BioNanoScience (2024) 14:429–446
https://doi.org/10.1007/s12668-023-01250-7

# Biosensor-Based Nanodiagnosis of Carcinoembryonic Antigen (CEA): an Approach to Classification and Precise Detection of Cancer Biomarker

**Maryam Hariri[1] · Vahid Alivirdiloo[2] · Nastaran Safavi Ardabili[3] · Sarah Gholami[4] · Sajjad Masoumi[5] · Mohammad Reza Mehraban[4] · Mahsa Alem[6,7] · Reza Shah Hosseini[8] · Ahmad Mobed[9,10] · Farhood Ghazi[10] · Iraj Alipourfard[11]**

Accepted: 15 November 2023 / Published online: 6 December 2023


**Abstract**
The precise detection of cancer biomarkers is a principal aspect of effective diagnosis, monitoring, and therapeutics. Carcinoembryonic antigen (CEA) is a protein normally found in very small amounts in the blood of adults. CEA blood levels can be elevated in benign diseases and certain types of cancer. The CEA test is most commonly used to identify a significantly frequent cancer, colorectal cancer. It has decisive clinical value in monitoring, differential diagnosis, disease, and assessment of therapeutic effects. Therefore, it is important to develop a sensitive and simple CEA detection method to diagnose cancer and improve patient survival accurately. Biosensing has great advantages for early disease detection due to its rapid response, high sensitivity, and convenient operating characteristics. Based on several studies, biosensors seem to be new and promising paths in the future of medical oncology. The main purpose of this study is to introduce and discuss the recent nanodiagnostic biosensors developed since 2018. Therefore, the readers of this study will be introduced to the latest biosensors, the various nanomaterials used in them, and their analytical characteristics.

**Keywords** Carcinoembryonic antigen (CEA) · Biosensors · Cancer · Nanodiagnosis · Oncology

## 1 Introduction

As the third most frequent malignancy and the second most fatal cancer, colorectal cancer (CRC) is expected to cause an estimated 1.9 million incidences and 900,000 deaths worldwide in 2020 [1, 2]. The incidence of CRC is high in developed countries and is decreasing in low- and middle-income countries due to Westernization [1, 2]. However, the incidence of early-onset CRC is increasing following the change in diet and environmental factors. For the first time, carcinoembryonic antigen (CEA) was isolated from human

Maryam Hariri and Vahid Alivirdiloo are co-first authors.

✉ Iraj Alipourfard
ialipourfard@ichf.edu.pl

[1] Department of Pathobiology, Auburn University, Auburn, AL 36832, USA

[2] Mazandaran University of Medical Sciences, Ramsar, Iran

[3] Department of Midwifery, Ardabil Branch, Islamic Azad University, Ardabil, Iran

[4] Young Researchers and Ellie Club, Babol Branch, Islamic Azad University, Babol, Iran

[5] Department of Medical Biotechnology, National Institute of Genetic Engineering and Biotechnology, Tehran, Iran

[6] Department of Microbiology, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran

[7] Digestive Disease Research Center, Ardabil University of Medical Sciences, Ardabil, Iran

[8] Faculty of Medicine, Istanbul Medipol University, Istanbul, Turkey

[9] Neuroscience Research Center, Tabriz University of Medical Sciences, Tabriz, Iran

[10] Stem Cell Research Center, Tabriz University of Medical Sciences, Tabriz, Iran

[11] Institute of Physical Chemistry, Polish Academy of Sciences, Marcina Kasprzaka 44/52, 01-224 Warsaw, Poland

CRC tissue by Gold and Freedman in 1965 [1, 2]. CEA is a fetal glycoprotein typically not produced in large quantities after birth and can increase in many diseases. CEA is a protein normally found in very small amounts in the blood of adults [3]. Blood levels of CEA can be elevated in certain types of cancer, such as the breast, lungs, pancreas, stomach, liver, ovaries, and non-cancerous (beneficial) conditions. The CEA test is most commonly used for colon cancer [3, 4]. The normal CEA concentration in healthy people is fewer than 5 ng $L^{-1}$, but serum CEA levels above 20 ng $L^{-1}$ indicate the presence of cancer [5, 6]. After birth, CEA expression would be significantly suppressed, then slightly increase to the normal plasma levels in healthy adults [3, 7]. CEA is generally overexpressed in many cancers, including pancreatic cancers, ovarian, lung, gastric, breast, and, mainly, colon cancer [3, 7]. As one of the critical tumor markers, the identification of CEA is very important in the monitoring, differential diagnosis, treatment surveillance, and prognostic evaluation of the disease. Traditional CEA tests are typically based on the immunoassay methods. However, immunoassays require complex and expensive equipment and specialists to perform. In addition, using radioactive elements in some radioimmunoassay methods can cause certain harm to the human body, limiting their widespread use. Enzyme-linked immunosorbent assay (ELISA) was first introduced by Van Weemen and Schuurs in 1971 and has been widely used in biological analysis for decades [8, 9]. Due to the efficient biocatalysis of the enzyme and the strong and lasting binding due to the specificity of the antibody and antigen, ELISA is decisive for its high sensitivity, fast detection, low cost, easy computerization, and high selectivity [8, 9]. This technique extended applicable advantages to many fields, such as environmental monitoring, clinical diagnosis, and food inspection [9]. In recent years, nanomaterial-based methods, particularly synthetic biosensors, have received much attention due to their excellent selectivity and accuracy, high sensitivity, fast response, and low costs [10, 11]. This review categorizes and briefly describes the advances in electrochemical and optical biosensors for CEA detection and also explains their strengths and weaknesses.

## 2 Carcinoembryonic Antigen (CEA)

Carcinoembryonic antigen (CEA), first named by Gold and Freedman in 1965, is an antigen that appears to be present in both the adenocarcinoma and fetal-derived cancer of the colon [3]. This protein was named CEA because it is merely raised in the cancer and embryonic tissue [3]. CEA is basically a fetal glycoprotein and is usually not produced in large quantities after birth unless there are some growth and development defects. The most determined clinical application of CEA is not rather diagnosis but the monitoring of CRC recurrence [3]. In colorectal cancer, serum CEA stabilizes 6 weeks after the tumor resection. Accordingly, insistently elevated CEA levels may specify residual tumor due to partial resection or recurrence of the tumor. Staging malignancy should be considered if there is insistently rising CEA above baseline, potentially suggesting cancer progression. Preoperative CEA levels of 2.35 ng/mL and above, which are still within the normal range, can be used to recognize patients with a poor diagnosis of stage I and II colorectal cancer [12].

As shown in Table 1, the rise of antigen level is in the range of nanograms and requires appropriate techniques for accurate detection. Due to the limitations and disadvantages of routine techniques, the development of nanomaterial-based methods has been considered by researchers in recent years, one of the most important of which is biosensor development.

## 3 Methods and Mechanisms in the Detection of CEA

Besides being an important biomarker in tumor detection, CEA is also important in non-malignant disorders, specifically pancreatitis, hepatitis, renal failure, and intestinal diseases. Consequently, the detection of CEA is of great importance in the identification of various diseases in the medical field. For that reason, quantitative detection of low concentrations of tumor markers such as CEA is critical for the early diagnosis of cancers [13, 14]. A variety of immunoassay methods have been advanced for detecting CEA in serum, such as radioimmunoassays, enzyme-linked immunosorbent assays (ELISA), fluorescence immunoassays, chemiluminescence immunoassays, and amperometric immunoassays. However, a common problem of these testing approaches is that multiple washing steps are required. These repeated washing steps can increase measurement errors, reducing efficiency while demanding complex instrumentation. An immunoassay is an assessment that relies on biochemistry to measure the presence and/or concentration of an analyte. The analyte can be large antibodies, proteins that an individual has produced due to an infection, or small

**Table 1** The rise of CEA levels in some chronic and cancer diseases

| Diseases | CEA level | Ref |
|---|---|---|
| Colorectal cancer | 11.8 (9.8–17.0) (ng/mL) | [13] |
| Lung cancer | 16.9 (11.1–24.2) (ng/mL) | [14] |
| Diabetes | 6.3 (5.4–7.6) (ng/mL) | [15] |
| Diabetes and colonic polyp | 7.5 (5.8–11.5) (ng/mL) | [16] |
| COPD* or inflammatory lesion in the lung | 5.7 (5.5–7.0) (ng/mL) | [16] |

*COPD**, chronic obstructive pulmonary disease

molecules. For example, ELISA testing relies on the inherent ability of antibodies to bind to a specific structure of a molecule. To optimize the ELISA and achieve the required sensitivity and dynamic range for the specific assay being developed, all of the different components of the assay must be evaluated [15, 16]. The components vary depending on the immunoassay format chosen. A schematic illustration of antigen detection mechanisms in ELISA and similar methods is presented in Fig. 1. In the recent decade, researchers have focused on developing sensitive biosensors that depend on the antigen-antibody interaction as its specific affinity-based recognition approach and detect tumor biomarkers with high sensitivity.

## 4 What Is Biosensor Technology?

Conventional biomarker detection techniques require skilled personnel, dedicated laboratory space, laboratory-based equipment, and time invested [11]. Detection techniques based on microscopy, cell culture, proteomics, or molecular biology are very sensitive but can take several days to detect the analysts [17]. These technologies and equipment are too large to be shipped to remote locations and unreliable for emergency detection [18]. Today, there is a need for portable devices and detection methods that can be used for rapid and sensitive detection. Biosensors are analytical devices that can detect a nanoscale of biomarkers with the support of nanomaterials and surface engineering [19]. The International Union of Pure and Applied Chemistry (IUPAC) terms a biosensor as: "A tool that uses specific biochemical reactions mediated by isolated whole cells, immune systems, enzymes, tissues, and organelles or to detect chemical complexes generally by optical, electrical and thermal signals" [19, 20]. Biosensors typically consist of biological components, such as molecular recognition elements and physicochemical detector components or transducers [20, 21]. The recognition element of the biosensor is immobilized on the surface of the transducer and can interact with the target molecule without adding reagents to the sample solution. A schematic illustration of biosensors working principals and detection mechanisms is presented in Fig. 2 [21, 22].

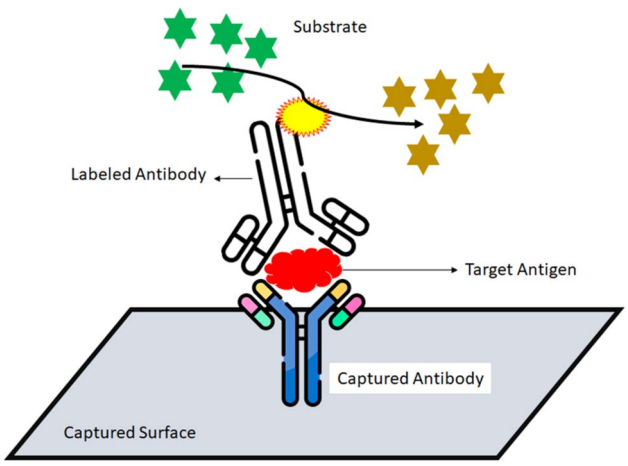


**Fig. 1** Schematic illustration of antigen detection mechanisms in ELISA and similar methods

Biosensors that allow sample analysis for the existence of a specific compound are called sensors. Sensors that use biological materials to interact with analytes specifically are called biosensors [23, 24]. Analyte refers to the compound that must be "detected" or whose presence must be determined. The interaction between the analyte and the biosensor is measured and converted into a signal, which in turn is amplified and displayed [23, 24]. Therefore, biosensing involves converting a chemical information stream into an electrical signal. The biological materials used in biosensors are mainly enzymes, antibodies, nucleic acids, lectins, whole cells, etc. [23, 24]. Hormones, antibodies, enzymes, nucleic acids, tissues, and whole cell structures can be used in biosensors as biological components [25]. The biological part can interact specifically with the target analyte and the output of the biochemical reaction is converted into a quantifiable signal by the transducer [25, 26]. Transducer systems can be optical, electrochemical, piezoelectric, magnetic, thermometric, ion-sensitive, and acoustic [27, 28]. An important part of biosensor manufacturing is the immobilization of bio-components. The performance of an immobilized molecular biosensor also depends on factors such as physical conditions (pH, impurities, temperature), chemistry, stability, and material thickness [28, 29]. Several biosensors have been developed that can detect a wide range of biomarkers associated with different types of cancer.

Nanomaterials have recently attracted much interest due to the increasing requirement to control desirable molecules present in the human body and the environment. Nanomaterials include nanoparticles (NPs) with dimensions less than 100 nm in at least one dimension [30]. The term "nanotechnology" refers to small materials when their size is less than a nanometer or several hundred nanometers [31, 32]. The controlled synthesis and modification of the properties of nanomaterials require knowledge of different disciplines such as chemistry, physics, electronics, computer science, biology, engineering, and agriculture, which may lead to the emergence of new and multifunctional nanotechnologies [30, 31]. Among, biosensors are one of the most widely developed nanomaterial-based technologies. According to several studies, nanomaterials in biosensor structures play a critical role in sensitivity stability augmentations [30, 31, 33]. Due to nanomaterials' importance in biosensor development, used nanocomposites are introduced in the next section and summarized in Table 2.

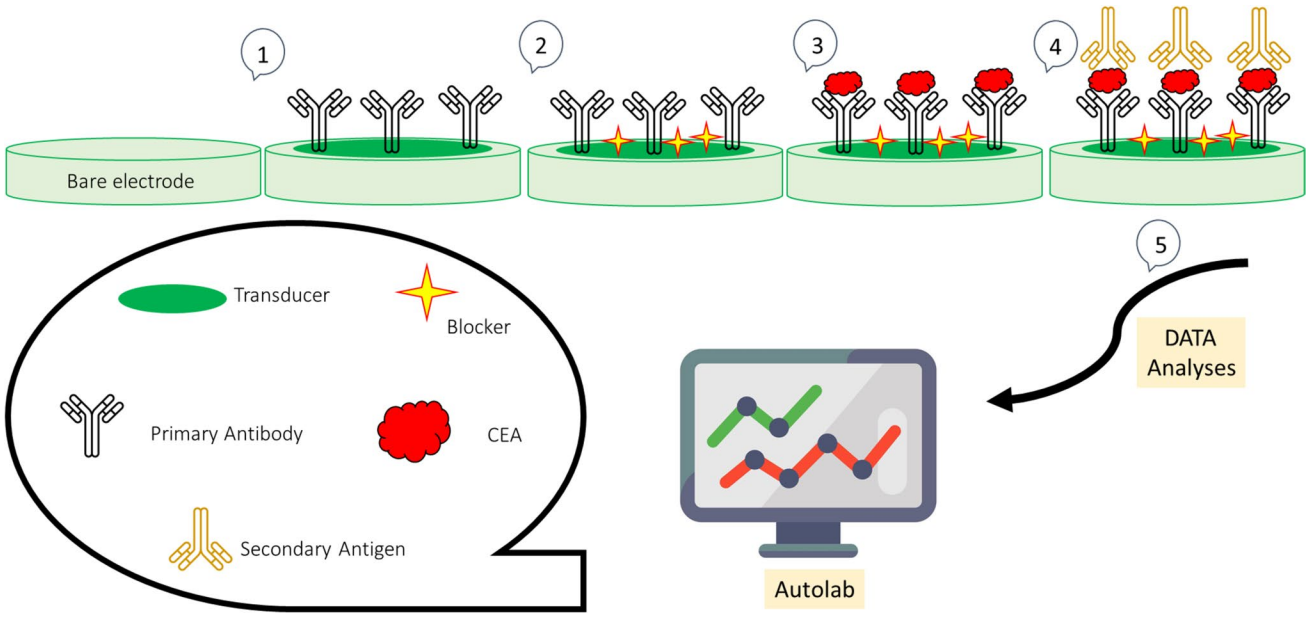


**Fig. 2** Schematic illustration of biosensor technology

## 5 Recently Developed Biosensors for the Detection of CEA

### 5.1 Electrochemical Biosensors

Electrochemical biosensors have seen encouraging progress in clinical diagnostics, food processing quality control, and environmental monitoring [96, 97]. Specifically, nano-inspired electrochemical biosensors for monitoring biomolecular interactions occurring at the label-free nanoscale have enabled reagent-free, non-invasive, in situ measurements of on-site, and online parameters of interest in different mediums or environments [96, 97]. In a new approach, a well-organized three-dimensional (3D) DNA-based nanosystem was self-assembled by label-free DNA nanotweezers and used as a probe to interact with the target. When the target molecule is identified by the 3D DNA nanoprobe (3DDNT), the DNA nanotweezers start to emit the target correspondent (T1). This platform enables CEA's sensitive and convenient detection and provides a reference for further manufacturing 3D DNA nanotweezer-based electrochemical biosensors [34]. Presently, CEA detection methods are based totally on antigen-antibody binding, and these techniques are restrained due to the excessive fee and long latency in tumor screening. In the experiment of DNA nanotweezer-based biosensors, adjustments inside the secondary shape of fluorescently modified guanine-rich DNA dramatically modified the fluorescence signal [35]. This technique is still being investigated to provide a fast, simple, inexpensive sensor platform for detecting biomarkers. It has great potential for a group screening of tumors and is expected to be very potent in tumor treatment [35]. Within the research examination, as a dual reactant of the CdS quantum dots loaded into the organic steel framework (CdS QDs-MOF) and triethanolamine changed to gold nanoparticles (TEOA-Ru (bpy) three 2 + ECL) gadgets become mounted for sensitive detection of CEA. Based on this result, a label-free ECL biosensor changed into an efficient and advanced device and used to detect CEA with an appropriate LOD. The proposed ECL platform (Fig. 3) has shown brilliant analytical overall performance in detecting CEA in human serum samples (Fig. 3) [36].

Single-walled carbon nanotubes (SWCNTs) can be formed as single long coiled graphene sheets. Nanotubes are generally considered to be a nearly one-dimensional structure because they have a length-to-diameter ratio of about 1000. SWCNTs are generally close to 1 nm in diameter and are thousands of times longer [98]. In recent research, nanocomposites of chitosan (CS) and carbon nanoonions (CNOs), gold nanoparticles (AuNPs), single-walled carbon nanotubes (SWCNTs), (AuNPs/CNOs/SWCNTs/CS) are organized to develop an ultra-sensitivity electrochemical immuno platform for CEA detection [37]. A label-free and double recognition-amplification (LDRA) plan was proposed for CEA determination, based on a newly designed dual-function messenger probe (DMP) combined with hybridization chain reaction (HCR) and DNA tetrahedron probes (DTPs). The use of DNA tetrahedral probes improved the possibility of hybridization and reduced background signals [99]. A new sandwich immunosensor has been introduced based on the surface-enhanced Raman scattering (SERS) method.

**Table 2** Recently developed biosensors for CEA detection

| Transducer | Techniques | Matrix | Nanocomposite | Linear range | LOD/LLOQ | Ref |
|---|---|---|---|---|---|---|
| Electrochemical | Aptasensor/CVs, EIS | Serum | Nanotweezers | 1 µM–500 nM | 4.88 fg $mL^{-1}$ | [34] |
| Optical | Fluorescence detection | Blood and serum | _ | 1 to 30 ng/mL | 0.961 ng/mL | [35] |
| ECL | Aptasensor/CVs, EIS | Serum | TEOA-AuNPs | $1.0 \times 10^{-4}$~10 | $8.5 \times 10^{-5}$ | [36] |
| Electrochemical | CV/SWV | Real samples | AuNPs/CNOs/SWCNTs/CS | 0.0001–400 ng $mL^{-1}$ | 100 fg $mL^{-1}$ | [37] |
| Electrochemical | CV/DPV | Real clinical samples | Magnetic nanoparticles (MB) | 0.0001–50 ng $mL^{-1}$ | 18.2 fg $mL^{-1}$ | [38] |
| SERS | Sandwich type immunosensor | Plasma | $MoS_2$ NFs-Au NPs/MBA | 0.0001–100.0 ng $mL^{-1}$ | 0.033 pg $mL^{-1}$ | [39] |
| Electrochemical | Immunosensor/LSV | Clinical | PTh-Au | 0.1–120 ng/mL | 0.055 ng/mL | [40] |
| Electrochemical | CV/DPV | Human serum | AuNPs | 0.05–20 ng $mL^{-1}$ | 0.01 ng $mL^{-1}$ | [41] |
| Optical | FRET | Human serum | GO | 0.03–6 ng/mL | 7.9 pg/mL | [42] |
| Electrochemical | Immunosensor/EIS | _ | Polypyrrole (PPy)/AuNPs | $10^{-10}$ to $10^{-6}$ g/mL | 0.033 ng/mL | [43] |
| Electrochemical | Immunosensor | Human serum | PPI/GO/GCE | 0.001 to 2000 ng/mL | 0.3 pg/mL | [44] |
| Electrochemical | Genosensor | Human serum | MOFs | _ | 0.63 fg/mL | [45] |
| Electrochemical | CEA-aptasensor | Human serum | Ng-PCL | 5 pg/mL and 200 ng/mL | 0.675 pg/mL | [46] |
| Electrochemical | CEA-aptasensor | Biological | MOF/rGO | 0.0025–0.25 ng $L^{-1}$ | 0.8 pg $L^{-1}$ | [47] |
| Electrochemical | Immunosensor | Biological | CuFe PBA/MoS2 | 0.23 µM and 0.01 ng $mL^{-1}$ | 0.01 ng $mL^{-1}$ | [48] |
| Electrochemical | Immunosensor | Biological | AuNPs-rGO | 0.001–500 ng $mL^{-1}$ | 0.35 pg $mL^{-1}$ | [49] |
| Electrochemical | Immunosensor | Human serum | L-Cys-Fc-Ru-$Ab_2$ | 1.0 pg $mL^{-1}$ to 100.0 ng $mL^{-1}$ | 0.5 pg $mL^{-1}$ | [50] |
| Electrochemical | Immunosensor | Human serum | β-homoserine | .0 fg $mL^{-1}$–1.0 ng $mL^{-1}$ | 0.33 fg $mL^{-1}$ | [51] |
| Electrochemical | Aptasensor | Human serum | MBs | _ | fg $mL^{-1}$ level | [52] |
| Electrochemical | Microfluidic immunosensor | Human serum | MNPs | 100 pg $mL^{-1}$ to 100 ng $mL^{-1}$ | 14.347 pg $mL^{-1}$ | [53] |
| ECL | Electrochemiluminescent | Human serum | SA-AuNPs | 0.1 to 5 pg $mL^{-1}$ | 58 fg $mL^{-1}$ | [54] |
| Electrochemical | Immunosensor | Human serum | Au NPs/PB-PANI | 1.0 pg $mL^{-1}$ to 100.0 ng $mL^{-1}$ | 0.35 pg $mL^{-1}$ | [55] |
| Electrochemical | Immunosensor | Human serum | Cu–CN/SPCE | 0.01 to 50 ng $mL^{-1}$ | 2.4 pg $mL^{-1}$ | [56] |
| ECL | Immunosensor | Human blood samples | AuNPs-Ir-Zr-MOL | .00 pg $mL^{-1}$ to 100 ng $mL^{-1}$ | 0.200 pg $mL^{-1}$ | [57] |
| Optical | Fluorescence spectra | Human serum | MSNs | 1 fg mL−1 to 0.1 ng mL−1 | 0.7 fg mL−1 | [58] |
| Optical | Fluorescence/CHA/HCR | Real samples | _ | 1 pg $mL^{-1}$–2 ng $mL^{-1}$ | 0.3 pg $mL^{-1}$ | [59] |
| Optical | SPR | Biological | AuNPs | 0.40 and 20 ng $mL^{-1}$ | 0.12 ng $mL^{-1}$ | [60] |
| Optical | SPR | Blood plasma | Bio-AuNPs | 750 mM | 17.8 pg/mL | [61] |
| Electrochemical | TDNs-CHA/DPV | Clinical | AuNPs | 1 to 30000 pg $mL^{-1}$ | 0.04567 pg $mL^{-1}$ | [62] |
| Optical | CL | Human serum | NS-CDs/Au-AgNPs | 0.3–80 ng $mL^{-1}$ | 94 pg $mL^{-1}$ | [63] |
| Electrochemistry | DPV/aptasensor | Human serum | _ | 10 pg $mL^{-1}$ to 100 ng $mL^{-1}$ | 0.84 pg $mL^{-1}$ | [64] |
| Optical | Fluorescence turn-on | Human serum | Cu/UiO-66 | 0.01–0.3 ng $mL^{-1}$ | 0.01 ng $mL^{-1}$ | [65] |
| Optical | Multi-color visual sensing | Human serum | $Ag_2S$ NPs-ZnO | 0.1–20 ng/mL | 0.05 ng/mL | [66] |
| Electrochemical | Immunosensor/CV | Blood | Polyoctopamine | 1 fM–100 nM | 11.76 fM | [67] |
| Electrochemical | Immunosensor/DPV | Human serum | PANI-HA | 0.01 to 10,000 pg $mL^{-1}$ | 0.0075 pg $mL^{-1}$ | [68] |
| Optical | FT-IR, UV-vis | Clinical | g-$C_3N_4$/CdSe | 10 ng $mL^{-1}$–100 µg $mL^{-1}$ | 0.21 ng $mL^{-1}$ | [69] |

**Table 2** (continued)

| Transducer | Techniques | Matrix | Nanocomposite | Linear range | LOD/LLOQ | Ref |
|---|---|---|---|---|---|---|
| Electrochemical | CVs, ChA | Human serum | MWCNTs-CoP | $10^{-4}$ to 10 ng $mL^{-1}$ | 12 fg $mL^{-1}$ | [70] |
| Optical | Colorimetric | Serum | - | - | 24.8 ng/mL | [71] |
| Optical | Colorimetric aptasensing | Clinical | AuNPs | 4 to 25 ng $mL^{-1}$ | 0.19 ng $mL^{-1}$ | [72] |
| Optical | Fluorescent/aptasensor | Serum | β-cyclodextrin/MBs | $1.0 \times 10^{-15}$–$1.0 \times 10^{-7}$ g $mL^{-1}$ | 6.76 ag $mL^{-1}$ | [73] |
| Optical | Colorimetric/ | Human serum | $CuNFs_2$-BiotinNHSAb2 | 0.05–40 ng/mL | 3.52 pg/mL | [74] |
| Optical | UV-vis spectra | Real samples | CuNFs2-$MoS_2$-AuNPs | 10 fg $mL^{-1}$–1 ng $mL^{-1}$ | 1.2 fg mL−1 | [75] |
| Optical | Aptasensor/labeled/DPV | Serum | ConA/HRP | 5 to 40 ng $mL^{-1}$ | 3.4 ng $mL^{-1}$ | [76] |
| Electrochemical | CV/EIS | Serum | rGO | 0.1–5 ng $mL^{-1}$ | 0.05 ng $mL^{-1}$ | [77] |
| Optical | Aptasensor/fluorescence | Serum | AuNPs | 0.01 to 9.0 ng/$mL^{-1}$ | 7.5 pg $mL^{-1}$ | [78] |
| Electrochemical | Amperometry | Serum | SPCE/NanoCaptors/JNP3 | 1–5000 ng $mL^{-1}$ | 210 pg $mL^{-1}$ | [79] |
| Optical | Fluorometric immunodevice | Serum | QDs | 1.0–40 ng $mL^{-1}$ | 0.3 ng mL−1 | [80] |
| Optical | Immunosensor | Tumor | RENPs | 1 to 320 ng $mL^{-1}$ | 0.37 ng $mL^{-1}$ | [81] |
| Optical | SPR | Serum | MWCNTs-AgNPs | 0.0002–20000 pM | 0.07 fM | [82] |
| Electrochemical | Immunosensor | Serum | GO/AgNPs | 100 fg/mL to 5 pg/mL | 75 fg/mL | [83] |
| ECL | CVs/EIS | Serum | GR-IL-Pt | 0.1 pg $mL^{-1}$–10 ng $mL^{-1}$ | 34.58 fg $mL^{-1}$ | [84] |
| Optical | SERS | Complex | AuNSs | 1 to 1000 ng/mL | 1.0 ng/mL | [85] |
| Optical | CVs/EIS | Serum | N, S-GQDs-Au-PANI | 0.5 to 1000 ng $mL^{-1}$ | 0.01 ng $mL^{-1}$ | [86] |
| Optical | Aptasensor/nanoprobe | Complex | $Fe_3O_4$-AuNPs | 0.01–1 nM | 0.6 ng/mL | [87] |
| ECL | Aptasensor | Serum | MOL | 1 fg/mL to 1 ng/mL | 0.63 fg/mL | [88] |
| Optical | Immunosensor | Serum | PB-PDA | 0.001–100 ng $mL^{-1}$ | 54.9 fg $mL^{-1}$ | [89] |
| Optical | Immunoassay | Serum | $BiOCl/CuBi_2O_4$ | 0.01–40 ng $mL^{-1}$ | 3.5 pg $mL^{-1}$ | [90] |
| Optical | Immunoassay | Serum | $FeO_x$-C-CS/CEA/AuNCs-keratin-BA | 12.5 fg $mL^{-1}$–37.5 pg $mL^{-1}$ | 4.3 fg $mL^{-1}$ | [91] |
| QCM | Immunoassay | Serum | GO-AuNPs | 0.1 to 120 ng $mL^{-1}$ | 0.09 ng $mL^{-1}$ | [92] |
| QCM | SAW | Serum | AuNPs | 0.1 to 30 ng/mL | 0.31 ng/mL | [93] |
| QCM | UV-vis absorption spectra | Serum | HRP NPs | f 0.01–100 ng $mL^{-1}$ | 7.8 pg $mL^{-1}$ | [94] |
| High-electron-mobility transistors | _ | Serum | AlGaN/GaN | | 1 fg $mL^{-1}$ | [95] |

*MSNs*, mesoporous silica nanoparticles; *TDNs*, tetrahedral DNA nanostructures; *CHA*, catalytic hairpin assembly; *CL*, chemiluminescence; *PANI-HA*, polyaniline nanowires, hyaluronic acid; *TEOA-AuNPs*, triethanolamine modified on gold nanoparticles; *GR-IL-Pt*, graphene-ionic liquid platinum; *ECL*, electrochemiluminescence; *MOL*, metal-organic layer; *SAW*, surface acoustic wave; *RENPs*, rare earth doped nanoparticles; *Ng-PCL*, naringin initiated poly(ε-caprolactone) polymer

This includes $MoS_2$ nanoflowers with Au nanoparticles ($MoS_2$-NFs-Au-NPs/MBA) labeled with 4-mercaptobenzoic acid as CEASERS tags. $Fe_3O_4$ with Au nanoparticles functionalized by delamination $Ti_3C_2Tx$. ($Fe_3O_4$ NPs-Au NPs)/$dTi_3C_2TX$ MXene) is used as a magnetic SERS carrier substrate for detecting CEA (Fig. 4) [39].

A new electrochemical gold-labeled silver-spot immunosensor, polythionin gold nanoparticles (PThAu), with a modified glassy carbon electrode (GCE) as a platform, and a secondary antibody-labeled Au NP as an immunoassay was engineered for CEA detection based on Ab2Au. This immunoassay device has the advantage of excellent reproducibility, stability, and selectivity. Therefore, this immunodetection protocol is a potential candidate for clinical use [40]. Another sandwich-type electrochemical immunosensor was assembled and recently verified. In contrast to other sensors that use exterior electrochemical species in the electrolyte to produce electrochemical signals, ferrocene derivatives are incorporated into the sensor design, allowing CEA detection in biological and buffer samples. In this biosensor, AuNP

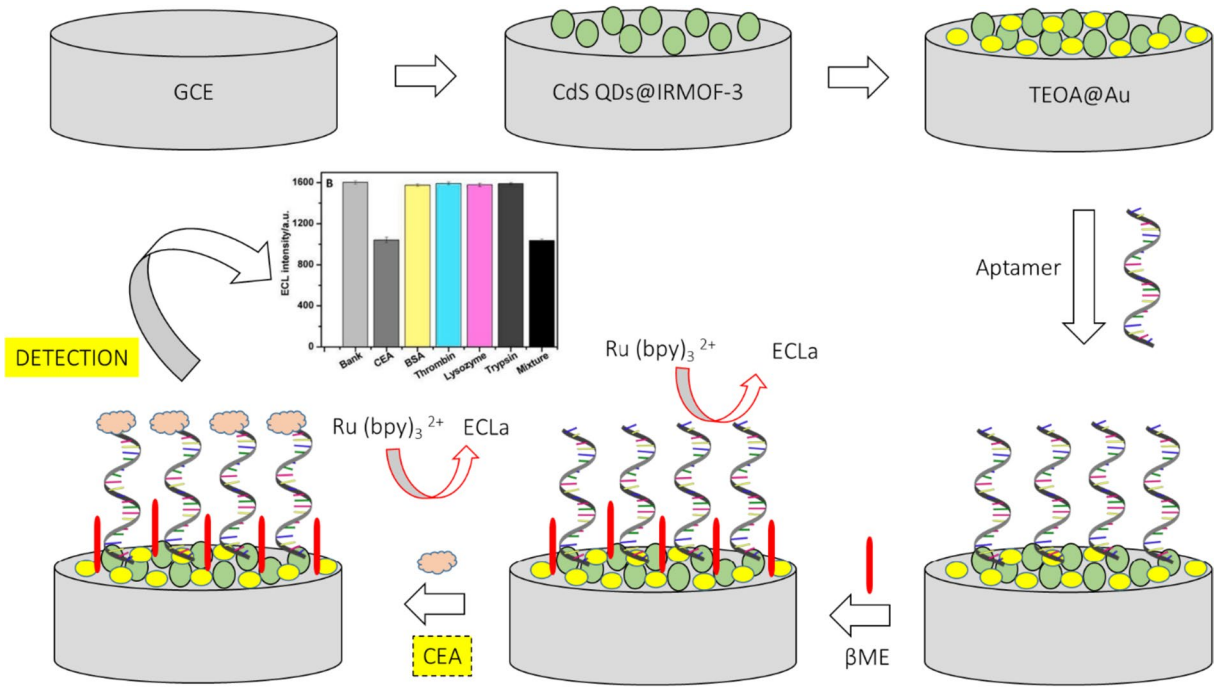


**Fig. 3** Schematic illustration of aptasensor construction, adapted from Ref (36)

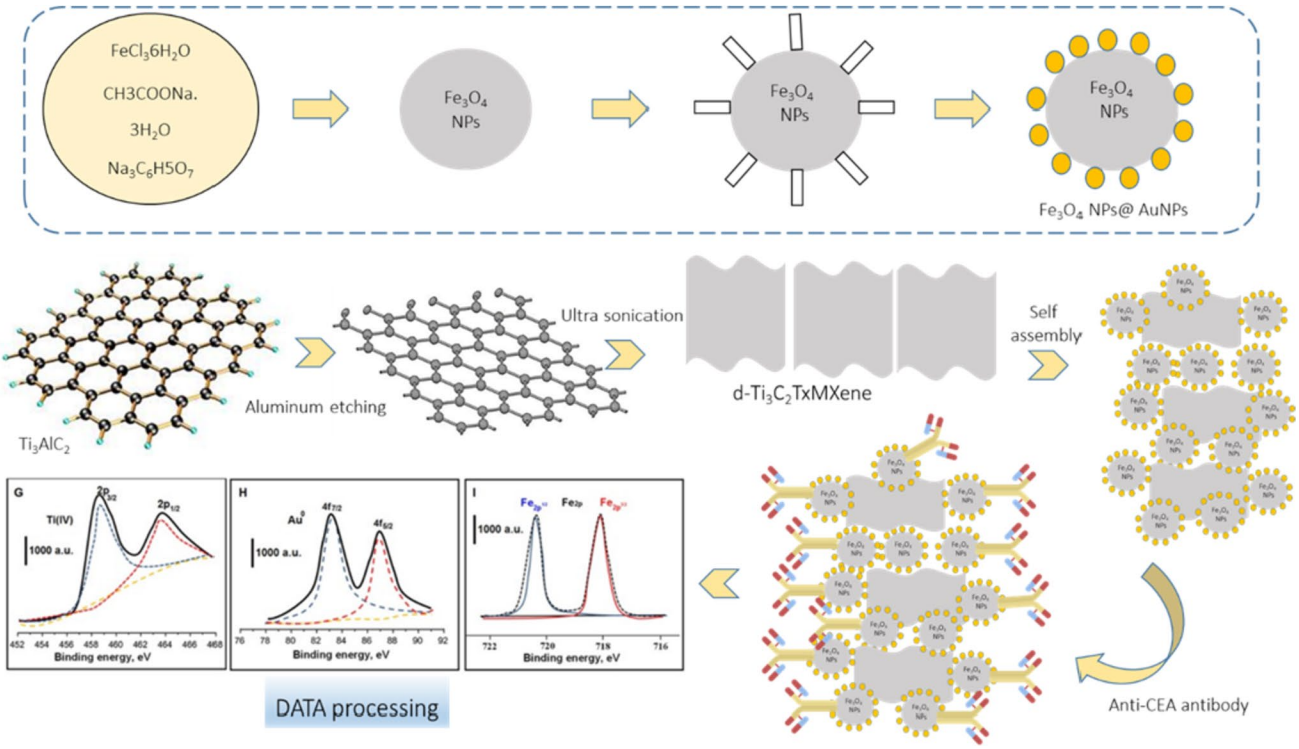


**Fig. 4** Prepared sandwich-type immunoassay for CEA detection, adapted from Ref (39)

is used to improve the conductivity of the sensor surface and contains immobilized secondary anti-CEA and ferrocene derivatives [41]. An ultra-sensitive uniform adapter sensor has been developed based on fluorescence resonance energy transfer (FRET) between graphene oxide (GO) and up-conversion nanoparticles (UCNP). Aptasensor is able to

monitor CEA levels in human serum directly, and the results are strongly comparable with commercially available chemiluminescent kits. The achieved analytical characteristics of this biosensor are promising for future clinical applications [42]. A flexible free-standing electrochemical immunosensor for the determination of CEA has been designed based on a conducting polypyrrole (PPy) nanocomposite film electrode. In this CEA biosensor, gold nanoparticles (AuNPs) are immobilized on a PPy composite film by electrodeposition and self-assembled to attach to the CEA aptamer. It can build a self-contained electrochemical biosensor that separates from additional soft substrates and current collectors (Fig. 5) [43].

Fluorescent resonance energy transfer (FRET) between two molecules is a critical physical phenomenon and is imperative for understanding some biological systems. It can be applied in the development of thin film and optoelectronics devices. Applying FRET technology to a light microscope can determine an approach between two molecules within a few nanometers [100]. An innovative ultra-sensitive immunosensor was created for the rapid detection of CEA based on a GO nanocomposite and polypropylene-imine-dendrimer (PPI) on GCE (PPI/GO/GCE) [44]. Metal-organic frameworks (MOFs) were employed for CEA biosensor construction. The developed sensor showed rapid operation and acceptable sensitivity [45]. Label-free ELC biosensor based on Ng-PCL polymer signal amplification was settled for the detection of CEA. Good stability, satisfactory reproducibility, and high specificity are important reported features. Thus, this strategy will offer a novel path for the detection of other biomarkers in early clinical diagnosis [46]. GNP-modified metal-organic framework/reduced graphene oxide (MOF/rGO) hybrid was applied to propose a novel nanosensor for CEA quantification in biological samples [47]. A new ECL biosensor based on CuFe PBA/$MoS_2$ nanocomposites was fabricated for constant and sensitive detection of CEA. The high sensitivity of this worldwide sensor offers an original approach for simultaneously detecting multiple cancer biomarkers [48]. AuNPs modified reduced graphene oxide nanosheets (AuNPs-rGO) based dual-quencher for highly sensitive detection of CEA [49] and NPs as the substrate to ensure accurate capture of primary antibody (Ab1), l-cysteine-ferrocene-ruthenium nanocomposites (L-Cys-Fc-Ru) to immobilize secondary antibody (Ab2) and a signal-on sandwich-like immunosensor was created for detection of CEA [50]. Anti-enzymolysis peptides conjugated with anti-fouling silk sericin-inspired beta-homoserine were settled for ELC detection of CEA in human serum. The planned biosensor was able to correctly assay CEA in clinical serum samples when compared with the frequently approved ELISA method, which shows promising potential for practical applications [51]. A novel immobilization-free ELC homogeneous genosensor was engineered for highly sensitive detection of CEA by a dual amplification approach. The developed biosensor has the potential for commercial applications and the creation of other target boards in the future [52]. A microfluidic immunosensor for spontaneous determination of CEA was based on droplet arrays and

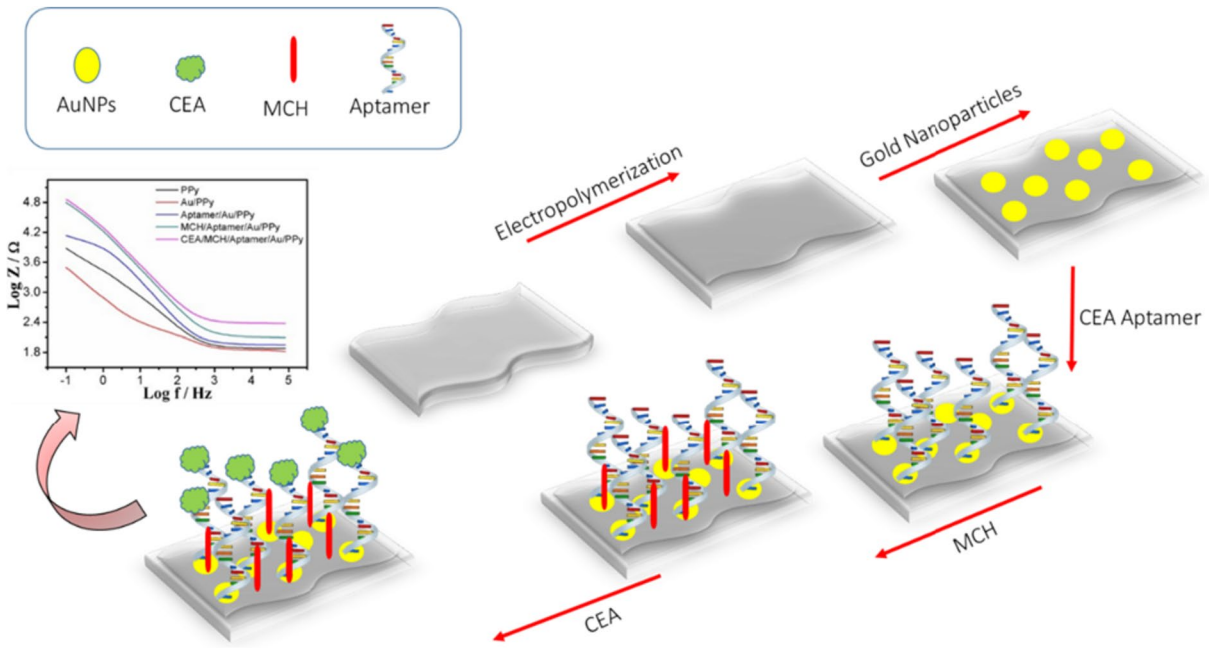


**Fig. 5** Representation of the process of constructing a self-supporting electrochemical biosensor based on PPy composite film, adapted from Ref (43)

immunomagnetic separation. The developed platform has the benefits of automation, cost-effectiveness, high throughput, low sample consumption, and encouraging applications in biochemistry [53]. ELC immunosensor for rapid determination of CEA using luminol-coated AgNPs was fabricated. The created biosystem showed acceptable sensitivity and can be considered for the detection and checking of CEA in the clinic [54]. AuNPs dotted Prussian blue-polyaniline core-shell nanocubes (Au NPs/PB-PANI) were fabricated for sensitive detection of CEA. The planned sensor showed a suitable result when the core-shell structure nanoprobe-based immunosensor was functional to determine CEA in real human serum samples [55]. An electrochemical biosensor based on an atomically Cu-dispersed nitrogen-doped carbon electrode (Cu-CN/SPCE) for screening CEA. The developed system was promising the future progress of electrochemical immunoassay in the field of tumor biomarkers [56]. An ECL immunosensor based on functionalized metal-organic layers (MOLs) as a reporter for sensitive detection of CEA [57].

### 5.2 Optical Biosensors

Optical biosensors provide distinct advantages over old-style analytical methods in providing label-free real-time detection of biological and chemical materials in a highly sensitive, specific, and low-cost manner. The benefits include small size, high specificity, sensitivity, and cost-effectiveness [101, 102]. Optical biosensing is advanced and complemented by multidisciplinary methods like microelectromechanical systems (MEMSs), microelectronics, micro/nanotechnologies, molecular biology, biotechnology, and chemistry [101, 102]. A potential rapid and sensitive detection method is based on the fluorescence assay through a combination of internal filter effect (IFE) and fluorescence resonance energy transfer (FRET). Due to their exclusive porous structure and large specific surface area, mesoporous silica nanoparticles (MSNs) can load a huge number of CdTe quantum dots (QDs) [58]. The fluorescent aptamer used in optical biosensors can match CEA and run a cascade nucleic acid amplification, which is induced by a combination of hybridization chain reaction (HCR) and catalytic hairpin assembly (CHA). In this method, a specifically planned single-stranded DNA (ssDNA) containing a CEA-specific aptamer sequence (AS) is hybridized with an inhibitor strand (IS) to form double-stranded DNA (dsDNA) (IS-AS) [59]. In addition, the biosensors developed using surface plasmon resonance (SPR) have been shown as sophisticated and advanced label-free optical biosensing tools. SPR optical biosensors are influential detection tools with extensive applications in biotechnology, environmental protection, food safety, medical diagnostics, and drug screening [103]. Nanoparticles have important roles in enhancing the sensor response of SPR-based biosensors while recognizing specific biological molecules [104, 105]. The functionalization process of these nanoparticles is very important as they affect their interaction with the environment and determine their applicability for the detection of biomolecules in complex matrices [104, 105]. The surface plasmon resonance immunosensor (SPRi) comprises a cysteamine linker attached to a gold chip and a mouse monoclonal anti-CEA antibody engineered according to the "EDC/NHS protocol." The association linearity regarding CEA measurements in both cancer and healthy subjects was acceptable for SPRi immune biosensors (Fig.6) [60].

To specifically measure CEA, biofunctional nanoparticles were developed with a surface bound to a certain amount of peptide (antibody or streptavidin against CEA). One study dedicated to the specificity of sensor response, colloidal stability, and zeta potential of included bio-functional nanoparticles and considered the reproducibility of SPRs [61]. Recently, a new electrochemical platform based on tetrahedral DNA nanostructures and a catalytic hairpin assembly was advanced for CEA detection. This projected genosensor has proven to be simple and fast with good portability, low cost, high stability, and specificity [62]. A further developed unique chemiluminescence (CL) technique co-doped with sulfur carbon dots, $H_2O_2$, and nitrogen using functional HRP-Au-Ag for signal enhancement. In this method, complementary DNA and horseradish peroxidase (HRP) were co-immobilized on the surface of Au-Ag NPs to form a functional nanoprobe (HRP-Au-Ag-cDNA) for signal amplification. In addition, an effective application of this optical biosensor for measuring CEA in human serum samples has been achieved, promising rapid tumor detection and treatment monitoring [63]. An electrochemical aptasensor designed for ultrasensitive determination of CEA based on HCR and exonuclease III dual signal amplification. This biosensor showed excellent analytical performance with a wide linear range and acceptable LOD. Therefore, this sophisticated sensor offers a new and easy way to detect CEA and early stages of cancer [64]. In another biosensor, Cu/UiO-66 was used as a bimetallic organic framework combined with a "fluorescence turn-on" technique for a susceptible detection of CEA. This analytical system offers excellent reproducibility, selectivity, and serum applicability within a novel platform for direct detection of diagnostic markers [65]. A novel multicolor visual immunoassay system was settled for visual inspection of CEA based on $Ag_2S$ NPs-ZnO NTs and observation of color changes in PANI/PB. This optical sensor carrier also provides new ideas for the visual design of wearable platforms in clinical diagnostics [66]. Polyoctopamine (POct), an amine-functionalized non-conductive polymer, has been provided as a transducer layer for electrochemical biosensors. This polymer provides a multipurpose covalent bond via either a thiol-linker bond, an aldehyde, or a carboxyl functional

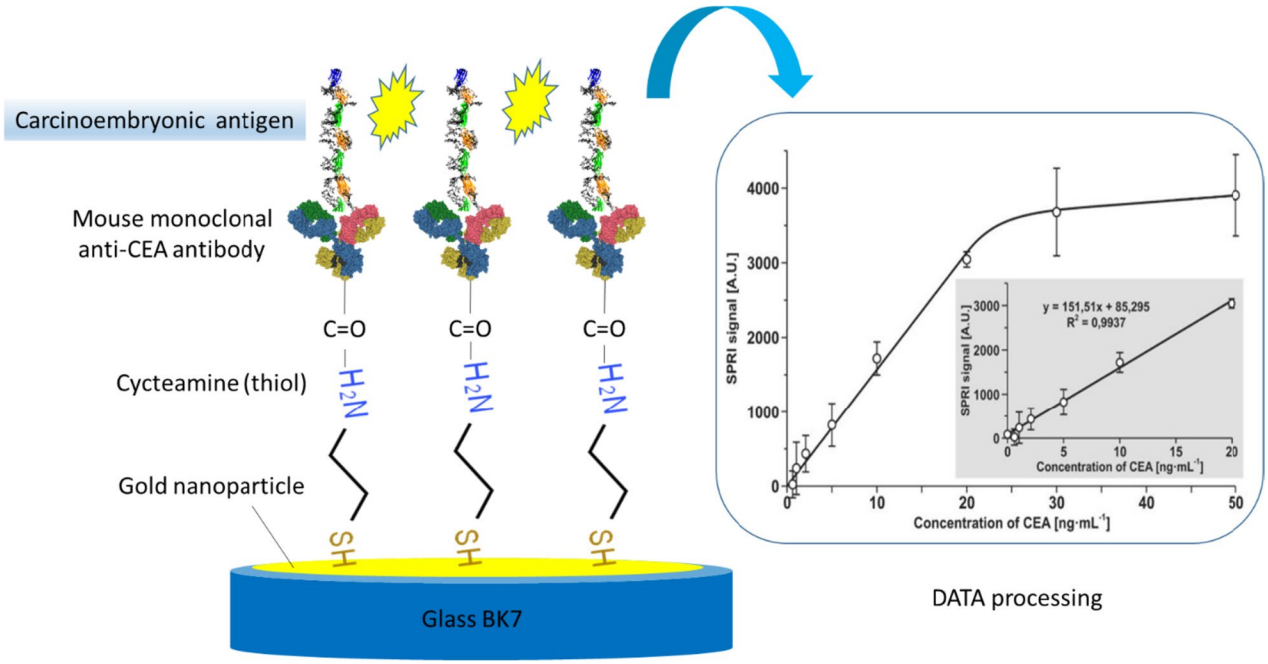


**Fig. 6** SPR-based biosensor for detection of CEA, adapted of Ref (60)

group without needing post-surface or pre-surface activation [67]. All electrochemical assessments can be performed in a short time with a small amount of sample. Therefore, POct facilitates the manufacture of impedance biosensors using amine functionalization technology. This increases sensitivity, enables label-free detection, and reduces response time [67]. A novel electrochemical immunosensor is designated to detect CEA based on composite wires, including hyaluronic acid (HA), onto polyaniline (PANI) nanowires. In this approach, coating the electrode with HA can significantly reduce the non-specific adsorption of proteins on the electrode surface and improve sensitivity and selectivity [68]. Recently, a label-free photoelectrochemical immunosensor based on gC3N4/CdSe nanocomposites was used to identify CEA. These immune sensors provide suitable detection devices for sensing other protein targets that are promising for early diagnosis (Fig. 7) [69].

In a pilot study, a dual-signal electrochemical immunosensor was fabricated using multi-walled carbon nanotubes (MWCNTs) and cobalt phosphate (CoP) as the electrocatalytic label. MWCNTsCoP showed excellent electrode-catalyst activity for bulk water separation in neutral media. The dual-signal immunosensor using MWCNTsCoP as an electrode-catalyzed label can exhibit good detection limit, wide linearity range, acceptable stability, and excellent selectivity for CEA detection (Fig. 8) [70].

A colorimetric aptasensing method has been launched with modifiable color mutants [71]. The clinical diagnosis showed both high sensitivity and specificity. However, the targeting aptamer can be easily modified; this platform also offers the possibility of threshold detection for other disease markers [72]. An experiment for the first time described a highly sensitive and accurate biosensor for CEA identification based on atom transfer radical polymerization (ATRP) using 2-bromoisobutyryl bromide (BIBB) as macroinitiators and β-cyclodextrin (βCD). Fluorescent biosensors are basically excellent analytical tools in physiological fluids analysis. This recommended biosensor has an outstanding anti-interference property, which has great potential in the field of bioanalytical applications [73]. The bifunctional chicken egg white-copper phosphate is a hybrid with organic-inorganic nanoflower (CuNF), can combine signal amplification and biological recognition, and produces a simple one-pot method. CuNF exhibits exceptional biocatalytic activity of polyphenol oxidase and peroxidase. In addition, the interaction between the protein avidin and biotin was used to prepare a biotin-labeled secondary antibody-encapsulated $CuNFs_2$ ($CuNFs_2$-BiotinNHS$Ab_2$) capture probe [74]. Besides, a triple signal amplification strategy using $MoS_2$-based nanocomposites has been developed for CEA analysis. Molybdenum disulfide nanocomposites (MoS2AuNPs) were decorated with gold nanoparticles to construct modified electrodes and nanoprobes. Due to their high catalytic capacity and large surface area, they can efficiently amplify electrochemical signals [75]. An electrochemical sandwich bio-system was appropriately advanced based on the Concanavalin A (ConA) and CEA DNA aptamers. Horseradish peroxidase (HRP) labels the

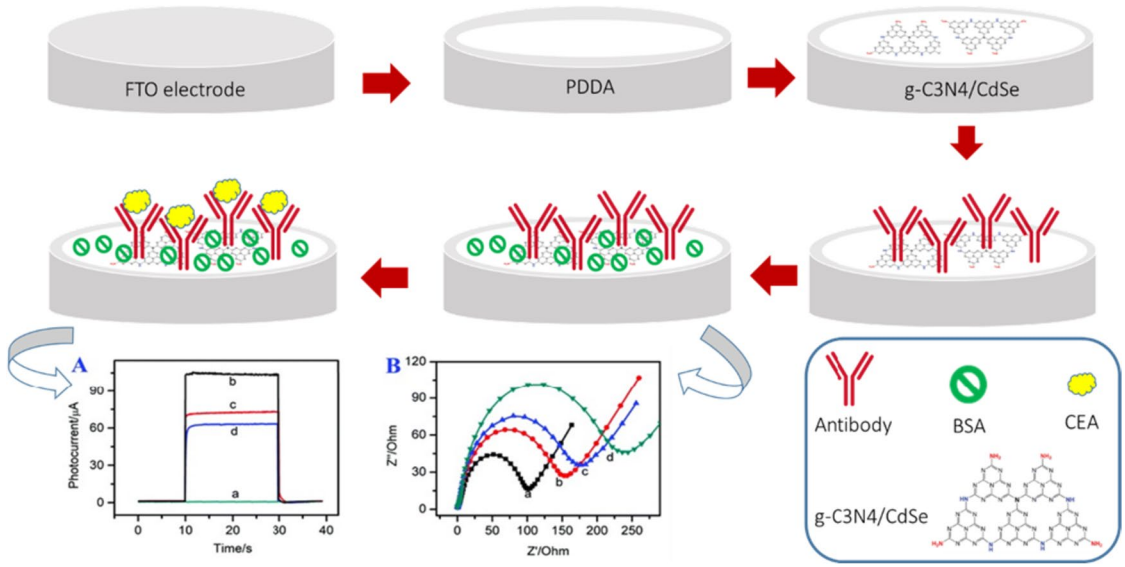


**Fig. 7** Representation of the intricate steps in the fabrication of the photoelectrochemical immunosensor, adapted from Ref (69)

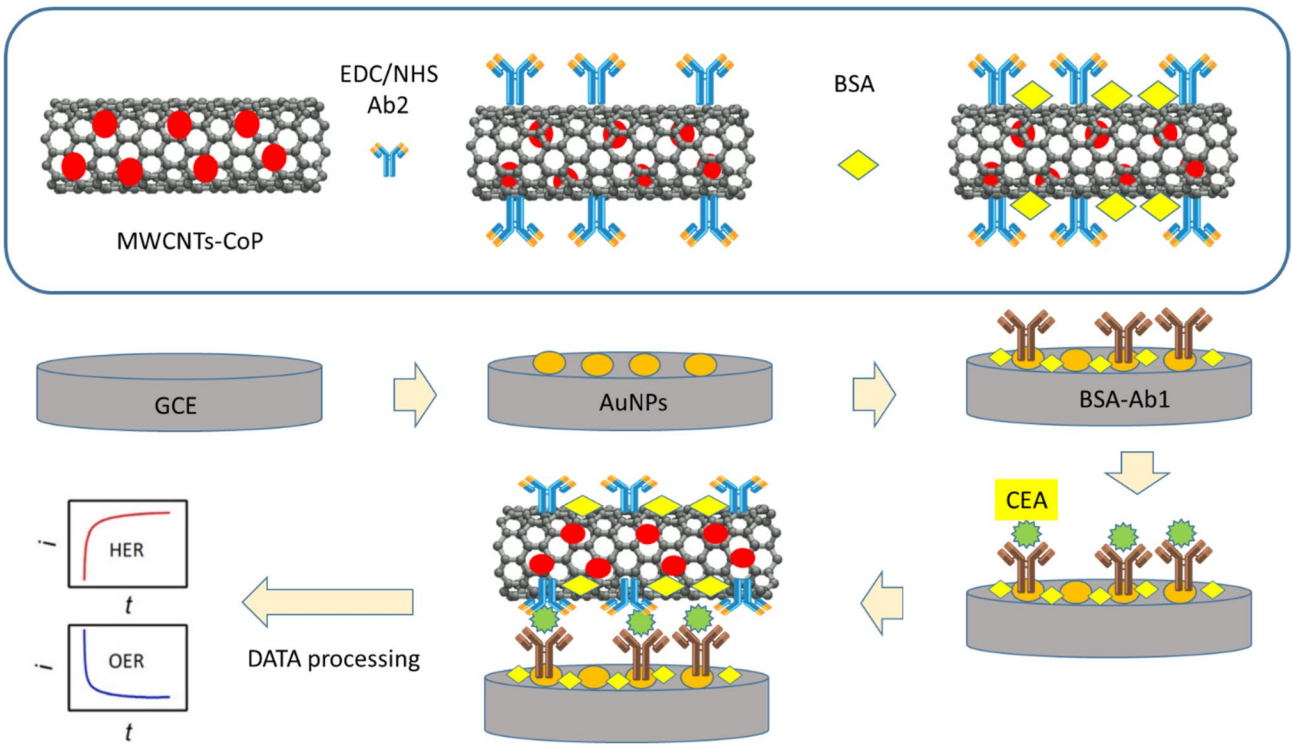


**Fig. 8** Illustration of the training of MWCNTs-CoP label and the construction of the dual-signal electrochemical immunosensor, adapted from Ref (70)

sandwich assembly for signal generation and amplification. Simple and inexpensive aptasensors may potentially serve as a general tool for detecting other glycoproteins of interest [76]. An original graphene-based label-free electrochemical immunosensor was settled for sensitive and rapid detection of CEA. In this work, rGO was used to bind Ab1 on the surface of GCE. This planned immunosensor showed a good amperometric response to CEA within good linearity and acceptable LOD. The evaluation of this biosensor

technology in CEA detection introduced comparable results to standard ELISA references (Fig. 9) [77].

A novel technology in which salt-induced AuNP aggregation lights up the fluorescence of dual-color DNA-AgNCs-apta is introduced to simultaneously monitor carbohydrate antigen 125 (CA125) and CEA. This aptasensor was self-possessed from red-emitting DNA-AgNCs with CA125 aptamer ($rDNA_2$-AgNCs-$apta_2$) and green-emitting DNA-AgNCs with CEA aptamer ($gDNA_1$-AgNCs-$apta_1$) in the ratio of 1:1 in volume [78]. Janus nanoparticles are anisotropic nanomaterials that are particularly appropriate for biosensor development. The existence of two different surfaces with well-defined specific functionalized chemistry allows signaling systems to be placed at different sites within the same nanomaterial and affinity-based biometrics [106]. Biotin-thiol-modified anti-CEA DNA hairpin aptamers on the Au surface assembled as a bio-recognition element for ultra-sensitive detection of CEA. Janus nanoparticles were functionalized with HRP on the silica surface and acted as signaling elements. The engineered method was based on the first specific recognition of CEA by bio-functionalized Janus nanoparticles, unfolding the DNA hairpin structure and unmasking biotin residues on the aptamer chain [79]. CEA was determined simultaneously with a prostate-specific antigen (PSA) by an innovative paper-based fluorometric immunoplatform with quantum-dot labeled antibodies. This method could simultaneously measure different types of other substances on a single paper-based multi-channel chip [80]. Coordination polymers (CPs) are one of the most used compounds that can be considered a natural addition of coordination compounds for polymerization [107, 108]. Therefore, CP is designed by the assembly of two different construction blocks, a cross-linking ligand and a metal moiety (metal complex fragment or metal ion). To date, CP applications have been explored in many areas, including drug delivery, chemical sensing, and heterogeneous catalysis [107, 108]. A zinc (II)-based CP (ZnCP) containing adenine as a cross-linking ligand was used and integrated with alkaline phosphatase (ALP) and CEA antibodies to construct an ultrasensitive biosensor. This immunoassay is used to restore normal levels of CEA in serum samples. In addition, this method provides a new approach to CEA detection, paving the way for rational design and manufacture of multifunction composites [81]. As discussed before, surface plasmon resonance (SPR) has emerged as the primary method for in situ bio-affinity assays of various targets without the need for enzyme or fluorescent labeling. Nanomaterial-enhanced SPR sensors have evolved rapidly, expanding the scope of SPR sensor technology. Utilizing a $Ti_3C_2$MXene-based sensor platform and multi-walled carbon nanotube (MWCNT), polydopamine (PDA), and Ag nanoparticles (AgNPs) signal enhancers, SPR biosensors were manufactured to detect CEA in real-world samples [82]. Using many nanohybrids, the latest reliable electrochemical immunosensors have been developed to detect CEA with ultra-high sensitivity with extremely high specificity and accuracy. The glassy carbon electrode (GCE) was replaced with thiolated graphene oxide (T-GO) to increase the active surface area of the electrode. Artificial immune sensors show excellent specificity in detecting CEA even in real human serum and have been proposed for primary detection and monitoring of CEA in the clinic [83]. The ratiometric electrochemiluminescence (ECL) assay is gaining widespread attention in biosensing because it can be measured accurately by eliminating environmental interference. However, two suitable chromophores were needed in most cases, complicating the system and limiting the actual application. Otherwise, this strategy demonstrates excellent utility for detecting CEA in human serum and is promising in ECL bioanalysis [84]. Surface-enhanced Raman scattering (SERS) contains fingerprint information, has a very small sample size, and produces sensitive data that can be multiplexed. In general, SERS enhances the Raman properties of molecules adsorbed on the surface of precious metals [109]. This can be caused by electromagnetic and chemical effects. The presence of local high electromagnetic fields at

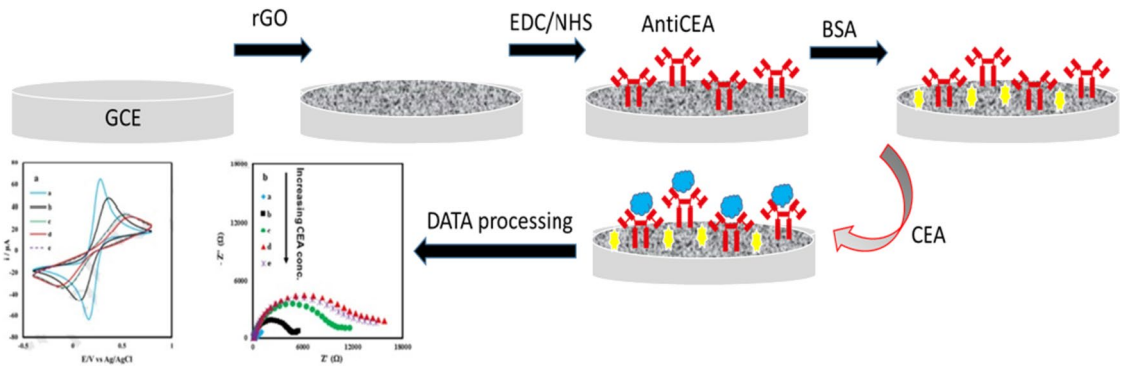


**Fig. 9** Developed immunosensor for detection of CEA, adapted from Ref (77)

the contact sites of metal nanoparticles (NPs), commonly referred to as hotspots, is the main cause of signal amplification [109]. A new biosensor based on a combination of molecular imprint polymer (MIP) and native antibody was manufactured to accurately detect ACE by surface-enhanced Raman spectroscopy (SERS). The MIP material acts as a pre-concentration scheme for the target protein, and the native antibody signals the presence of CEA on the MIP platform (Fig. 10) [85].

The nanowires N, S-graphene quantum dots with Au-polyaniline (N, S-GQDs-Au-PANI) were used for measurable detection of CEA. In addition, the nanowires used were excellent conductive materials that accelerated electron transfer and provided a highly stable label-free immunoassay approach for CEA investigations [86]. Nanopores are basically the pores with a diameter of 1 nm. For instance, it can be produced by proteins that form pores or as existing holes in synthetic materials like silicon and graphene. If the nanopores exist, then an electrically insulating sheet can be employed as a single-molecule detector [110]. The unique affinity between CEA and $Fe_3O_4$-Au magnetic nanoparticles (MNP) modified with aptamers has recently led to the development of a new technique. After magnetic separation, the residual Apt-MNP can be transported through nanopores by applying a positive potential, forming CEA-Apt-MNP. The amount of the current drop allows for the complete differentiation of each blocking signal because of the apparent particle size difference between CEA-Apt-MNP and AptMP [87, 110]. A unique electrochemiluminescence platform has been developed successfully using a deposited two-dimensional (2D) ultrathin metal-organic (MOL) layer based on aggregation-induced emission (AIE) for sensitive detection of CEA [88]. NIR-II fluorescent nanoprobe-labeled lateral flow biosensor was advanced for sensitive determination of CEA. The created platform showed high sensitivity, specificity, and accuracy, and but it also has features of quickness, simplicity, and low cost [111]. PB-PDA nanocomposites as signal reporters and nanolabels for separate-type cathodic PEC immunosensors for the determination of CEA were developed. The planned immunosensor showed decent operability and high sensitivity for CEA detection, demonstrating its potential for the identification of other tumor markers [89]. Smartphone-based PEC immunosensor based on $BiOCl/CuBi_2O_4$ heterojunction was employed for ultrasensitive detection of CEA. The advanced system was portable and presented an implementable method for the development of miniaturized PEC detectors and on-site detection equipment [90].

### 5.3 Mechanical Biosensors

Mechanical biosensors' variations in mechano-physical factors, such as motion, forces, and masses, are measured after the bio-molecular interaction [112, 113]. Mechanical biosensors provide some advantages over other biosensors. For instance, mechanical biosensors such as quartz crystal microbalance (QCM) are capable of excellent mass resolution, which can be used to develop bio-affinity-based biosensors to detect the lowest amount of analyte [112, 113]. QCM sensors are widely used nowadays. QCM sensors are the primary tool for biomolecular analysis due to their unique advantages, such as fast response time, high sensitivity, ease of use, portable devices, and cost-effectiveness [114]. The QCM electrodes comprise a quartz electrode sandwiched between metal layers (Ti, Au, and Al) for electrical contact. As mass builds up on the electrodes, the frequency response of the crystal tends to change [115, 116], and a precision analysis system can detect this alteration. QCM-based

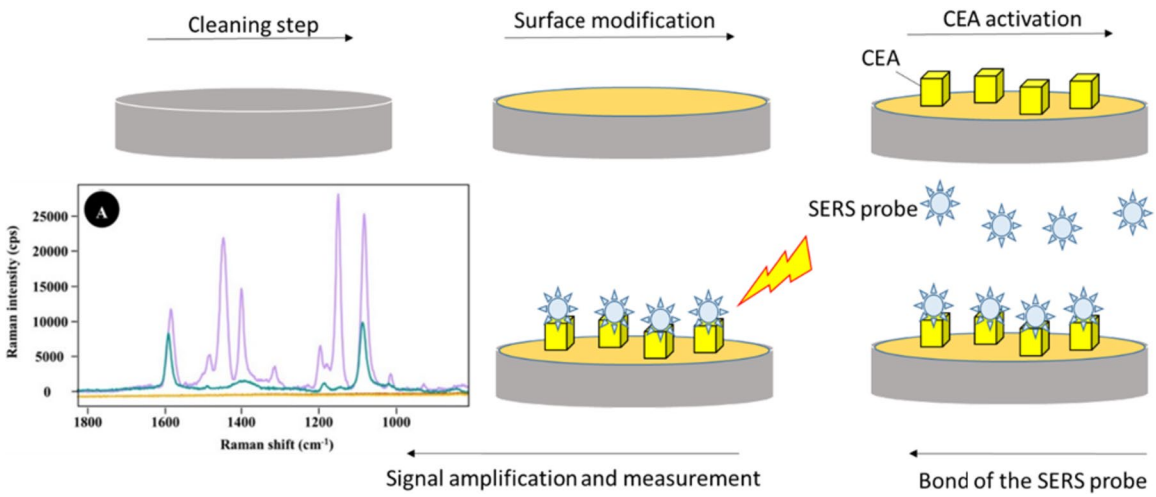


**Fig. 10** SERS-based biosensor for detection of CEA, adapted from Ref (85)

biosensors are highly applicable for analyzing proteins and DNA molecules, diagnosing cancer, detecting tumor markers, and differentiating normal and cancerous cells [115, 116]. Through new research, the label-free QCM has been developed for real-time and selective measurement of CEA. The mouse monoclonal anti-CEA antibody was covalently immobilized on this layer as a bioreceptor for CEA, and graphene oxide Au nanoparticles (GO-AuNPs) were produced in situ on the surface of the QCM electrode [92]. Surface acoustic waves (SAW) have some advantages, such as label-free and real-time detection capabilities, remote and wireless control capabilities, and ease of use. The attractiveness of SAW devices is also due to the ease of data analysis, small size, high sensitivity, and affordability [93]. A research study has developed an innovative QCM immunodetection method that enables highly efficient detection of CEA from serum samples of patients. It contains HRP-nanoparticles as an enhancer and is associated with enzymatic biocatalyst precipitation (EBCP) against 4-chloro-1-naphthol (4-CN) on an anti-CEA-coated capture QCM probe. In addition, the advanced QCM immunoassay has shown long-term storage stability, high specificity, and constant results in human serum samples compared to those obtained from commercially available ELISA analysis methods [94]. An AlGaN/GaN high-electron-mobility transistor was employed for highly sensitive recognition of CEA. The developed method has the benefits of integration, low cost, miniaturization, and rapid detection compared to other challenging approaches for human pathologies like cancer and could be used in forthcoming medical diagnostics [91, 95, 117, 118]. AuNCs-keratin-based electrochemical/fluorescent and magnetic copper silicate boronic acid-conjugated dual-sensing was advanced for the detection of CEA [119].

## 6 Conclusion and Future Prospective

It is important to diagnose and treat human illnesses, especially cancer, at the preliminary stages of progression, permitting a hit remedy for the sufferers. Therefore, biosensors as simple, sensitive, and cost-effective diagnostic tools are crucial to coming across illnesses efficaciously. Biosensors have potential applications that assist medical oncologists with numerous functions, including manipulating disease, scientific care, preventive remedies, and ailment reviews. Most of the biosensors described in this review are still recently developed and propose to evaluate CEA in the lowest and earliest possibility. In their construct, different nanomaterials, technologies, and complementary structures like aptamers are used in a dynamic, in vivo friendly, and automatic detection system for CEA biomarkers. However, the CEA biosensors are sophisticated and promising in future clinical approaches, as the state-of-art technology, their performance characteristics such as stability, reusability, and compatibility with body fluids should be further trialed. Despite the efforts of the scientific community to develop portable and minimally invasive electrochemical biosensors for continuous and in vivo self-monitoring, the basic theory behind the development of biosensors remains to be determined. On the other hand, in the future prospective of biosensors technology, the commercialization, miniaturization, and application of developed biosensors should be addressed so that despite numerous publications in recent years, unfortunately, very few of these developed systems have been industrialized in a real and practical approach.

**Acknowledgements** This study was supported by the Physical Medicine and Rehabilitation Research Center and Aging, Research Institute, Faculty of Medicine, Tabriz University of Medical Sciences, Iran.

**Author Contribution** Maryam Hariri: data curation; Ahmad Mobed: conceptualization and supervision; Nastaran Safavi Ardabili, Reaza Shah Hosseini: investigation; Vahid Alivirdiloo, Sarah Gholami, Sajjad Masoumi, Mohammad Reza Mehraban, Mahsa Alem, Farhood Ghazi: writing manuscript; Iraj Alipourfard: supervision, writing, worked out the technical details, edited and submitted the manuscript as the correspondence. All authors have reviewed and approved the final manuscript.

**Data Availability** Not applicable

## Declarations

**Ethics Approval and Consent to Participate** Not applicable

**Consent for Publication** Not applicable

**Competing Interests** The authors declare no competing interests.



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