A Highly Sensitive Graphene-based Field Effect Transistor for the Detection of Myoglobin

B. Vamsi Krishna 1 · A. Gangadhar 2 · S. Ravi 3 · D. Mohan 4 · Asisa Kumar Panigrahy 5 · V. Raja Rajeswari 6 · M. Durga Prakash 7

Received: 6 February 2022 / Accepted: 24 February 2022 / Published online: 27 April 2022
© The Author(s), under exclusive licence to Springer Nature B.V. 2022

Abstract
Biomedical applications adapt Nano technology-based transistors as a key component in the biosensors for diagnosing life threatening diseases like Covid-19, Acute myocardial infarction (AMI), etc. The proposed work introduces a new biosensor, based on Graphene Field Effect Transistor (GFET), which is used in the diagnosis of Myoglobin (Mb) in human blood. Graphene-based biosensors are faster, more precise, stronger, and more trustworthy. A GFET is created in this study for the detection of myoglobin biomarker at various low concentrations. Because graphene is sensitive to a variety of biomarker materials, it can be employed as a gate material. When constructed Graphene FET is applied to myoglobin antigens, it has a significant response. The detection level for myoglobin is roughly 30 fg/ml, which is quite high. The electrical behavior of the GFET-based biosensor in detecting myoglobin marker is ideal for Lab-on-Chip platforms and Cardiac Point-of-Care Diagnosis.

Keywords
Graphene · FET · Myoglobin · Limit of detection · Biosensor · GFET · Acute myocardial infarction

1 Introduction
Graphene is a single-atom thick element with honeycomb structure. Varieties of Biosensors are being implemented using Graphene FETs and are suitable for low power and high frequency applications [1]. In addition to electronic properties such as smaller bandgap, high electron mobility, high transconductance, etc., GFETs exhibit superior optical and mechanical properties [2]. Across the globe, increase in the number of patients with cardiovascular disease (CVD) has become a major threat. In particular, Acute myocardial infarction (AMI), a severe health issue caused because of impaired blood flow in human heart. It is affected by larger people leading to higher death rates. Diagnosis of cardiovascular diseases is carried out using biomarkers such as Myoglobin (Mb), Cardiac troponin T (cTnT), Co-peptin, Creatine kinase Myocardial Band (CK-MB), Cardiac troponin I (cTnI), setc [3]. However, early detection of CVD mandates the use of Mb detection than cTnI and CK-MB due its fastest response, with a delay of 90 min [4].

Myoglobin, an Oxygen-bound protein found in heart, increases its level when there is heart or muscle damage. Depending on the increase in the level of myoglobin the condition of the patient may lead to death in 2 to 3 h. In the proposed work, a novel GFET structure has been designed and its electrical characteristics are verified with the help of semiconductor module of COMSOL

1 Department of ECE, Koneru Lakshmaiah Education Foundation, Guntur 522502, Andhra Pradesh, India
2 Department of ECE, University College of Engineering Narasaropet, Narasaropet 522601, India
3 Department of ECE, Gudlavalleru Engineering College, 521356 Gudlavalleru, Andhra Pradesh, India
4 Department of ECE, Sreenidhi Institute of Science and Technology, 501301 Hyderabad, Telangana, India
5 Department of ECE, Gokaraju Rangaraju Institute of Engineering & Technology, 500090 Hyderabad, Telangana, India
6 Department of ECE, VR Siddhartha Engineering College, 520007 Vijayawada, Andhra Pradesh, India
7 Department of ECE, SRM University-AP, 522240 Mangalagiri, Andhra Pradesh, India
Multiphysics software simulation tool. The 2D and 3D structure of the proposed GFET are shown in Fig. 1(a) and (b), respectively. The GFET structure consists of two different gate terminals named as top gate and bottom gate.

The device is designed and simulated in the following procedure. Firstly, consider a silicon substrate of 2.5 μm length and 300 nm thickness. A silicon dioxide layer of 6 nm is grown on the surface of silicon substrate which acts as dielectric for the bottom gate. A layer of 20 nm Hafnium dioxide (HfO₂) is imposed on the Silicon dioxide layer which acts as dielectric material for the top gate. Afterwards, a graphene layer of thickness 0.65 nm is positioned on the HfO₂ layer, as graphene has zero band gap. Myoglobin (Mb) is a kind of biomarker, and it is used in the detection of cardiovascular diseases. The target Mb can be captured with the help of sensitive material which has immobilized with anti-Mb antibody. Myoglobin is an oxygen-binding hemeprotein which supplies oxygen to cardiac muscles. When there are problems with heart muscles, myoglobin is swiftly discharged from necrotic myocardium before the arrival of cytoplasmic enzymes such as creatine kinase or its isoenzyme creatine kinase-MB. In the routine, myoglobin concentrations are determined by radioimmunoassay or immune-nephelometry [5, 6].

This paper is organized as follows: Literature survey on previous research works towards myoglobin detection is reviewed in Section 2. Section 3 of the paper explains the proposed biosensor design and its structural details. Section 4 illustrates the experimental results obtained through the proposed work and comparison with previous work carried out in this domain. Section 5 concludes the paper with possible future work that can be carried out in this direction.

2 Exiting Work on GFET Biosensor

A survey on various biomarkers involved in the detection of cardiovascular diseases (CVD) using electrochemical approach is presented in [7]. This paper elaborates various biomarkers in the plasma or blood serum, like C-reactive protein, myoglobin, interleukins, cardiac troponins, Tumor necrosis factor alpha, Creatine kinases, and fatty acid binding protein. This paper also narrates various nanomaterials used in the design of biosensors for the detection of biomarkers. Some of the nano materials discussed includes Carbon nano materials, noble metal nanoparticles, and platinum nano particles. Usage of Carbon Nanotubes (CNTs) and Graphene in biosensor applications is presented as a survey in [8]. Graphene based biosensors are faster, accurate, stronger, and reliable. These biosensors are suitable for optical sensors, electrochemical sensors, and FET based sensors. These sensors are useful in solving issues in food industries, human health, and environmental safety. Thermally reduced Graphene oxide (TRGO) based biosensor is a type of FET based biosensor, having the lower detection limit as 0.2 ng mL⁻¹. Another type of graphene sensor using portable sensor has been designed for the detection of lead content in child blood, is designed, which is having the lower detection limit as 37.5 ng mL⁻¹ [9]. Biosensors are also designed for the detection of myoglobin (Mb), which is one of the essential processes in the diagnosis of myocardial damage. Another direction of GFET biosensor for Escherichia Coli (E-Coli) bacteria detection is carried out in [10]. This bacteria lives in human and animal intestines. The proposed technique has lower detection limit as 10 cfu mL⁻¹. Another approach using nano structured porous silicon (nanoPS) based biosensor, for the detection of E-coli bacteria is described in [11]. Two nano PS structures namely NoCr/nanoPS/Au-NiCr and Al/nanoPS/Al had been fabricated for the detection of E-coli bacteria and glucose.

Detection of myoglobin in the diagnosis of cardiac problems using Mn-doped ZnO nanoparticles is proposed in [12]. It is an electrochemical approach using nano materials as screen printed electrodes (SPEs). The electrical property of
the channel, for instance its conductance, varies due to the presence of biomarker, which makes the detection of myoglobin possible. The lower limit of detection in this approach is 0.35 nm mL\(^{-1}\). A cardiac detection approach using Myoglobin detection and C-reactive protein is proposed in [13]. Unlike finding the changing in conductance of the channel, in the presence of biomarker, this technique uses magneto impedance (MI) as a changing parameter. This technique claims that identifying the two biomarkers such as C-reactive protein as well as Myoglobin are equally important in order better analyse the cardiac problems. While the proposed method has the lower limit of detection for myoglobin as 0.01 pg mL\(^{-1}\), but its value for C-reactive protein is 1 pg mL\(^{-1}\).

### 3 Proposed Design and Methodology of GFET Biosensor

In the proposed design work, COMSOL Multiphysics tool has been used to specify the physical dimensions and properties of the materials involved in the GFET transistor such as, Graphene, HfO\(_2\), SiO\(_2\), gold electrodes, etc. The structure developed is analyzed with and without mesh, according to the requirement of the accuracy in results. Figure 2(a) and (b) shows the simulation structures of MOSFET designed for the proposed work. In this approach, to save simulation time, the meshing is done automatically, and the same can also be done manually if one wants to reduce computation time. Table 1 summarizes the design parameters of the graphene FET.

The proposed approach involves different steps like sample collection from patients, keep it in a cold storage to preserve, and this samples are applied on the designed biosensors using GFET. This work mainly aims to detect Myoglobin in the human blood. The principle of detection of myoglobin through Graphene based biosensor is as follows: Sensor designed using Graphene at the micrometer levels can detect molecules that are attached to or detached from a surface.

![Fig. 2 Designed structure of (a) Graphene FET (b) Graphene FET with Mesh](image)

| Table 1 Graphene FET device parameters for COMSOL Multiphysics simulation tool |
|-----------------------------|-----------------------------|
| Device Parameter             | Value                      |
| Gold contact thickness (thick_Au) | 50 nm                      |
| Thickness of HfO\(_2\) (thick_HfO\(_2\)) | 20 nm                      |
| Thickness of SiO\(_2\) (thick_SiO\(_2\)) | 6 nm                       |
| Drain voltage (Vd)           | 10mV                       |
| Thickness of Silicon (thick_Si) | 300 nm                     |
| Length of Gold contact (Len_contact) | 500 nm                    |
| Top gate voltage (V\(\text{tg}\)) | 0 V                        |
| Bottom gate voltage (V\(\text{bg}\)) | 0 V                        |
| Source voltage (V\(s\))     | 0 V                        |
| Width (W)                    | 4 \(\mu\)m                  |
| Relative permittivity (\(\varepsilon_r\)) | 4.2                      |

The attachment and detachment change the one electron in the Graphene structure results in change in surface resistance of the biosensor. Measuring this contact resistance experienced by the electrons when moving across the metal-Graphene junction results in the detection of Myoglobin. The proposed work uses Graphene material with 0.65 nm thickness which can detect Myoglobin with high accuracy.

GFET equipped myoglobin binding receptor particles to identify the presence of myoglobin in the sample serum under consideration. Typical binding process using 3-Aminopropyltriethoxysilane (APTS) [14] based receptor is shown in Fig. 3.

Myoglobin GFET sensor is modeled with an aqueous solution-gated FET and it is simulated to analyse its electrical characteristics for different values of Gate and Drain voltages. The structure contains a Graphene channel added with Myoglobin spike antibody, over which phosphate-buffered Saline (PBS with a pH value of 7.4) is coated. This structure provides high performance gating function. Now, if a sample serum is applied over the modified Gate terminal, in the presence of myoglobin antigen, the conductivity of the channel varies, and hence its surface potential also varies.
To overcome this limitation the band gap can be increased using many like doping graphene surface with transition metals and passivation with foreign molecules are to change the chemical properties which help to binding between graphene and biomarker [15]. There is a change in the graphene physical variants in the presence and absence of biomarker. The sensitivity of graphene not high in presence of biomarker compared with graphene doped with transition metals. The surface volume may be possibility to absorb the more. The design process with doping impurities to graphene like aluminium, arsenic, gallium like these which used to make graphene as semiconducting material. Using also helpful in increasing the bandgap the best transition material is Pd which has high stability. The properties graphene is in presence and absence of biomarker is important to determine the overall performance [16]. Metal contacts of 50 nm thickness are positioned at the respective positions of source, gate and drain terminals. The metal contact at the gate terminal acts as top gate. The top gate and bottom have their specific characteristics with respect to their applied voltages and resultant currents.

For the effective characteristics, the dimensions of designed GFET terminals were fixed. Now, the determination of presence or absence of the biomarker becomes easy as the devices dimensions are fixed. Immobilized antibodies like Horseradish Peroxidase-anti-myoglobin, Mouse anti-human myoglobin (MCA5874G), etc., are required to identify the myoglobin in blood samples. On the graphene film, antibodies are immobilized for the detection of respective biomarkers, which results in ease of computation and reduced time [17]. Several researchers have designed various devices which can be useful in biosensing applications [18–21].

4 Results and Discussion

4.1 Graphene FET Characterization

Figure 4 illustrates the scheme used by graphene FET for sensing myoglobin. The target Mb is functionalized when the Probe Mb is anchored on graphene channel FET sensors by a linker molecule. Figure 4a shows the fabrication process of the FET. The graphene channel (1 × 50 mm) was shift on to silicon substrate using traditional method. In the process of producing the graphene FET device, the source and drain metal electrodes were formed after annealing the graphene on silicon substrate at 110 °C for 6 h. When measuring the fluid biomolecules, the gate voltage is applied directly on the buffer solution by placing it on the graphene sensing area of the device. Figure 4b illustrates the quality of graphene by Raman spectroscopy analysis. The sheet resistance of graphene was ~ 380 Ω when its measured using van der pauw
method. As shown in Fig. 4c, the resistance of graphene was found to be in the ranges of 3 to 8 kΩ when the source-drain current was measured in order to confirm that graphene’s electric prosperities would not be adversely affected by electrical conducting.

The morphology of the graphene was characterized by tunneling electron microscope (TEM) shown in Fig. 5. The structure was disorderly, and the size of the large fold was a few hundreds of nanometers; however, when magnified, finer folds as small as a few tens nanometers were observed.

4.2 Target Myoglobin (Mb) Functionalization and Detection on Graphene FET Biosensor

In Fig. 6(a), the target Mb was incubated by dropping complementary and negative control strands at the concentrations indicated in the legends with and without functionalization. For 1 h, the graphene FET chip was functionalized in a solution. The chip was then gently rinsed with 1 PBS. The chip was incubated in, and rinsed with serum for the human serum test. Human plasma was micro-filtered to create the serum. A total of 20 µL of samples are treated.
A semiconductor characterization system (SCS) with a probe station was used to measure the resistance and current-voltage (I-V) curves. The tests for the human serum sample test were carried out in serum. Due to the wettability of hydrophobic graphene, the graphene FET biosensor was immersed in PBS overnight for Mb absorption. The graphene FET biosensors were incubated in serum overnight for the serum test, and the blank measurements were repeated until there was no shift with serum. The sensor was then used to treat the target and Mb in serum. Vg was varied between 0 and 1 V, and the drain–source voltage (Vd) was set between 0.03 and 0.1 V. At a given Vds, the drain–source current (Id) was measured.

The Drain characteristics of GFET with myoglobin biomarker are shown in Fig. 6(b). It can be observed that the output current is in the order of micro-amperes. Further it can be noted that the Drain voltage is varied for a small range i.e., from 0 to 1 V, which produces different values of drain current under the presence of myoglobin concentration. For instance, the myoglobin biomarker with 30 fg/mL produces a Drain current as high as 1.3 µA, which is an easily differentiable value for myoglobin with a concentration thickness of 2pg/mL, having maximum Drain current as 0.9 µA. The Drain characteristics of GFET, for the Drain voltages ranging from –0.1 V to +0.1 V has been plotted in Fig. 6. It is observed before functionalization, that is Graphene without combining with solvent or antibody, the Drain current is ranging from –50 µA to +50 µA (Black coloured line), Fig. 6(b). When it is combined with PBASE solvent the current ranges between –40 µA to +40 µA (Red coloured line), Fig. 6(b). When the GFET becomes functionalized i.e., Graphene conjugated with PBASE solvent and Myoglobin antibody, the drain current ranges from −17 µA to +17 µA (Blue coloured line). From these results it can be concluded that the functionalized GFET produces reduced output current, and hence the detection of presence of the myoglobin in the blood sample. Furthermore, limit of detection of myocardial FET immunosensors is compared well with available literatures as depicted in Table 2.

5 Conclusions

The best quality level indicative device of acute myocardial infarction (AMI) in clinical settings remains ELISA-based test. Graphene based Field Effect Transistor biosensors are widely chosen for the identification of different biomarkers in the recent years. Reliability, accuracy, and speed are the major features enforced to use GFETs. In this work proposed a graphene based field effect transistor as a high sensitive point-of-care myoglobin testing assay. The proposed electrical sensor accomplished myoglobin discovery at a ten times lower fixation than the 500 fg/mL cut-off for recognizing myocardial injury. The electrical characteristics of the GFET are seen to fluctuate in response to the concentration of myoglobin in the blood. The developed biosensor has higher sensitivity and a lower detection limit of 30 fg/mL. This would

| Virus Type | Type of Immunosensor | Limit of detection | Ref. |
|------------|----------------------|--------------------|------|
| myoglobin  | Electrochemical       | 0.33-0.01mmol/L    | [22] |
| myoglobin  | Electrochemiluminescence | 0.0492 ng/mL | [23] |
| myoglobin  | Electrochemical       | 0.22 ng/mL         | [24] |
| myoglobin  | Aptaasenor FET based  | 0.1 µg/mL          | [25] |
| myoglobin (Mb) | electrochemistry and electrocatalysis | 0.2 to 350.0 µmol/L | [26] |
| myoglobin  | Graphene FET based    | 30 fg/mL           | This Work |
consequently, work on the fitting of preventive measures and medicine in a customized way. Additionally, we firmly accept that the execution of quick and sensitive myoglobin point-of-care sensors in emergency offices will be valuable for patients.

Acknowledgements The authors are thankful to SRM University-AP, Mangalagiri-522240, Andhra Pradesh for their cooperation and support during this research work.

Author Contributions B. Vamsi Krsihna, Asisa Kumar Panigrahy, V. Raja Rajeswari, and M. Durga Prakash: Conceptualization; M. Durga Prakash and B. Vamsi Krsihna: investigation; D. Mohan, Asisa Kumar Panigrahy, V. Raja Rajeswari, and M. Durga Prakash: resources; B. Vamsi Krsihna, (A) Gangadhhar, S. Ravi, V. Raja Rajeswari, and M. Durga Prakash: data curation; D. Mohan, Asisa Kumar Panigrahy, and M. Durga Prakash: writing—original draft preparation; M. Durga Prakash, (B) Vamsi Krsihna: writing—review and editing; Asisa Kumar Panigrahy, and M. Durga Prakash: visualization; M. Durga Prakash: supervision.

Data Availability No supplementary materials.

Declarations

Conflict of Interest The authors declare that they have no conflict of interest.

Research Involving Human Participants and/or Animals This article does not contain any studies with human or animal subjects.

Consent to Participate Additional informed consent was obtained from M. Durga Prakash identifying information is included in this article.

Consent for Publication Author(s): Dr. M. Durga Prakash.

Author’s signature: Dr. M. Durga Prakash (M D Prakash)

Date: 05-02-2022.

References

1. Pumera M (2011) Graphene in biosensing. Mater Today 14(7-8):308–315
2. Krsihna BV, Ravi S, Prakash MD (2021) Recent developments in graphene based field effect transistors. Mater Today Proc 45:1524–1528
3. Prakash MD, Tripathy S, Vanjari SRK, Sharma CS, Singh SG (2016) An ultrasensitive label free biosensor platform for the detection of cardiac biomarkers. Biomed Microdevices 18(6):1–10
4. Haque M, Foad H, Seo H-K, Othman AY, Ansari ZA (2020) Cu-doped ZnO nanoparticles as an electrochemical sensing electrode for cardiac biomarker myoglobin detection. IEEE Sens J 20(15):8820–8832
5. Wang Q, Liu F, Yang X, Wang K, Wang H, Xin, Deng (2015) Sensitive point-of-care monitoring of cardiac biomarker myoglobin using aptamer and ubiquitous personal glucose meter. Biosens Bioelectron 64:161–164
6. Baum H, Booksteegers P, Steinbeck G, Neumeier D (1994) A rapid assay for the quantification of myoglobin: evaluation and diagnostic relevance in the diagnosis of acute myocardial infarction. Eur J Clin Chem Clin Biochem 32(11):853–858
7. Bakirhan NK, Ozcelikay G, Sibel A (2018) Ozkan. “Recent progress on the sensitive detection of cardiovascular disease markers by electrochemical-based biosensors. J Pharm Biomed Anal 159:406–424
8. Zhu Z (2017) An overview of carbon nanotubes and graphene for biosensing applications. Nano-Micro Lett 9(3):1–24
9. Wang C, Cui X, Li Y, Li H, Huang L, Bi J, Luo J et al (2016) A label-free and portable graphene FET aptasensor for children blood lead detection. Sci Rep 6(1):1–8
10. Huang Y, Dong X, Liu Y, Li L-J, Chen P (2011) Graphene-based biosensors for detection of bacteria and their metabolic activities. J Mater Chem 21(33):12358–12362
11. Recio-Sánchez G, Torres-Costa V, Manso M, Gallach D, López-García J, Martín-Palma RJ (2010) Towards the development of electrical biosensors based on nanostructured porous silicon. Materials 3(2):755–763
12. Haque M, Foad H, Seo H-K, Othman AY, Kulkarni A, Ansari ZA (2020) Investigation of Mn doped ZnO nanoparticles towards ascertaining myocardial infarction through an electrochemical detection of myoglobin. IEEE Access 8:164678–164692
13. Yang Z, Wang H, Guo P, Ding Y, Luo Y (2018) A multi-region magnetoeimpedance-based bio-analytical system for ultrasensitive simultaneous determination of cardiac biomarkers myoglobin and C-reactive protein. Sensors 18(6):1765
14. de Dios AS, Díaz-García ME (2010) Multifunctional nanoparticles: analytical prospects. Anal Chim Acta 660(1–2):1–22
15. Yang H, Shan C, Li F, Han D, Zhang Q, Li Niu (2009) Covalent functionalization of polydisperse chemically-converted graphene sheets with amine-terminated ionic liquid. Chem Commun 26:3880–3882
16. Singh P, Sohi PA, Kahrizi M (2021) Finite element modelling of bandgap engineered graphene FET with the application in sensing methanethiol biomarker. Sensors 21(2):580
17. Krsihna BV, Ahmadsaidulu S, Tarun SST, Jayanthi D, Navaneetha A, Reddy PR, Prakash MD (2021) Design and development of graphene FET biosensor for the detection of SARS-CoV-2. Silicon 1–9
18. Prakash MD, Nelam BG, Ahmadsaidulu S, Navaneetha A, Panigrahy AK (2021) Performance analysis of ion-sensitive field effect transistor with various oxide materials for biomedical applications. Silicon. https://doi.org/10.1007/s12633-021-01413-9
19. Prakash MD, Krsihna BV, Satyanarayana BVV, Vignesh NA, Panigrahy AK, Ahmadsaidulu S (2021) A study of an ultrasensitive label free silicon nanowire FET biosensor for cardiac Troponin I detection. Silicon. https://doi.org/10.1007/s12633-021-01352-5
20. Moriga C, Ponnuri RT, Satyanarayana BVV, Gadivada AAC, Panigrahy AK, Prakash MD (2021) A novel teeth junction less gate all around FET for Improving Electrical Characteristics. Silicon. https://doi.org/10.1007/s12633-021-00983-y
21. Prakash MD, Nihal SL, Ahmadsaidulu S, Swain R, Panigrahy AK (2022) Design and modelling of highly sensitive glucose biosensor for Lab-on-chip applications. Silicon. https://doi.org/10.1007/s12633-021-01543-0
22. Shao B, Chen W, Yan L, Huang Y, Wang B, Zou Q, Xuan Y, Sun W, Niu Y (2021) Preparation and electrocatalytic study of myoglobin biosensor based on platinum-gold-three dimensional graphene modified electrode. Int J Electrochem Sci 16(211039):2
23. He S, Zhang P, Sun J, Yi Y, Huang C, Jia N (2022) Integrating potential-resolved electrochemiluminescence with molecularly imprinted immunosassay for simultaneous detection of dual acute myocardial infarction markers. Biosens Bioelectron 201:113962
24. Al Fatase A, Haque M, Umar A, Ansari SG, Alhamhoom Y, Muhsinah AB, Mahnashi MH, Guo W, Ansari ZA (2021) Label-free electrochemical sensor based on manganese doped titanium
dioxide nanoparticles for myoglobin detection: biomarker for acute myocardial infarction. Molecules 26(14):4252

25. Surya SG, Sanjit SM, Agarwal DK, Lahcen AA, Yuvaraja S, Chappanda KN, Salama KN (2020) A label-free aptasensor FET based on Au nanoparticle decorated Co 3 O 4 nanorods and a SWCNT layer for detection of cardiac troponin T protein. J Mater Chem B 8(1):18–26

26. Wang W, Dong L, Gong S, Deng Y, Yu J, Dong H, Wang T, Sun W (2019) Electrochemistry of myoglobin on graphene–SnO2 nanocomposite modified electrode and its electrocatalysis. Arab J Chem 12(8):3336–3344

**Publisher’s Note** Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.