode	Paper-based system	Charge-coupled device	3.13	Human blood serum	[32]
Choline	Bipolar electrode	PDMS glass substrate-based hybrid chip	Charge-coupled device	43.19	Human blood serum	[33]
Choline	Bipolar electrode	Large size	Photo multiplier tube	50	NA	[34]
Glucose	Closed bipolar	Paper-based system	NA	70	NA	[35]
Glucose Choline	Six channel closed bipolar electrode	3D-printed	Smartphone	Glucose = 24 Choline = 10	Human blood serum	This work

for 10 min before applying an external voltage to the ECL device. Choline detection was carried out by obtaining linear range 0.1 to 5 mM with LOD 10 μM. For each different concentrations of choline, ECL signal intensity was calculated and a related linear fit graph was plotted which is shown in Fig. 4d. To test the workability of the six-well CBPE-ECL device, simultaneous detection of varied concentrations of glucose and choline was performed, and it was found that the fabricated ECL device effectively sensed both analytes at the same time. The simultaneous detection of glucose and choline was accomplished by pipetting three different concentrations of each (glucose = 0.1, 5, and 10 mM and choline = 0.1, 3, and 3 mM) into separate channels and calculated ECL intensity as indicated in Fig. 4e and f. Table 1 shows the comparative study for different ECL biosensing platforms with presented 3D-printed ECL platforms to sense glucose and choline.

Real sample analysis of glucose and choline

Finally, a real sample analysis was performed to assess the practicability of the fabricated ECL device. The original glucose concentration was estimated by intersecting the extrapolation of the fitting line with the concentration axis [33]. The original values of glucose before the sample being spiked were found to be 3.9 mM. Standard spiking (addition) method was adopted for the real sample analysis of glucose and choline. Following method was adopted to do the real sample analysis of glucose.

An unknown blood sample was collected from the medical center (BITS Pilani Hyderabad Campus, Hyderabad, India) and diluted to ten times to avoid interference. In our case, ten times dilution was performed by adding 100 μL of real sample into 900 μL of DI water. Real sample analysis was performed using the standard spiking method for different concentrations of glucose and choline, and yielded a satisfactory recovery rate.

Following method was adopted to do the real sample analysis. The real sample was diluted to 10 times i.e. In present study, 100 μL of real sample was added to 900 μL DI water. Then, known concentration of glucose (1000 μM) and diluted real sample having equal volume (30 μL each) added into well along with glucose oxidase (10 mg/mL having 20 μL volume) and ECL intensity was calculated. Recovery rate was calculated using the following formula [36].

$$R = \frac{PracticalConcentration(found)}{TheoriticalConcentration(added)} * 100$$

In a similar way, real sample analysis was carried out for different concentrations of choline. In spite of rigorous literature survey, no proven clinical method to detect choline

Table 2 Summary of the outcome from the real sample analysis

Analytes	Clinical method (mM)	Using 3DP-CBPE-ECL device (mM)	Added (mM)	Obtained (mM)	% Recovery
Glucose	4.2	3.9	1000	4980	95.7
			2000	5988	96.6
			3000	7050	97.9
Choline	-	-	100	96.5	96.5
			500	477	95.5
			3000	2890	96.3

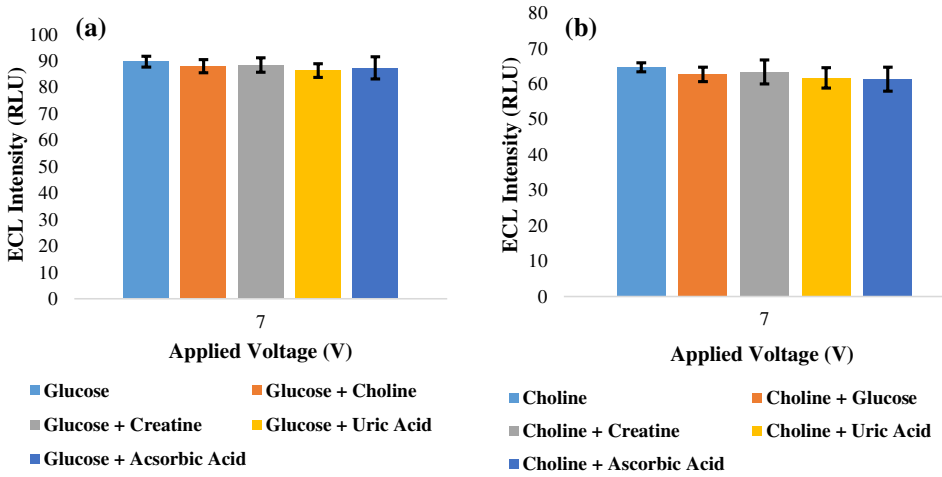

Fig. 5 Interference study: **a** interference study of glucose with choline, creatine, uric acid, ascorbic acid: glucose = 5 mM, choline = 1 mM, creatine = 0.5 mM, uric acid = 0.5 mM, ascorbic acid = 0.1 mM, luminol = 4 mM, GOx = 10 mg/mL, and applied voltage = 7 V. **b** Interference study of choline with glucose, creatine, uric acid, ascorbic acid: choline = 2 mM, glucose = 1 mM, creatine = 0.5 mM, uric acid = 0.5 mM, ascorbic acid = 0.1 mM, luminol = 4 mM, COx = 10 mg/mL, and applied voltage = 7 V, $n = 3$

was found. Hence, herein, no clinical data for choline could be provided. Multiple experiments were carried out with the 3DP-CBPE-ECL device to detect choline in real samples but we could not get convincing results. This could be due to the fact that choline concentrations in real serum are much below the detection limit. As a result, the conventional additive approach was used to detect choline, and the results were satisfactory. The recovery rate with standard deviation for various concentrations of glucose and choline is tabulated in Table 2.

Interference study of glucose and choline with other biomolecules

The interference study of glucose and choline with other biomolecules such as ascorbic acid, uric acid, and creatinine was carried out to prove the selectivity of the device. Following method was adopted for the interference study of glucose with other biomolecules. First, known concentrations of glucose (5 mM in 30 µL), luminol (4 mM in 30 µL), and glucose oxidase (10 mg/mL in 20 µL) were pipetted into well and ECL intensity was calculated. Then, interfering compound such as uric acid (0.5 mM in 30 µL) was added to same well and ECL intensity was calculated. Less than 5% change was observed in ECL intensity which indicated that the fabricated device provided good selectivity. Similarly, an interference study of glucose with other interfering compounds (ascorbic acid, choline, and creatinine) was carried out, and the selectivity of the device was confirmed. Figure 5a and b show the interference study of glucose and choline with other interfering compounds.

Conclusion

In this study, a portable and compact, 3D-printed six-well closed bipolar device was fabricated and validated by performing single-step detection of glucose and choline. After performing several optimizations, the single-step detection of glucose and choline was accomplished for the linear ranges of 0.1 to 10 mM and 0.1 to 5 mM, respectively, with a LODs of 24 µM and 10 µM. Following are the main key advantages of fabricated six-well 3DP-CBPE-ECL device: (1) single-step, low-cost fabrication (3D-printing) approach was used to fabricate ECL devices, (2) most popular luminol/H_2O_2-based enzymatic chemical reactions were used to detect glucose and choline, and (3) the smartphone was leveraged to its full potential by executing many activities such as capturing ECL images, powering the ECL device, and calculating the ECL intensity of the obtained ECL signal. Hence, based on the results and other key important functions, the fabricated six-well 3DP-CBPE-ECL device has shown potential to employ in a variety of biochemical applications.

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Declarations

Conflict of interest The authors declare no competing interests.

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