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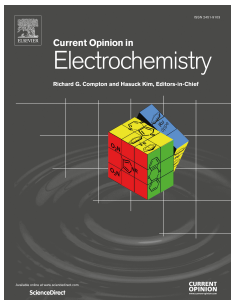

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PII: S2451-9103(21)00100-9

DOI: https://doi.org/10.1016/j.coelec.2021.100786

Reference: COELEC 100786

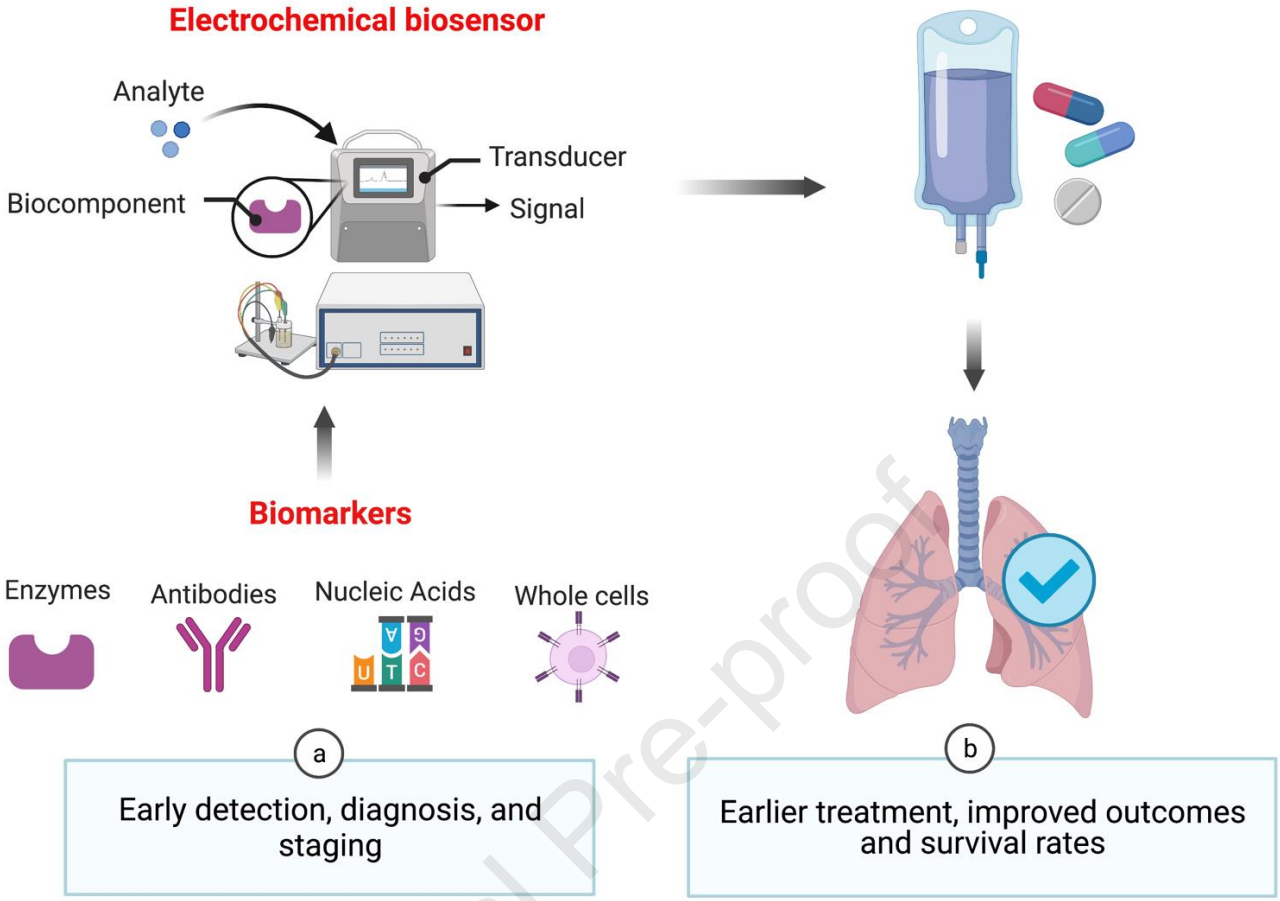
To appear in: *Current Opinion in Electrochemistry*

Received Date: 31 March 2021

Revised Date: 13 May 2021

Accepted Date: 27 May 2021

Please cite this article as: Tabish TA, Hayat H, Abbas A, Narayan RJ, Graphene quantum dot-based electrochemical biosensing for early cancer detection, *Current Opinion in Electrochemistry*, https://doi.org/10.1016/j.coelec.2021.100786.



Graphene quantum dot-based electrochemical biosensing for early cancer detection

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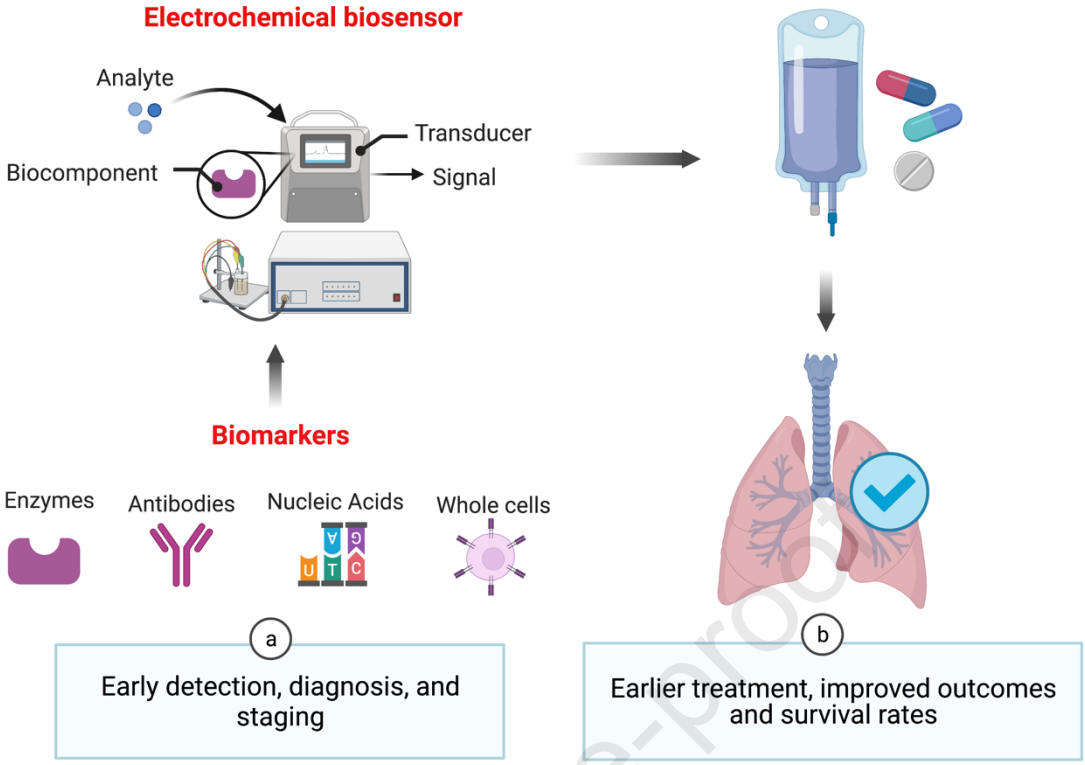
Abstract

Electrochemical biosensing systems coupled with graphene quantum dots (GQDs) have demonstrated suitability for cancer diagnostic strategies, particularly to identify the changes facilitating the early phases of tumorigenesis as well as to detect ultralow concentrations of biomarkers that distinguish between normal and malignant cells. GQDs, known as a novel class of zero-dimensional semiconductor nanocrystals, are tiny graphene particles arranged in a honeycomb structure with a size range of 1-50 nm. The size of these GQDs is comparable to the size of biomolecules, thereby providing an ideal platform to study biomolecules such as proteins, cells, and viruses. GQDs are a superior platform for specific and sensitive recognition of cancer biomarkers; they are highly synergistic with electrochemical sensors. This review will shed light on the recent advancements made in the field of GQDs-based electrochemical sensors for early cancer detection, with the aim of highlighting the prospects for further development in cancer diagnostics.

Graphical abstract



Introduction

Cancer is one of the main causes of human mortality, with 19.3 million new cancer cases and almost 10.0 million cancer deaths in 2020 **[1]**. Cancer exacts a tremendous toll on patients, their families, and society and is associated with a devastating economic burden. Cancer survival heavily relies on the tumor type and disease stage. Late diagnosis of cancer at advanced stage are among key drivers for cancer mortality. Standard therapeutic modalities have limited success for the treatment of cancer, in part, because of late diagnosis, when cancer cells have metastasized in the distant tissues/organs **[2]**. Cancer cells grow and invade into surrounding healthy tissues and eventually can reach distant organs. The spreading process of cancer cells and their distant colonization accounts for 90 % of cancer deaths **[3]**. The concept of early detection of cancer has the potential to revolutionize approaches to drug discovery, therapeutic intervention, and prevention of human cancers. The identification of specific genetic and protein activities involved in the early make-up of cancer allows for the assessment of the presence of disease at an early stage, detection of new targets for therapeutics, and monitoring of therapeutic responses. Innovative biosensing technologies to identify such specific and reliable biomarkers for early cancer detection offer unique opportunities to accelerate drug development strategies in fighting against cancer **[4]**. Most notably, electrochemical biosensing provides high sensitivity and selectivity for critical biomarkers responsible for crucial molecular events in tumor formation and progression **[5]**.

Biosensors are diagnostic devices encompassing a biological recognition element (for example, enzymes, DAN, mRNA, antibodies, receptors) and a transducer (electrochemical, optical, acoustic, thermal) **[6]**. Since the advent of electrochemical biosensors in 1960, many approaches have been established for real-time, rapid, and sensitive monitoring and identification of target biomolecules **[7]**. Electrochemical sensing is generally performed in three-electrode configuration comprising working (sensing), counter, and reference electrodes.

The primary requirement of electrochemical sensing is an electrolyte, which interacts with a specific conducting material. In cyclic voltammetry (CV), the potential of a working electrode is measured in connection with the reference electrode at a fixed voltage **[8]**. While electrochemical impedance spectroscopy (EIS) is employed to evaluate the corrosion of materials as well as the adsorption and desorption from an electrode surface **[9]**. Linear sweep, cyclic, hydrodynamic, stripping, and square-wave are extensively employed CV methods for the electrochemical recognition of cancer receptors **[10]**. A detailed description of these methods along with electrochemical biosensing for disease detection have been reviewed elsewhere **[11-13]**. Electrochemical biosensors offer several advantages over conventional sensing and screening systems such as rapid readout, selective, sensitive, ease-of-fabrication, minimally trained personnel, in-field utility, non-invasiveness, low background to noise ratio, simple instrumentation, and ease of miniaturization. These point-of-care devices can also be used at home. The incorporation of nanomaterials into electrochemical biosensing has significantly improved the real-time, rapid, cost-effective, sensitive, and specific detection of biomarkers at ultralow concentrations. Nanomaterials are used to modify the electrode, which in turn enhances the adsorption of biomarkers owing to their high surface-to-volume ratio and high specific surface area. Nanomaterials exhibit size-dependent physiochemical features, optical properties, quantum confinement effects, mechanical flexibility and electrochemical characteristics. To date, numerous nanomaterials have been used for the development of electrochemical biosensing such as nanoparticles **[14]**, magnetic beads **[15]**, carbon nanotubes **[16]**, graphene **[17]**, polymeric nanostructures **[18]** and quantum dots (QDs) **[19]**. Recently graphene quantum dots (GQDs) have received significant attention due to their vast majority of exceptional properties, including size-dependent photoluminescent and electrochemiluminescent properties, electro-catalytic features, large surface area, functional groups, edge effects, multiplexing capabilities, photo-stability, water-solubility, biocompatibility and minimal toxicity.

GQDs, known as a novel class of zero-dimensional semiconductor nanocrystals, are graphene particles arranged in a honeycomb structure with a size range of 1-50 nm **[19]**. The graphene structure in GQDs significantly improves their electrical conductivity, electron mobility/transport and mass diffusion of analyte, which in turn accelerates the electron transport between analyte and electrode, thereby leading to an excellent electrochemical response. GQDs have extensively been explored for applications in biomedical imaging **[20]**, drug-loading for chemotherapy **[21]**, and photosensitization in photodynamic and photothermal therapies **[22]**. GQDs are synthesized by either bottom-up or top-down approaches. Top-down approaches include exfoliation, oxidative cleavage, hydrothermal, solvothermal methods, ultrasonic-assisted or microwave-assisted methods, electrochemical oxidation; bottom-up approaches include carbonization and pyrolysis **[23-28]**. The optical properties of GQDs can precisely been modified based on their size, shape, number of layers, edge defects, band gap, and degree of carboxylic functionalization moieties present at the surface of the GQDs **[29]**. A wide variety of approaches are available for the construction of GQDs-based electrochemical sensors. Most studies involve the use of GQDs as a modified electrode surface **[30]**. The modified electrode surface facilitates electrochemical biosensing of biomolecules. For example, In 2011, Zhu et al. **[31]** reported the design of GQDs-modified electrochemical biosensor for the detection of ssDNA and target proteins with known aptamers; they demonstrated that GQDs havde the potential to improve the specificity of biomolecules via electron transfer to the surface electrode. They demonstrated that GQD can be functionalized onto the surface of pyrolytic graphite electrode; the probe ssDNA can be easily halted due to the interface between the nucleobases and GQDs. Furthermore, the interplay between size and photoluminescence of GQDs also facilitates the charge transfer between carboxyl functional moieties and GQDs, which in turn increases the electron density and mobility of GQDs. The increase in electron density of GQDs exhibits a strong interacrtion between the

GQD-modified electrode and the biomolecule (analyte). The electrochemical charge transfer ability of GQDs offers an innovative way for detecting molecules at ultralow concentrations owing to their high electron mobility and functional groups. The quantification of the interaction between the modified electrode and the analyte is monitored by a measurable charge transfer (via potentiometry and conductometry). In the case of conductometric biosensors, variations in the electrical conductivity of the sample are measured. In potentiometric biosensors, biosensors are often functionalized with enzymes; the sensing approach is based on the formation of detectable ions.

This opinion article for the first time discusses the advances in GQDs-based electrochemical biosensing for early cancer detection via representative studies, recent innovations and technical challenges.

GQDs in electrochemical biosensing for cancer detection

Cancer is a complex disease that is governed by a wide range of molecular events through genetic alteration and protein profiling. Decades of cancer research have produced a series of markers that are typically derived from the molecular characteristics of type, localization, and dissemination of tumor as well as disease stage. Generally, cancer biomarkers are divided into three classes: (i) diagnostic – used to detect cancer with high specificity; (ii) prognostic – used to find disease recurrence and (iii) predictive – used to assess the tumor response towards a specific drug. Cancer biomarkers are generally found in fluids (e.g., plasma, serum, urine, saliva) and tissues at molecular (e.g., DNA and receptors/protein) **[32]**, organelle (e.g., microvesicles such as exosomes) **[33]**, cellular (phenotypes, apoptosis, angiogenesis and proliferation) levels **[34, 25]**. Specific examples of biomarkers are carcinoembryonic antigen (CEA), cancer antigens (e.g., CA-125, CA-145, and CA-199) prostate-specific antigen (PSA), IL-13 soluble receptor Rα2 (IL-13sRα2), cadherin-17 (CDH-17), hydrogen peroxide (H_2O_2) and interleukin 6/8 (IL-6/8) **[36]**.

The specific biomarker for prostate cancer is PSA; its screening started in the USA in 1987 **[37]**. The sensitivity, specificity, and positive predictive accuracy of PSA have always been controversial. PSA and its counterpart genes play a crucial role in the development of prostate cancer at an early stage **[38]**. In 2011, Yang et al. **[39]** synthesized QDs functionalized graphene sheets for the identification of primary and secondary PSA antibodies in patient serum samples, revealing a broad-ranging of linear performance (0.005–10 ng/mL) with a low detection limit (3 pg/mL). The low detection limit and broad linear range of sensing are attributed to the enhanced adsorption of PSA antibodies and QDs on graphene nanosheets and good electrical conductivity. Figure 1 A shows the comparison of square wave voltammograms (SWV) reaction of QD onto pristine and graphene nanosheets electrodes, showing an obvious peak at −0.8 V (which depicts the oxidation of QDs). Figure 1 B illustrates the SWV performance of the sensor at of PSA in a concentration-dependent manner. The peak current increased with an increasing PSA concentration (as shown in Figure 1 C). Figure 1 D represents the sensitivity of the electrochemical sensor modified with graphene sheets functionalized QDs, which is fifty times higher than that of graphene oxide. Figure 1 E shows the plot of the PSA concentrations using two electrodes (based on QDs and graphene oxide) offered a straight line. In another study, Wu et al. **[40]** reported the ultrasensitive electrochemiluminescent biosensor for the detection of PSA in serum samples (with a detection limit of 0.29 pg/ml) using GQDs. They used Au/Ag-reduced graphene oxide to assemble two different types of GQDs (aminated GQDs and carboxyl GQDs) to significantly improve the electrochemiluminescence signals and to further improve the surface area of electrode, which in turn can increase electrochemiluminescence signals.

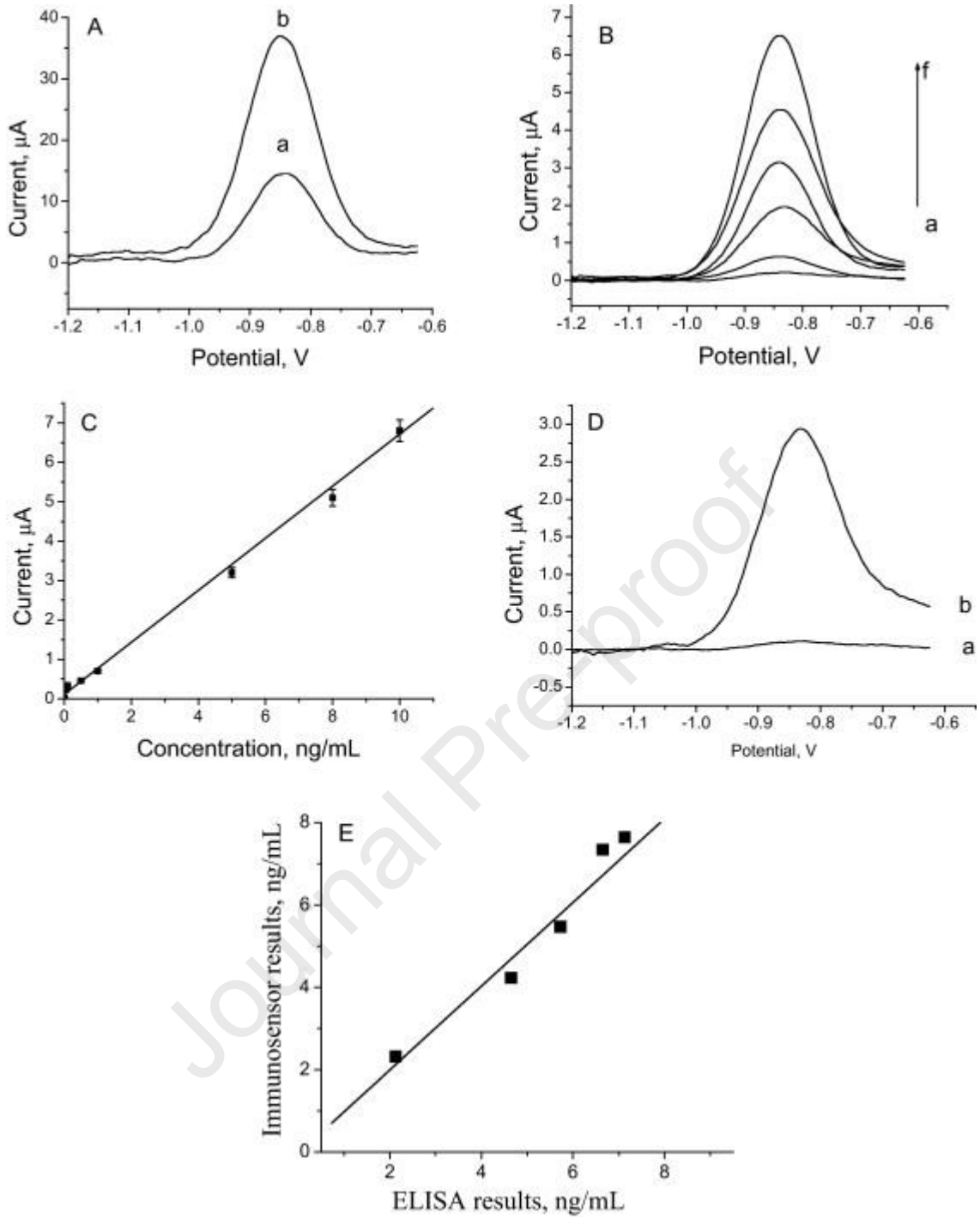


Figure 1. (A) Square wave voltammograms (SWV) performance of QDs on (a) bare and (b) graphene sheets-based electrode. (B) SWV performance of the sensor to PSA in a concentration-dependent manner: (a) 0.05 ng/mL, (b) 0.5 ng/mL, (c) 2 ng/mL, (d) 5 ng/mL, (e) 8 ng/mL, and (f) 10 ng/mL. (C) Calibration curve of the sensor with PSA. Error bar = RSD (n = 5). (D) SWV performance of the sensor based on graphene oxide (a) and graphene sheets (b) towards 5 ng/mL of PSA. (E) Comparison of the PSA concentrations in serum samples investigated using graphene sheets-based sensor and the enzyme-linked immunosorbent assay (ELISA) method. Reproduced with permission from reference **[39]**.

Carcinoembryonic antigen (CEA), an extensively studied glycoprotein, is overexpressed in colorectal, gastric, breast, ovarian, and lung cancers **[41]**. The average typical levels of CEA in normal cells are in the range of 3–5 ng mL^{-1}, while overexpression up to 10 ng mL^{-1} has been found in tumors **[42]**. CEA is also a standard screening biomarker advised by the National

Academy of Clinical Biochemistry and the American Society of Clinical Oncology for diagnosis, invasion, and monitoring of a specific therapy **[43]**. Nevertheless, the sensitivity and specificity of CEA detection in asymptomatic people is likely to be very low. A label-free electrochemical sensing approach using N, S-GQDs@Au-polyaniline nanowires has been reported, detecting CEA with a broad-ranging linear trend (0.5 to 1000 ng mL^{-1}) with a detection limit of 0.01 ng mL^{-1} **[44]**. In this work, GQDs-based nanowires revealed excellent electron transfer capabilities, thus providing a platform for the sensitive detection of CEA. EIS performance GQDs composite electrode at different concentrations of CEA is shown in Figure 2 a. Figure 2 b illustrates the calibration curve of CEA by GQDs composite using the EIS plot. Figure 2 c shows the total impedance once CEA has been adsorbed. Figure 2 d shows the variations in total impedance with a rising concentration of CEA. The rise in impedance is 2.28 folds in comparison to the pristine electrode, thereby indicating the high specificity GQDs composite sensor towards CEA detection. Yang et al. **[45]** reported nitrogen-doped GQDs conjugated with Pt-Pd bimetallic nanoparticles for the detection of CEA with a broad range of linearity (5 fg/mL to 50 ng/mL) and a low detection limit (of 2 fg/mL).

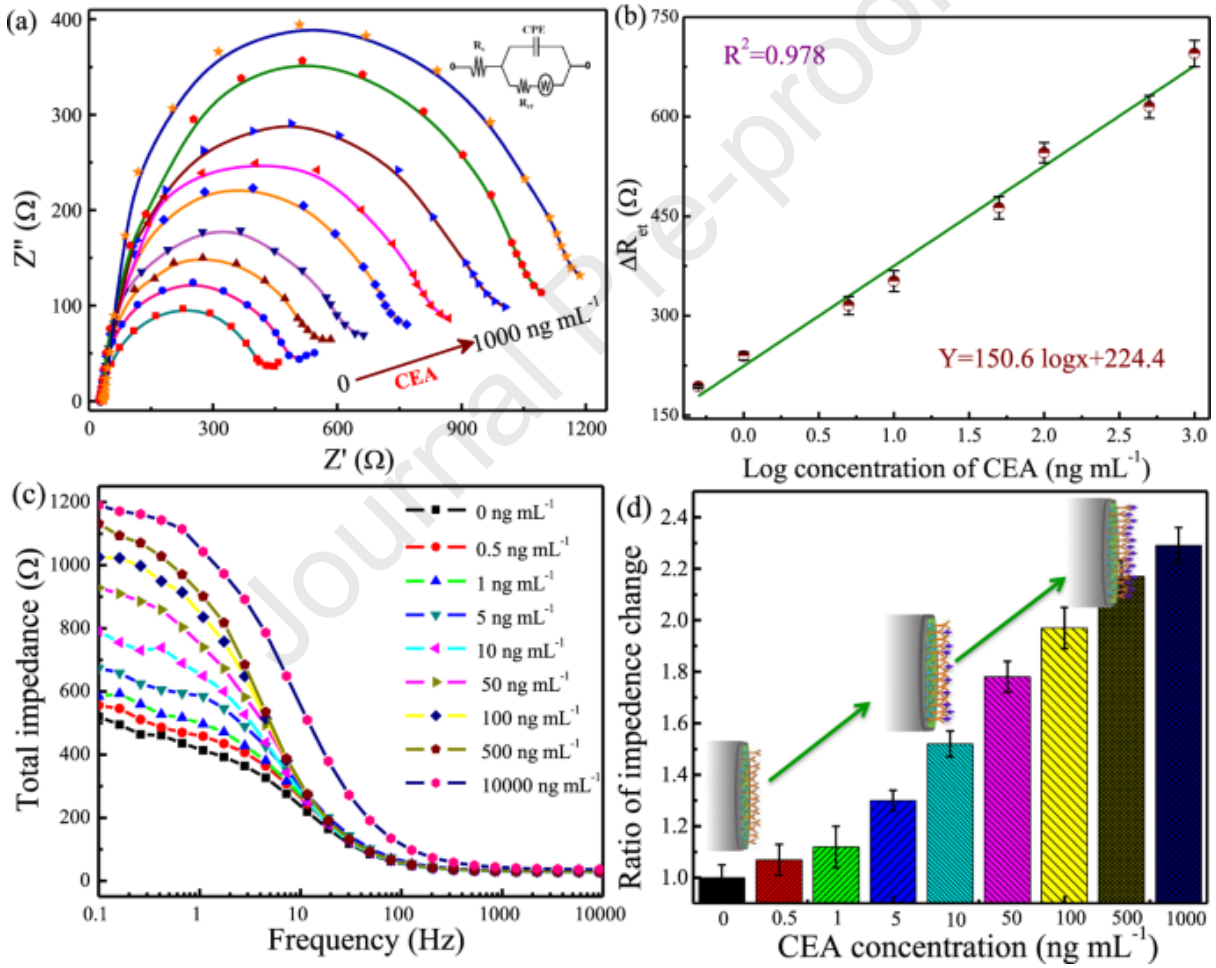


Figure 2: The (a) Nyquist plot of Pt-PANI-Au/N,S-GQDs/anti-CEA at different concentrations of CEA (0 to 1000 ng mL^{-1}), (b) calibration curve for the detection of CEA, (c) total impedance before and after the attachment to CEA and (d) ratio of change in impedance respecting the pristine Pt-PANI-Au/N,S-GQDs/anti-CEA electrode without CEA. Reproduced with permission from reference **[44].**

p53 in normal cells is expressed at low levels. While p53 mutations play a crucial role in tumor progression and generate auto-anti p53 antibodies **[46]**. It has been suggested that mutant p53 appears at the early stage of lung, skin, head and neck, and esophageal

cancer progression **[47]**. Hasanzadeh et al. **[48]** reported the immobilization of p53 onto a nanocomposite of poly l-cysteine (a conductive material) and GQDs/Au nanoparticles (amplification component), revealing that p53 concentration is linear up to a concentration of 0.000197-0.016 pM (by the SWV technique) and a concentration of 0.195–50 pM (by the DPV technique), with a low detection limit of 0.065 fM. This study was conducted using human plasma and cell lines (both normal and malignant cell lines – L929, colon cancer HCT, prostate cancer PC-3, and human breast adenocarcinoma MCF7). Figure 3 shows differential pulse voltammetry (DPVs) of Au/GQDs/ poly l-cysteine/Au nanoparticles in plasma followed by incubation with p53 at various concentrations (0, 0.0000219, 0.0000658, 0.000197, 0.00059, 0.144, 0.432, 1.296,and 11.66 pM).

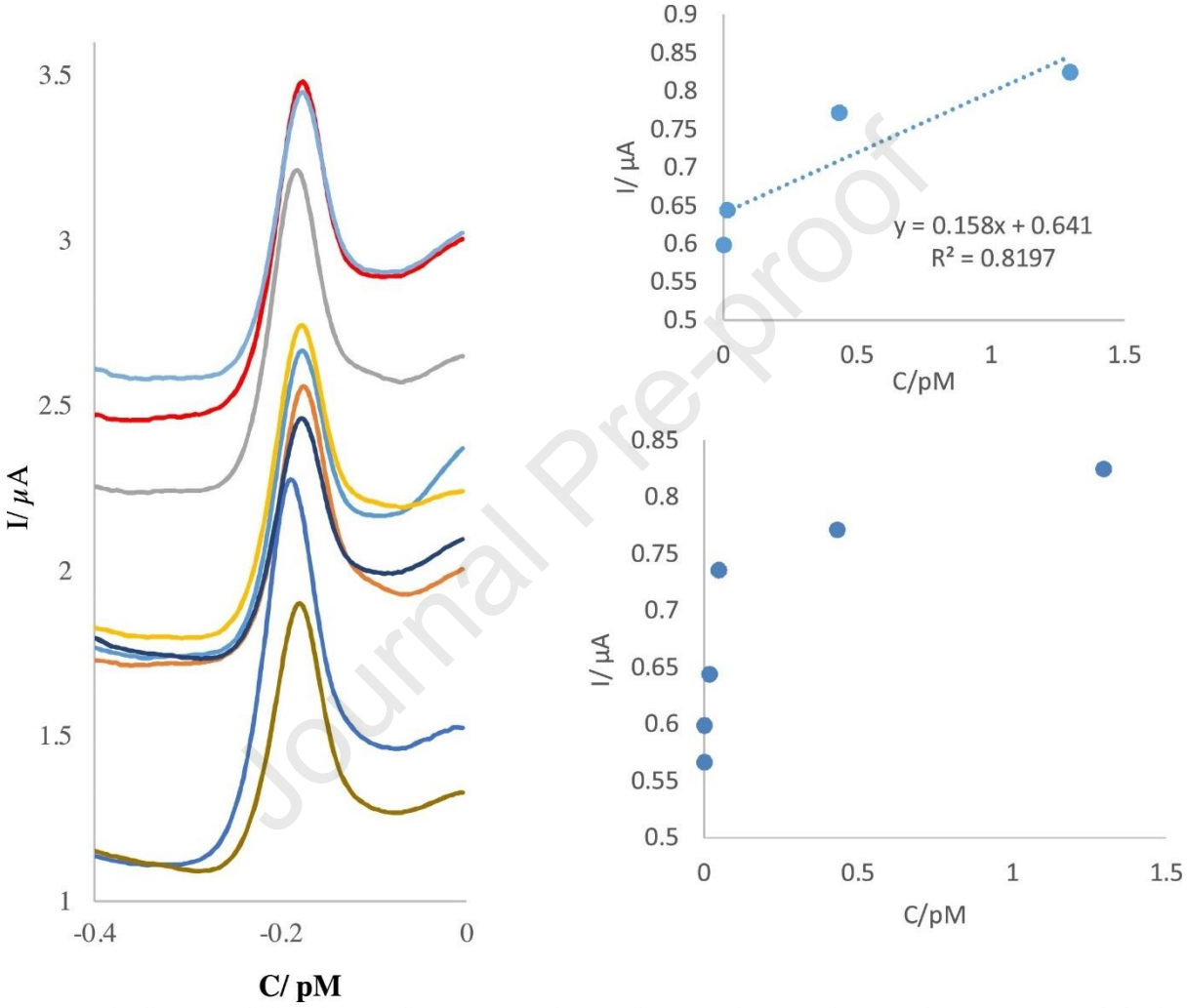


Figure 3: A) differential pulse voltammetry (DPVs) of Au/GQDs/P-Cys/Au nanoparticle-in human plasma samples followed by incubation with p53. Step potential = 0.005 V (Ag/AgCl), Modulation amplitude = 0.025 V (Ag/AgCl), Modulation time = 0.05s, Interval time = 0.1s, Scan Rate = 0.01 V/s. Inset; Calibration curve for detection of p53 antibodies (SD = 3.25, n = 5). Reproduced with permission from reference **[48]**.

Conclusion and future outlook

The exponential growth of early cancer detection research is a testament to the impact of innovative drug development. Electrochemical sensing technologies coupled with quantum

dots have achieved remarkable growth over the past decade and continue to refine the electrochemical assays for the non-invasive identification of the morphological and biochemical complex variations associated with the early- stage development of cancer. Importantly, graphene as a transducer in electrochemical sensor offer superior properties such as electrical and thermal conductivity, photoluminescence, mechanical flexibility and good biocompatibility. Studies discussed in this article offer a solid starting point that can trigger and navigate future investigations on translating these discoveries to the clinic. However, demonstration of the large-scale clinical efficacy of these approaches is needed to verify and optimize the diagnostic accuracy towards disease detection. To ensure the high reliability and real-time accuracy of such biosensors, signal amplification biorecognition/bioconjugation procedures are needed to be controlled precisely and sensitively in terms of size and shape for the ease of operation and on-site application, stability of both biomolecule and nanomaterials, electrode modification/conjugation compositions at correct ratios, instantaneous identification/adsorption of multiple proteins/biomarkers and corresponding detection limits. The design and development of multiplexed electrochemical sensing systems for detection of multiple receptors and biomarkers remains scarce. Such multiplexed systems can enable early cancer detection more reliable and accurate than existing screening and pharmacological tools. Multivalent nanomedicine can address these challenges; for example, the use of multifunctional nanoparticles can aid the development of multiplexed point-of-care devices. Furthermore, cancer-expelled microvesicles such as exosomes have received significant attention in recent years, which are in comparable size with GQDs. Exosomes contain mRNA, epidermal growth factor receptor (EGFR) dimers (e.g., HER1, HER2, HER3), and immune checkpoint dimers which are indicative of pre-malignant lesions. Exosomes can be isolated from liquid biopsies such as plasma, serum, urine, and breast milk. Therefore, electrochemical sensing technologies must be utilized to study and identify cancer-expelled exosomes. The solutions to these issues will not only help us understand the morphological and biochemical complex variations associated with early-stage development of cancer but also will improve patient quality of life through personalized medicine.

Acknowledgements

Tanveer Tabish acknowledges the financial support from the International Alliance for Cancer Early Detection, a partnership between Cancer Research UK [EICEDAAP\100007], Canary Center at Stanford University, the University of Cambridge, OHSU Knight Cancer Institute, University College London and the University of Manchester.

Authors contribution

Tanveer A. Tabish and Roger J. Narayan conceptualized and wrote the manuscript. Hasan Hayat and Aumber Abbas edited the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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Declaration of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.