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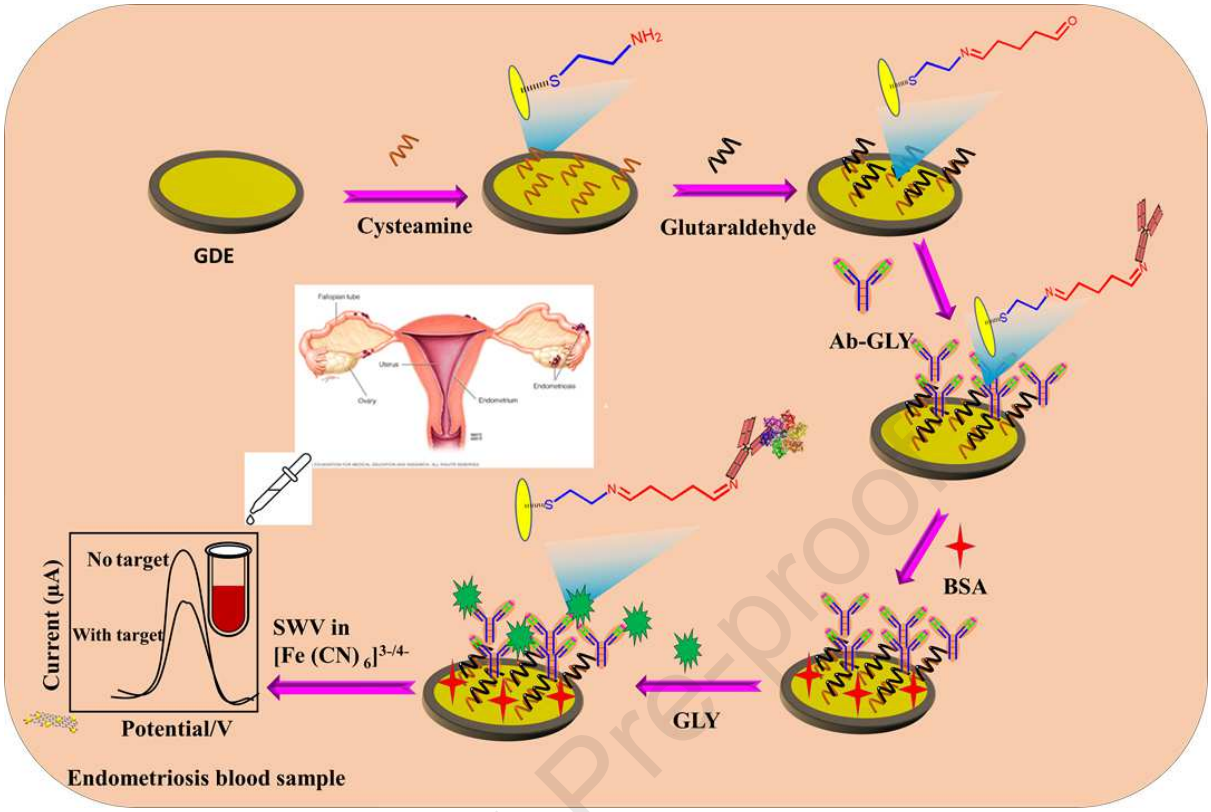
Thangapandi Kalyani: Conceptualization, Writing - original draft, Writing - review & editing, Visualization, Methodology, Validation. Amalesh Nanda: Writing - review & editing, Visualization, Methodology, Validation. Saikat Kumar Jana: Conceptualization, Validation, Supervision, Project administration, Funding acquisition, Writing - review & editing.

Graphical abstract



Schematic illustration of step by step fabrication of modified electrode and detection GLY

Detection of a novel glycodelin biomarker using electrochemical immunosensor for endometriosis

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Abstract

Endometriosis is one of the important issues in women worldwide, which decreases the quality of women's lives in their reproductive age. The diagnosis of endometriosis is carried out by the invasive procedure, which is expensive and painful. In the last few decades, researchers have given more attention to constructing a suitable biomarker-based biosensor for semi/non-invasive diagnosis of endometriosis. As a result, glycodelin (GLY) was found as a promising biomarker because of its selectivity and sensitivity. To the best of our knowledge, it was the first study that reported the detection of GLY biomarker using an electrochemical immunosensor. Briefly, a label-free electrochemical immunosensing platform was constructed through in-situ surface modification of cysteamine layer and immobilisation of antibody (anti-GLY) with help of glutaraldehyde. The interaction between antigen and antibody was measured using square wave voltammetry (SWV). The SWV signal could decrease proportionally with the increasing GLY concentration ranging from 1 to 1000 ng mL^{-1} (R^2 = 0.9981) and a detection limit (LOD) of 0.43 ng mL^{-1}. Moreover, an immunosensor could exhibit high sensitivity, selectivity, long-term stability, reproducibility and regeneration. Accuracy of the immunosensor was compared with enzyme-linked immunosorbent assay (ELISA), and satisfying results were obtained. The detection of GLY biomarker may be a new possibility for endometriosis diagnosis.

Keywords: Endometriosis; Glycodelin; Immunosensor; Cysteamine; Label-free

1. Introduction

Endometriosis is one of the common gynaecologic disorder where lesions of endometrial tissues grow on over the women's reproductive tract. The prevalence of endometriosis is associated with pain (pelvic, dysuria, dyspareunia, and dysmenorrhoea) and infertility which interfere with quality of life [1]. The population-based data illustrate that 10–15% of women are suffering from endometriosis, whereas 30–50% are infertile due to disease prognosis [2]. Early, non and semi–invasive tests for the diagnosis of endometriosis remains a challenge. Laparoscopy followed by biopsy is one of the gold standard surgical processes to diagnose [3–4]. Although non–invasive, other clinical examinations such as imaging techniques, transvaginal sonography, and magnetic resonance imaging are not very effective and efficient for apposite diagnosis [5]. Besides, cancer antigen 125 (CA 125) test is commonly used for endometriosis because of having the aberrant expression of CA 125 antigen in the serum (>35U.mL^{-1}) [6–7]. But, CA 125 is not considered as a suitable biomarker for endometriosis because of the lack of possibility to predict the early stages of the endometriosis [8]. Therefore, researchers have given more attention to identifying the suitable biomarkers for the early stage of an endometriosis diagnosis.

The glycodelin (GLY, MW: 47 kDa), a glycoprotein, a potential novel biomarker of endometriosis is reported as substantial by the researcher [9]. It is also known as progestogen-associated endometrial protein, which was used as prognostic and diagnostic biomarker. First, the GLY protein is secreted from the endometriosis tissues and then released into the bloodstream [10]. A recent study has found that the presence of GLY was significantly higher in endometriosis patients (>39 ng mL^{-1}) than in healthy women (5-31 ng mL^{-1}). The GLY concentration was reported to be 30% in stages I and II, whereas 40 – 50% in stage III and IV, which is higher than the normal level [11–12]**.** Hence, this study argues that this biomarker may be a suitable option for the early detection of endometriosis in stages I and/or II.

Various analytical techniques are used for the detection of protein level, including enzyme-linked immunosorbent assay [13–14], polymerase chain reaction [15], surface plasmon resonance [16–17], mass spectrometry [18–19]. Yet, these analytical instruments are quite expensive and required well–trained manpower. It is also very time consuming and difficult for assessing real-time detection. Compared to these analytical methods, electrochemical immunosensor has acquired considerable attention in recent years [20–21]. The reasons behind

this are as follows: cost-effectiveness, easy to operate, efficiency, low sample volume intakes and rapid detection of protein level in real samples [22–23]. In addition, choosing the electrochemical analytical method is an important role in the ultrasensitive of biomolecules/analyte [24–26]. Most of the electrochemical immunoassays reported that the voltammetric method rather is preferred than potentiometric and conductometric methods because of its sensitive and rapid analytical process [24–26]. The cyclic voltammetry (CV) [27–29], linear sweep voltammetry (LSV) [30,31], differential pulse voltammetry (DPV) [28,32], and square wave voltammetry (SWV) are frequently used in voltammetric techniques for detecting the biomolecules [29,33]. Hussain and Silvester [29] described that the DPV and SWV are more sensitive techniques compared to the linear CV and LSV techniques, because of their sampling method, which decreases the charging current [non-faradaic current as compared to faradaic current (in protic solvents)]. The increased ratio of faradaic to negligible capacitive current allows pulse methods to measure lower currents and higher sensitivity. The SWV is more advantageous including being sensitive and can be used in electrochemical experiments much faster than DPV, usually run at a scanning speed of 1→10 mV s^{-1}. SWV with an experiment scanning speed of up to 1 V s^{-1} or faster, allow much quicker determinations. Moreover, SWV is often used because of its ability to be worked at high frequencies and removal of oxygen from the target analyte solution may not be required due to the reduction in oxygen being included in the background current [29,33–35].

Consequently, numerous researches have focused on constructing electrochemical immunosensor for the rapid detection of target biomolecule [36]. Currently, label–free electrochemical immunosensor are more attracted by the researcher, because these methods are simple, low-cost, ultra-sensitivity, rapid analytical response while reducing the construction step compared to sandwich-type electrochemical immunosensors [37]. During analytical measurements, the interaction between antigen-antibody signal decreased the electron transfer due to the diffusion barrier increase as a result of insulating nature of biomolecules [38]. However, the most significant step in the construction of an immunosensor is the proper immobilisation of antibody on the sensing platform. This requirement is satisfied by the self-assembly method, thiol-linked self-assembled monolayers (SAMs) are commonly used modification technique to the immobilise antibody on the surface of the gold electrodes via formation of Au–S bonds for electrochemical biosensor development. Cecchetto et al., reported

an impedimetric immunosensor for the detection of dengue biomarker using 11-mercaptoundecanoic acid SAMs layer [39]. Grewal [40,41] reported an electrochemical immunosensor for the detection of an *Entamoeba histolytica* antigen using cell-free yeast-scFv probes. Arya et al., reported dithiobis(succinimidyl propionate) functionalised gold microelectrode array for the sensitive detection of cortisol using impedimetric immunosensor [36]. Cabral [38] reported hyaluronic acid–carbon nanotube hybrid film modified glassy carbon electrode for hepatitis B electrochemical immunosensor [38]. Carneiro et al., reported detection amyloid beta peptide using electrochemical immunosensor for Alzheimer's disease[37]. Figueiredo et al., reported cysteamine SAMs layer modified disposable gold electrode for the detection of NS1 protein [42].

In a recent year, many biomarkers (such as interleukin-8, interleukin-31 [43,44], CA 125 [45,46], CA 19-9 [45–47], apolipoprotein-1 [48], prealbumin [48], transferrin [48], yntaxin–5 [49], laminin–1 [49], etc.) have been reported by the several research groups for the detection of endometriosis, yet the performance of these biomarkers has not been adequate. More attention should be given to develop a single blood test that can detect endometriosis, which can help millions of women worldwide. In contrast, a novel GLY biomarker measurement is essential to clearly differentiate endometriosis patient (stage I and/or II) from healthy person. Moreover, the main advantage of immunosensor was simple, easy to construct and need no for expensive secondary antibody; enzyme or functionalized nanomaterials for signal amplification thus reduced the cost of manufacturing. After an extensive literature review, it was found that there is no research article published on detecting the GLY using electrochemical immunosensor. The above reasons, motivated us to construct an immunosensor for the detection of GLY in the diagnosis of endometriosis.

Scheme 1.

2. Experimental section

2.1 Pre-treatment of electrode

The gold disc electrode (GDE, 1.6 mm diameter) was immersed in a piranha solution for approximately 5 minutes (30% H_2O_2 and concentrate H_2SO_4, 1:3 v/v). After washing, the GDE

was polished using alumina powder (0.3 and 0.5 µm). After that, the GDE was rinsed with water and pure ethanol. Next, the GDE was electrochemically cleaned by CV in H_2SO_4 solution (0.1 M). The applied potential window was –0.2 to 1.6 V at a scan rate of 0.1 V/s (10 cycles). Finally, the GDE was cleaned by KOH solution using CV, cycled 15 times from –0.2 to 1.2 V at a scan rate of 0.1 V/s. Finally, the electrode was washed with water and ethanol.

2.2. Preparation of immunosensor

The construction of label-free electrochemical immunosensor each surface modification step is illustrated in Scheme 1. After pre-treatment, the GDE was immersed in 10mM Cys in an ethanolic solution for 16 hours at 25°C. Afterwards, the GDE was rinsed with absolute ethanol and water to eliminate unbound Cys molecules. Cys/GDE was dipped in a 2.5 % Glu for 45 minutes. Glu/Cys/GDE was immediately incubated with 10 µg mL^{-1} of anti-GLY solution for 1 h at 37°C. Next, ab-GLY/Glu/Cys/GDE was washed using phosphate-buffered saline (PBS), followed by 1 % BSA solution for 30 minutes at 25°C. The excess of BSA was removed using PBS. Finally, the immunosensor was used to quantify GLY using the following method. Finally, BSA/ab-GLY/Glu/Cys/GDE was incubated with 1 ng mL^{-1} of GLY antigen for 40 minutes at 25°C, and diluted to obtain the desired concentration from 2000 ng mL^{-1} GLY stock solution.

2.3. Blood sample collection

This women's health-related research project was conducted at the National Institute of Technology, Arunachal Pradesh, India which was approved by the Department of Science and Technology (DST), Science and Engineering Research Board (SERB) team. The institutional scientific, ethical committee was given approval [Proposal number: NIT (A.P)/R&D/Project/11-12/1/Vol.2/IEC-02, Approval date 27/09/2016] for this study and samples were collected from Pratiksha Hospital, Guwahati, Assam, India. Written consent from the patients selected for this study, had been taken at the time of blood sample collection. Women included in the study did not receive any medical or hormonal treatment during the last 3 months. Moreover, women with prior history of any gynaecological surgery, including chocolate cyst, lower pelvic and abdominal surgery with other possible causes of pain or pelvic pathology, including pelvic

tuberculosis, were excluded from the study. Venous blood samples collation, process and storage procedure were described previously [50].

3. Results and discussion

3.1. Electrochemical characterisation of the immunosensor

Herein, cyclic voltammetry (CV) and electrical impedance spectroscopy (EIS) displayed each step of the fabrication procedure of the proposed immunosensor. Figure 1 shows the PBS (10mM, pH 7.4) contain 10mM $[Fe(CN)_6]^{3-/4-}$ and 1 M KNO_3 were used as standard redox marker, potential range between –0.2V to 0.6V at a scan rate of 0.02V/s. After pre-treatment, the GDE was tested using CV. The obtained peak intensity was 0.043 mA, with an anodic peak potential (E_{pa}) of + 0.253 V, and a cathodic peak potential (E_{pa}) was 0.093 V, with a peak density of 0.027 mA. After Cys modification, Cys/GDE was compared with GDE. The peak current was decreased (rose colour) compared with GDE (black colour). These results consisted of coverage of the electrode surface by insulating Cys layer. Bi-functional linker Glu was introduced with Cys layers. Figure 1a demonstrates that the peak current (red colour) was deceased compare with Cys/GDE (rose colour). This observation ensures that the Glu is successfully bonded with the Cys layer. The Glu/Cys/GDE reacted with anti-GLY. Glu reacts with lysine residues on the antibody to form Schiff base linkages; as a results again peak current (green colour) was decreased due to antibody bind with Glu. The Glu/Cys/GDE was incubated in 1% of BSA for blocking to remain active site, as results peak current (pink colour) was decreased. Finally, BSA/anti-GLY/Glu/Cys/GDE was incubated with GLY antigen, and the peak current (blue colour) was continuously decreased, because of the formation of antigen-antibody immunocomplex.

Figure 1.

EIS can also give more information on the impedance in the immobilisation step and estimated the structure of equivalent circuit [51]. The impedance spectra consist of two portions: a semicircle portion controlled by the electron transfer resistance and a liner portion controlled by the diffusion-limited process [31-32]. The equivalent circuit of the proposed immunosensor

(Figure 1b) connects electron transfer resistance (Rct), double layer capacitance (Cdl), solution resistance (Rs) and Warburg impedance (Zw). All EIS data were obtained by Nyquist plots and fitted using Randle's equivalent circuit (obtained from EC Lab softer). The GDE (black colour) showed a small semicircle domain (R_{ct} = 10 Ω), suggesting that the GDE facilitated electron transfer rate of redox marker at the electrode surface. Afterwards, the GDE was absorbed by cysteamine molecule (rose colour), the semi-circular diameter (R_{ct} = 44.94 Ω) of the Cys modified GDE (rose colour) was slightly bigger than GDE (black colour). This result demonstrates the concerned blocking effect of Cys layer towards charge transfer. After Cys react with Glu, the semi-circular diameter (R_{ct} = 244 Ω) of the Glu modified GDE (red colour) was slightly bigger than Cys (rose colour). Finally, anti-GLY antibody (green colour, R_{ct} = 479.65Ω), BSA (pink colour, R_{ct} = 561 Ω), and GLY antigen (blue colour, R_{CT} = 709.9 Ω) were immobilised on the Cys/GDE. Impedance spectra clearly showed an increase in R_{ct}, because of the blocking of electron transfer by bioactive molecules on the Cys-modified electrode, which was consistent with CV results.

3.2. Optimisation step of immunosensor

The proposed immunosensor is optimised by different parameters such as glutaraldehyde incubation time, antibody incubation time, antibody concentration, incubation temperature and antigen incubation time in 10 mM $[Fe(CN)_6]^{3-/4-}$ and 1 M KNO_3 solution at a potential range between -0.2 to 0.6 V in SWV technique. First, the optimisation of the Glu incubation time for the Cys/GDE was discussed. Figure S1a shows that the peak current was decreased with an increased Glu incubation time. After 60 minutes peak current was almost the same. This result indicates that maximum Glu react with the electrode surface. Therefore, 60 minutes was selected for Glu incubation time for the immunosensor.

The effect of antibody incubation time on the response of the proposed immunosensor was investigated from 1 minute to 60 minutes using 2µg mL^{-1} of anti-GLY solution (Figure 2a). When antibody incubation time was increased from one minute to 40 minutes, the peak current of SWV decreased (Figure 2b). Afterwards, the peak current of SWV was an approximately constant value. As a result, 40 minutes was selected as an optimum antibody incubation time for the immunosensor construction.

The influence of antibody concentration on the response of the proposed immunosensor was investigated. Different concentrations of anti-GLY were tested in figure. 2b. The peak current of SWV (Figure 2b) was decreased when anti-GLY concentration was increased from 2 to 12 µg mL^{-1} at 25^{o} C. This is because of anti-GLY immobilisation on the electrode surface, which then almost kept constant with the further increased concentration of anti-GLY antibody figure 2b. The resulted suggesting electrode was saturated. Therefore, 10 µg mL^{-1} was selected as the optimal anti-GLY concentration for the immunosensor construction.

After anti-GLY immobilisation, the electrode was incubated with 2% BSA solution (0.05 M PBS, pH= 7.4) for 5, 10, 15,20, 30 and 40 minutes. The peak current of SWV was decreased (Figure 2c) up to 30 minutes. After that, the peak current of SWV remains unchanged, indicating maximum BSA immobilised on the electrode surface for 30 minutes.

The effect of temperature on the immunosensor performance was examined at the incubation temperature range from 5°C to 40°C (Figure S1b). The result displayed that the 37 ºC is a suitable temperature for antibody incubation temperature (Figure S1b). In high incubation temperature, peak current was increased. This result suggests that there may be denaturation of protein [54].

The effect of antigen incubation time on the response of the proposed immunosensor was investigated. The final modified electrode (BSA/anti-GLY/Glu/Cys/GDE) was incubated 10ng mL^{-1} of GLY solution for 5, 10, 20, 30, 40 and 50 minutes at 25^{o}C. Figure. 2d shows that the peak current of SWV decreased with incubation time up to 30 minutes. This result clarifies that the GLY antigen molecules fully react with immobilised anti-GLY. After increasing incubation time, the peak current SWV is unchanged. Therefore, 30 minutes was selected as the optimum superior incubation time of antigen and antibody.

The overall optimal experimental condition of this assay was as concluded as follows: 60-minute incubation time of Glu, 40 minute incubating time of anti-GLY, 10 µg mL^{-1} concentration of anti-GLY, 30 minute incubation time of BSA, 37 °C incubation temperature of anti-GLY and 30 minute incubation time of GLY.

Figure2.

3.3. Electroanalytical performance of the immunosensor

The analytical performance of the proposed immunosensor (BSA/anti-GLY/Glu/Cys /GDE) was evaluated at various concentration of GLY under the identical experimental condition. Figure -3a shows that the response of the proposed immunosensor decreased with increased GLY antigen, which visibly shows a “signal off” trend. GLY antigen was captured by the corresponding anti-GLY antibody to block the electron transport in the electrode surface. There is a linear plot between SWV peak current and logarithm of GLY concentration in a range from 1 to 1000 ng mL^{-1} (Figure 3b). The equation is Log (I) = -4.003[log C_{GLY}] + 16.265 (R^2 = 0.965) with a detection limit of (LOD) of 0.048 ng mL^{-1} (3Sb/m, where Sb and m refer to standard deviation of the blank value and slop value) at a signal-to-noise ratio of 3. To the best of our knowledge, no other electrochemical sensor for detection of the GLY biomarker has been reported. Moreover, the main advantage of immunosensor has evaded the need for expensive secondary antibody; enzyme and/or functionalised nanomaterials for signal amplification thus reduced the cost of manufacturing.

Figure 3.

3.4. Selectivity, stability, reproducibility and regeneration of the immunosensor

The important challenges in the development of immunosensor are attaining good selectivity, stability, reproducibility and regeneration in the analysis of real samples [55–60]. The presence of non-specific substances that can impact the signal attained for an analyte frequently turns out to be an issue because of the interference effects. So, it was essential to confirm the substrate selectivity of the proposed immunoelectrode [61]. The selectivity of the immunoelectrode was investigated by monitoring the SWV response of BSA/ab-GLY/Ghy/Cys/GDE, and GLY/BSA/ab-GLY/Ghy/Cys/GDE (10 ng) in the presence of the

interference's substances such as CA 125 (100 U mL^{-1}), CA 19-9 (100 ng mL^{-1}) and IL-10 (100 ng mL^{-1}) that were mixed with PBS, then, the SWV response was measured (Figures. 4-a and S2-a). There was no significant variation in the SWV response after the interaction of BSA/ab-GLY/Ghy/Cys/GDE, and GLY/BSA/ab-GLY/Ghy/Cys/GDE immunoelectrodes with interfering compounds. The relative standard deviation (RSD) was ± 0.243 and ± 0.219, respectively. To additionally demonstrate the selectivity performance of the process for clinical samples analysis, complex samples of GLY in serum were analysed. Final, GLY/BSA/ab-GLY/Ghy/Cys/GDE was incubated in human serum sample (undiluted) and verified by the same concentration of interference's substances. As shown in Figure 4a, the peak current was almost similar to control. There was a small variation in SWV signal response due to interfering biomarker. The RSD (± 0.365) of SWV response of sample with interfering substance was less than 5%. This result indicates that the proposed immunosensor is only selective to GLY detection.

The storage stability of the immunoelectrode (BSA/ab-GLY/Ghy/Cys/GDE) was also investigated. First, two different immunoelectrodes (GLY/BSA/ab-GLY/Ghy/Cys/GDE) were prepared in the same days under experimental conditions, which were used for measuring 10 ng of GLY and undiluted serum sample. After measurement, the immunoelectrode was stored at $4^{o}C$ in PBS solution (pH- 7.4) when not in use [55–60]. The proposed immunosensor was measured every week. After 1 month, the proposed immunosensor retained about 91.89% (for 10 ng of GLY) and 92.56 % (serum sample) of initial response value (Figure4b), indicating that the proposed immunosensor has acceptable stability. The good long-term storage stability of immunoelectrode could be ascribed to: (1) the covalent attachment of Cys layers to gold electrode surface, and (2) Fc region of anti-GLY was covalently immobilised onto the Cys layers.

The reproducibility was to certify the precision of the immunosensor [55–60]. The reproducibility was evaluated using four different BSA/ab-GLY/Ghy/Cys/GDE immunoelectrodes was estimated by three days under the optimum experimental conditions for the detection of 10 ng GLY and followed by serum GLY (Figure 4c). The RSD of the response current signals for immunosensor were found around 1.62%, 1.81%, and 1.86% for GLY antigen (day 1-3), and 1.75, 1.86 and 1.95 for serum GLY (day 1-3), respectively. Which is revealing good accuracy and reproducibility of the immunosensor.

Figure 4.

The regeneration of immunosensor was examined to demonstrate the further application of the immunosensor. The immunosensor can be regenerated by dissociation of antigen–antibody linkage using under drastic conditions such as acid or alkali solution from immunoelectrode [62–64]. The regeneration of a proposed immunosensors was achieved by immersing into a regeneration buffer containing of glycine-HCl (0.1 M, pH -2) for 2 minutes (Optimisation of regeneration process steps were given in supporting information Figure S3) [62,63], and rinsing with PBS (pH 7.0) to break the antigen–antibody bond. The glycine could well disconnect the antigen–antibody linkage, flaking the taken antigens off the sensing layers. The serial measurements were repeated four times, an average recovery of 91.8% and an RSD of 8.2% were acquired (Figures. 4d, and S2-b). The results indicate that the proposed immunosensor could be regenerated and used for minimum four times.

3.6. Real sample analysis

The proposed immunosensor was a challenge by detecting of GLY concentration from endometriosis patients. 0, 10, 20, 50 and 100 ng of recombinant GLY antigen was spiked into serum sample (serum sample was 2X diluted by PBS buffer (pH 7.4) and measured SWV for method validation (Table. 1).

Table 1.

After BSA/anti–GLY/Glu/Cys/GDE, GLY antigen was measured directly from control and endometriosis serum (serum samples were 2X diluted by PBS buffer (pH 7.4) solution). Three different endometriosis serum samples of each stage were measured by SWV electrochemical technique. Accuracy of the GLY detection was compared with ELISA (standard curve and data with the 4-stages concentration of serum samples were given in supporting information (Table. S1)). The GLY concentration obtained though the electrochemical method were slightly lower than the ELISA assay. This result representing possible electrochemical interference from serum sample. After calculating the average GLY concentration of each stage of endometriosis serum

sample, including control was shown in Table 2. Our proposed immunoassay results displayed parallel with ELISA data. This result was suggesting that proposed immunoassay has promising potential applications for diagnosis of endometriosis.

Table 2.

4. Conclusion

In this work, we had constructed a label-free electrochemical immunosensor based on cysteamine SAM via GLY antibody immobilisation for the ultrasensitive detection of novel GLY antigen. Based on this sensing platform, the proposed immunosensor exhibit an extremely high LOD (0.043 ng mL^{-1}) and wide range of linear response (1 - 1000 ng mL^{-1}), with favourable selectivity, reproducibility, repeatability and stability. The main advantages of the developed label-free immunosensor were straightforward measurement of GLY levels in the clinical sample, low-cost construction, and high-level sensitivity. Accuracy of the proposed immunosensor was successfully compared with ELISA assay. Summarily, the proposed method had the potential to be further developed for practical clinic for the diagnosis of endometriosis stage I and/or II. Moreover, our study aimed to make a panel of GLY and CA 125 for the detection of endometriosis stages I–IV in future.

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Figure of caption

Scheme 1. Schematic illustration of step by step fabrication of modified electrode and detection GLY

Figure 1. (a) CV and (b) EIS of different modified electrodes in 10 mM $[Fe(CN)_6]^{3-/4-}$/1 M KNO_3 solution at a scan rate of 20 mV s^{-1}.

Figure 2. Optimisation of antibody incubation time (a), antibody concentration (b), BSA incubation time (c), and antigen incubation time (d) in 10mM PBS contain 10 mM $[Fe(CN)_6]^{3-/4-}$ and 1 M KNO_3 solution. All measurements were repeated three times (n = 3).

Figure 3. (a) SWV response of different concentrations of GLY (1 – 1000 ng mL^{-1}).
(b) Linear plot between logarithmic concentration versus current of GLY. All measurements were repeated three times (n = 3).

Figure 4. (a) Selectivity: SWV response of the (i) BSA/ab-GLY/Ghy/Cys/GDE, (ii) GLY/BSA/ab-GLY/Ghy/Cys/GDE (10 ng GLY), (iii) serum + GLY/BSA/ab-GLY/Ghy/Cys/GDE (10 ng GLY) immunoelectrode with 100 U mL^{-1} CA 125, 100 ng mL^{-1} CA 19-9 and 100 ng mL^{-1} IL-10. (b) storage stability of (i) GLY/BSA/ab–GLY/Ghy/Cys/GDE (10 ng GLY), (ii) serum + GLY/BSA/ab-GLY/Ghy/Cys/GDE (10 ng GLY). (c) SWV response of corresponding reproducibility of 10 ng GLY and serum, (d) Regeneration of immunoelectrode (10 ng GLY). Error bar = RSD (n = 3).

Table of caption

Table 1. The electrochemical detection of GLY in human serum bloods

Table 2. The electrochemical detection of GLY in real blood samples (clinical bloods)

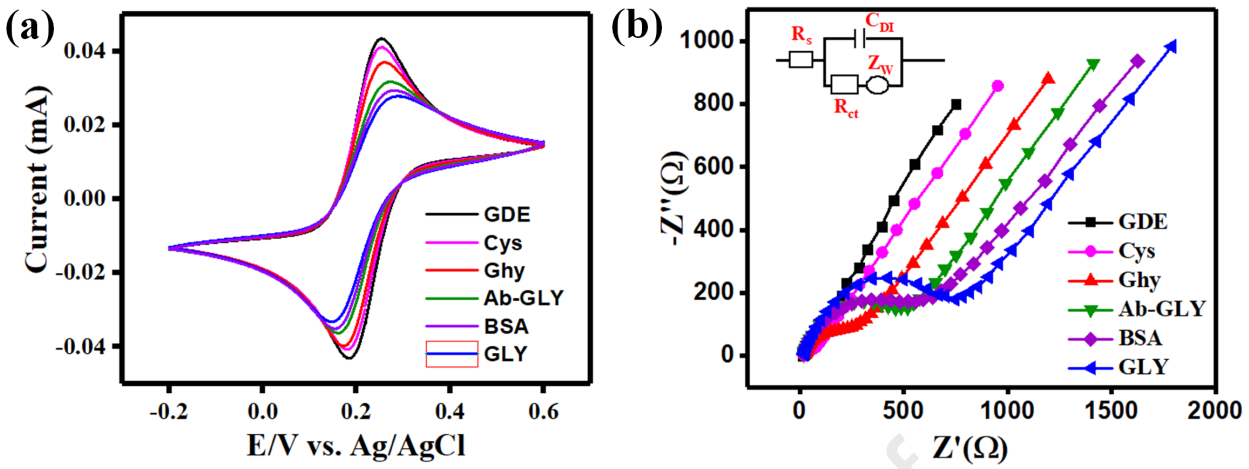
Table 1. The electrochemical detection of GLY in human serum bloods

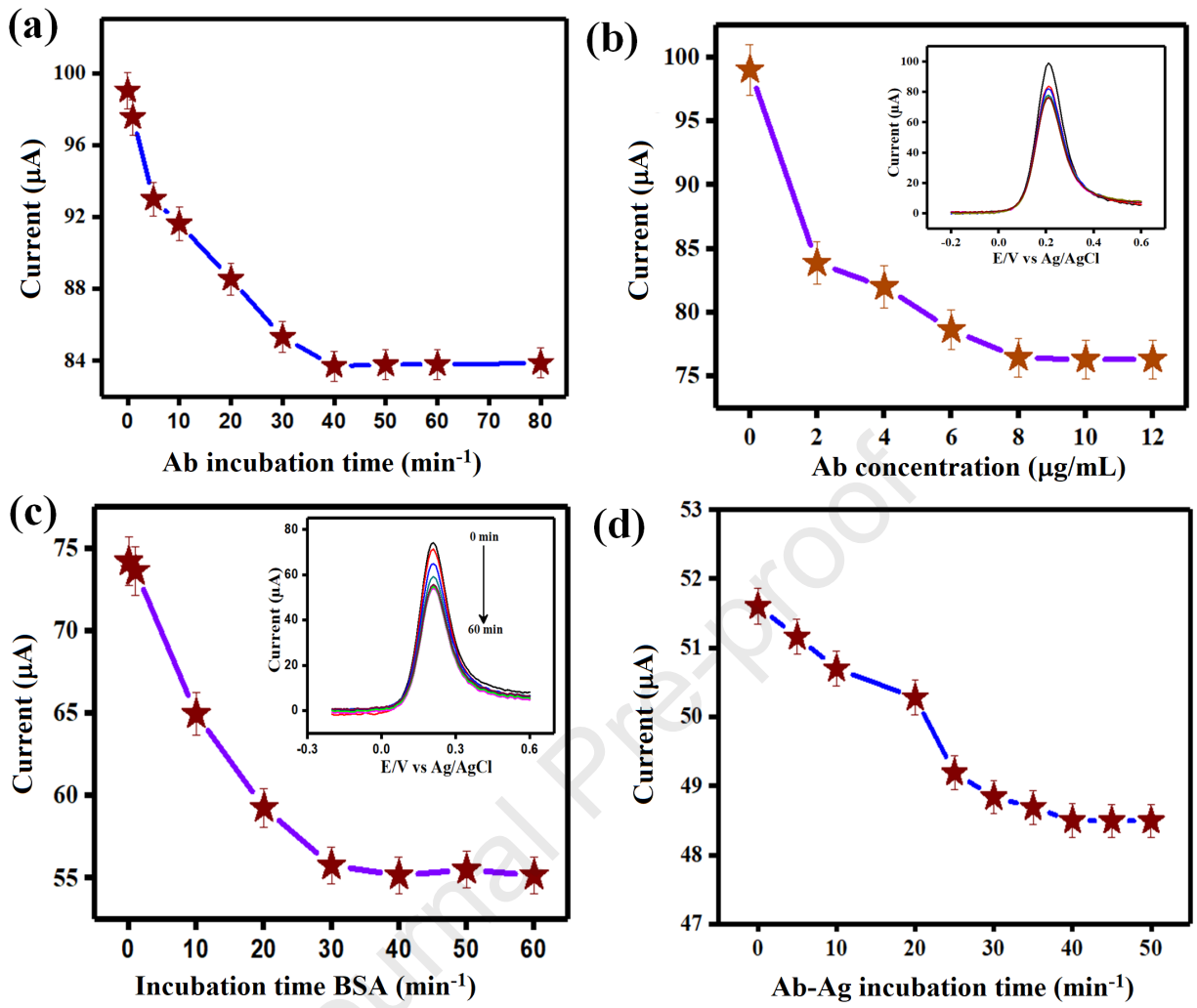
source	Proposed sensor			ELISA	
	Added ($ng\ mL^{-1}$)	Found ($ng\ mL^{-1}$)	SD (n = 3)	Found ($ng\ mL^{-1}$)	SD (n = 3)
	0	13	0.50	12	0.323
Healthy patient	10	22.5	0.65	22	0.42
serum sample	20	31	0.396	29.5	0.46
	50	57.5	1.03	58.3	0.90
	100	108	1.140	110	1.30

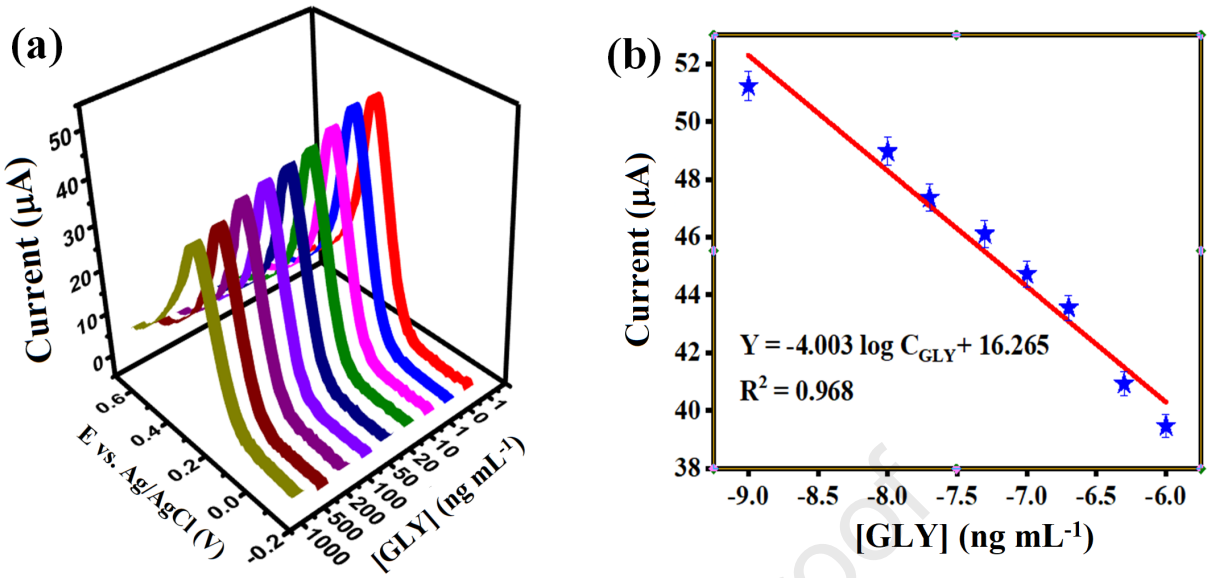
* SD, standard deviation.

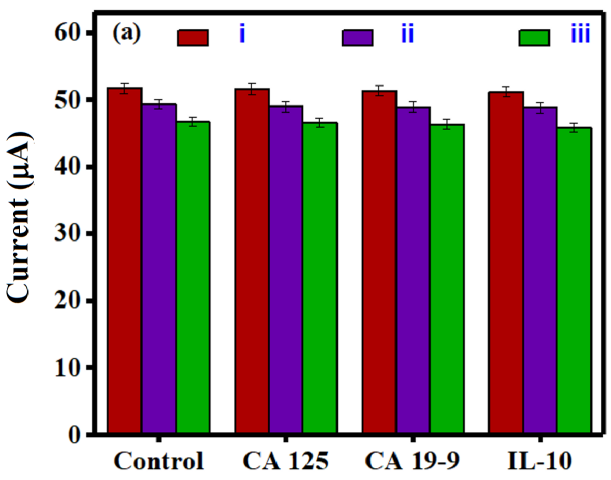
Table 2. The electrochemical detection of GLY in real blood samples (clinical bloods)

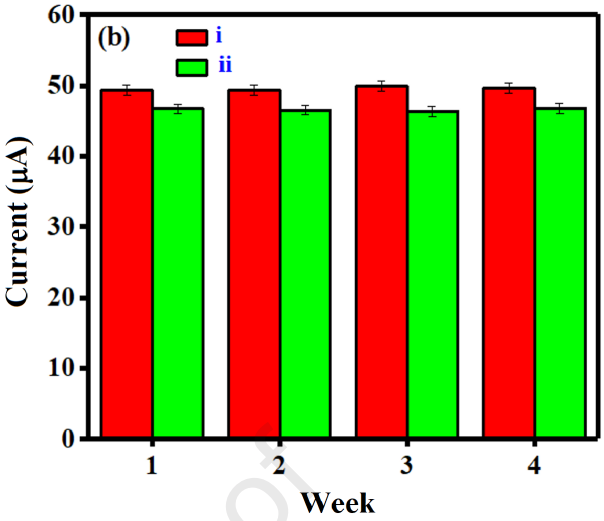
Source	Proposed sensor		ELISA	
	Found ($ng\ mL^{-1}$)	SD (n = 3)	Found ($ng\ mL^{-1}$)	SD (n = 3)
Healthy patient	11.45	0.425	10.6	0.064
Endometriosis patient (Stage 1)	17.23	0.624	16.22	0.598
Endometriosis patient (Stage 1I)	21.80	0.285	20.75	0.991
Endometriosis patient (Stage 1II)	42.39	0.665	40.341	0.706
Endometriosis patient (Stage 1V)	57.274	0.649	58.76	0.578

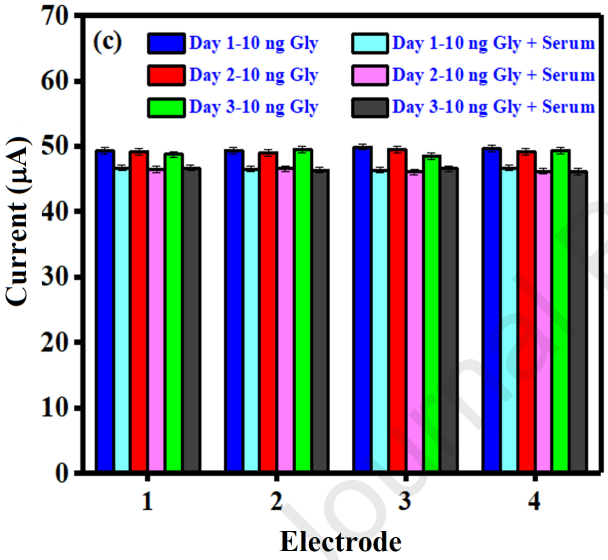


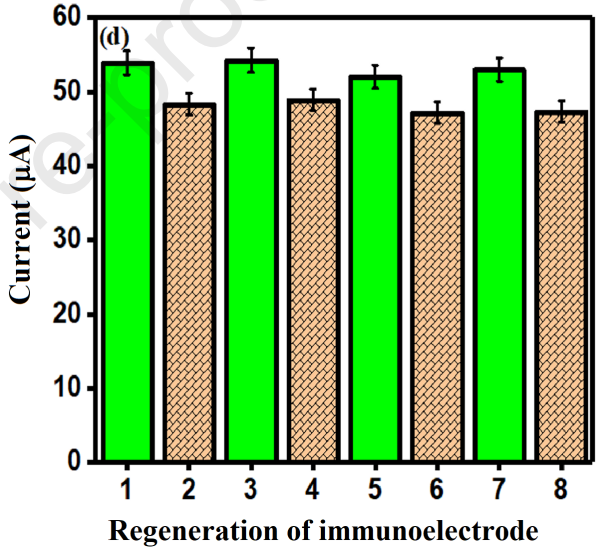


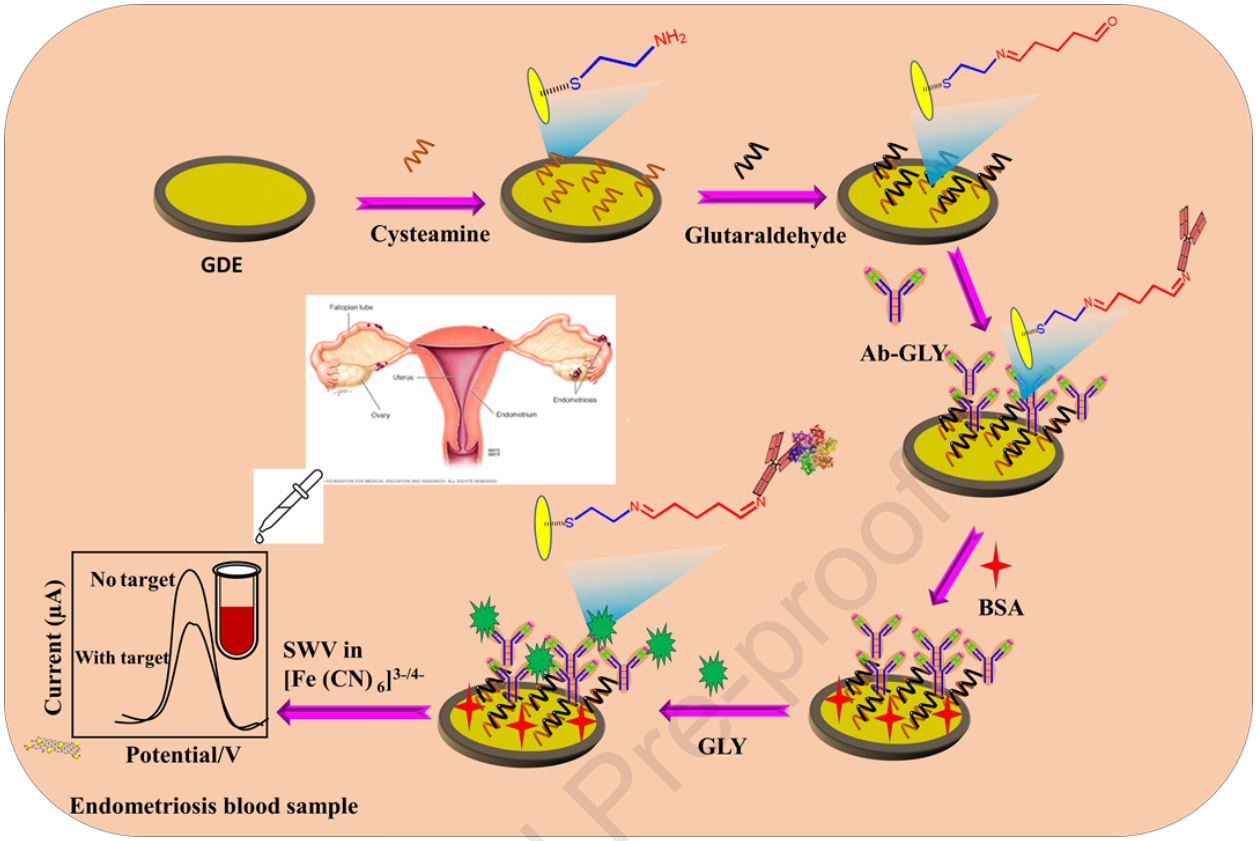












Highlights

- An electrochemical immunosensor for the detection of glycodelin biomarker in endometriosis is first time reported.
- The LOD was 0.43 ng mL^{-1} and the liner domain 1 - 1000 ng mL^{-1}.
- In addition, the sensor exhibited acceptable selectivity, good storage stability, and high reproducibility.
- The applicability of the fabricated sensor to actual human serum samples.
- The proposed immunosensor is very promising for clinical applications for endometriosis

Declaration of interests

The authors declare that they have no known competing for financial interests or personal relationships that could have appeared to influence the work reported in this paper. The authors also submitted the same study in the Indian patent office for consideration.