Disposable Paper based Screen Printed Electrochemical Immunoplatforms for Dual Detection of Esophageal Cancer Biomarkers in Patients' Serum samples

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Supplementary Information (SI)

2.1 | Chemicals and reagents

N-ethyl-N′ -(3-dimethyl aminopropyl) carbodiimide hydrochloride (EDC), Bovine serum albumin (BSA), and N-hydroxysuccinimide (NHS) were purchased from Sigma-Aldrich. Biomarkers such as CYFRA21-1, and TP53, along with their respective antibodies, including anti-CYFRA21-1 and anti-TP53, were procured from My BioSource company, USA, and stored at -20 °C. Potassium ferrocyanide ($K_4[Fe(CN)_6)\cdot 3H_2O]^{3-/4-}$), and potassium ferricyanide ($K_3[Fe(CN)_6)]^{3-/4-}$) were bought from Fisher Scientific, India. Sodium dihydrogen phosphate dihydrate (NaH_2PO_4, 99.5 %), disodium hydrogen phosphate dihydrate (Na_2HPO_4, ≥99.5 %), ascorbic acid ($C_6H_8O_6$, 99.7 %); urea (NH_2CONH_2, 99.5 %); uric acid ($C_5H_4N_4O_3$, 99 %) and sodium chloride (NaCl, 99.9 %) were obtained from SRL India Pvt. Ltd. Others antibodies of sperm Protein, i.e., SP17 (anti-SP17), and tumor necrosis factor-alpha (anti-TNF-α), were also procured from My BioSource, USA for interferent analysis. A rough paper-like substrate (electro-coated waterproof silicon carbide rough paper) was purchased from a nearby electrical appliances shop for fabricating SPE while graphite power was procured from Alfa Aesar. Different concentrations of anti-CYFRA21-1, anti-TP53 antibodies, and CYFRA21-1, TP53 antigens were made in PBS of pH 7.4, respectively. For this, 0.2 M NaH_2PO_4 and 0.2 M $NaHPO_4$ were mixed with each other in distilled water (DI) to prepare the electrolyte solution (0.2 M PBS of different pH 6.0, 6.6, 7.0, 7.4, and 8.0) and kept in the refrigerator for further

experiment. All the other reagents explored were of high analytical level and utilized without any treatment.

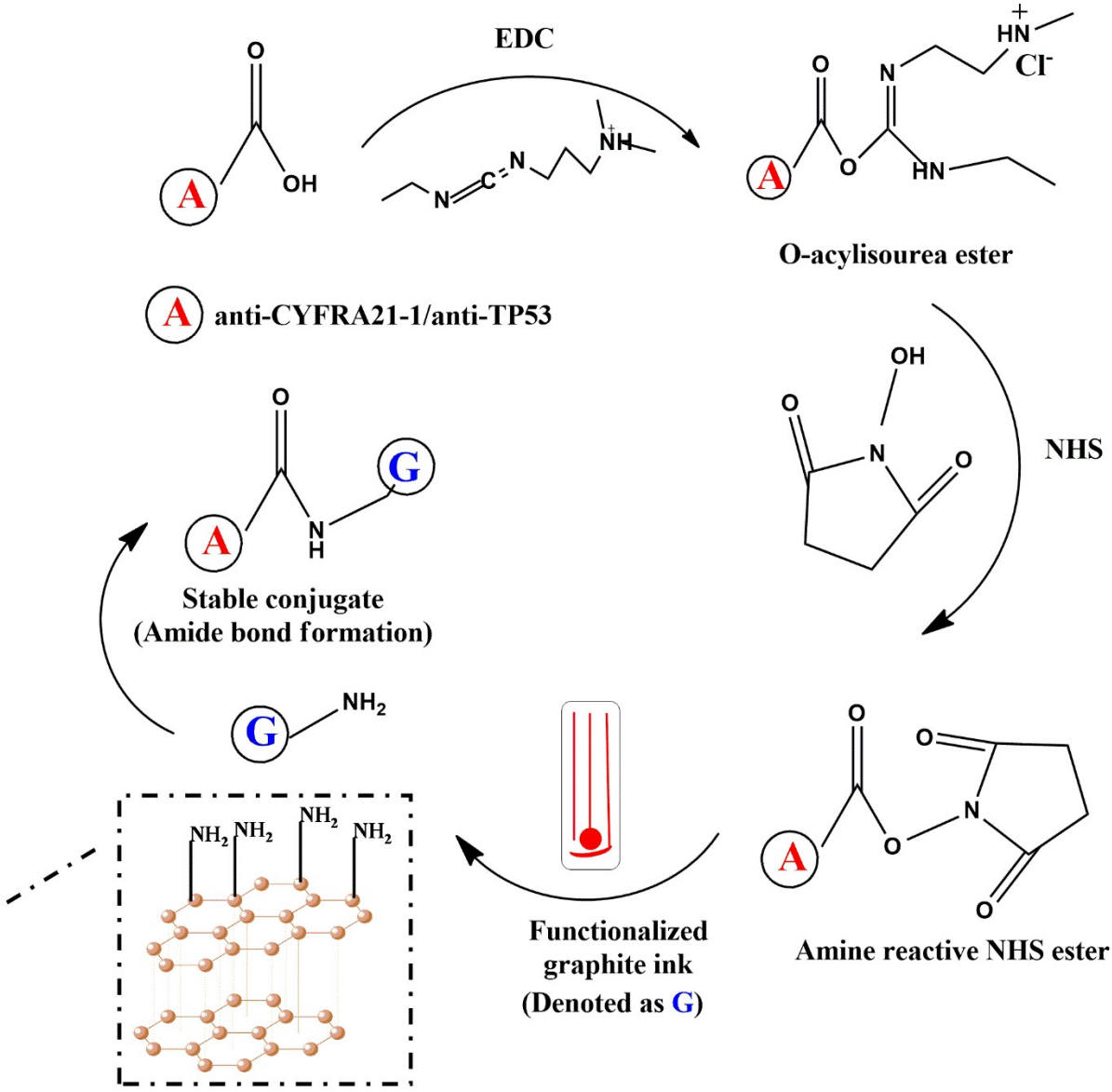
2.2 | Characterizations

The screen printing of electrodes was printed out using the GRAFICA Flex TRONICA machine. The functional group analysis was performed utilizing the Fourier transform infrared spectroscopy (FT-IR, Perkin Elmer) for monitoring the change on modified electrodes from 4000 to 400 cm^{-1} wavenumber range. Further, energy dispersive spectroscopy (EDS) and scanning electron microscopy (SEM) were done using JSM-IT 200 model to characterize the presence of elements and surface morphology change of the modified electrodes, respectively. In addition, the hydrophobicity and hydrophilicity of the developed electrodes were examined by employing a water-based contact angle *via* a drop shape analyzer [KRUSS, Germany]. The electrochemical characterization of different electrodes was done using cyclic voltammetry (CV) to study the process of immobilization and depict the immune-complex interaction occurring between the two antibodies (anti-CYFRA21-1 and anti-TP53) and antigens (CYFRA21-1, and TP53 antigens), respectively. The CV measurements of different modified electrodes were done using the Autolab, Potentiostat/Galvanostat electrochemical analyzer (EcoChemie, The Netherlands) employing disposable paper based SPE immunoplatforms having 1.10 version of NOVA software. The electrochemical responses with CV were performed in triplicate (n=3) in electrolyte PBS (0.2 M) carrying the potassium ferricyanide/ferrocyanide of 5 mM as the redox coupler as well as 0.9% NaCl. The potential window for CV measurements was kept wide, i.e., from -0.8 to +0.8 V at a 50 mVs^{-1} scan rate.

2.6 | Development of paper-based screen printed immunoplatforms

“The immobilization of antibodies (anti-CYFRA-21-1 and anti-TP23) onto gum arabic-coated screen-printed electrodes (SPEs) through the EDC-NHS mechanism is a meticulously orchestrated process aimed at creating a robust and selective biosensing platform. Initially, the carboxyl groups present on the gum arabic-coated electrode surface are activated using 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC). This activation forms an intermediate O-acylisourea compound. Subsequently, N-Hydroxysuccinimide (NHS) is introduced to react with the intermediate, resulting in the formation of stable NHS esters on the gum arabic surface. The activated gum arabic now provides a reactive substrate for antibody immobilization. Antibodies, being rich in primary amines, readily form covalent amide bonds with the NHS esters on the gum arabic-coated electrodes. This covalent linkage ensures the firm attachment of antibodies to the electrode surface, enhancing stability and longevity. To further refine the sensor surface, any remaining activated sites are typically blocked using a suitable blocking

agent. This meticulous process not only facilitates the specific binding of antibodies to the electrode but also minimizes nonspecific interactions, ensuring a highly selective and sensitive electrochemical sensing platform for the detection of CYFRA-21-1 and TP53 cancer biomarkers in serum samples".



Scheme S1: In general functionalization mechanism showing surface modification occurring during fabrication of SPE of different antibodies.

Electrochemical Sensing Studies of CYFRA-21-1 using DPV technique: -

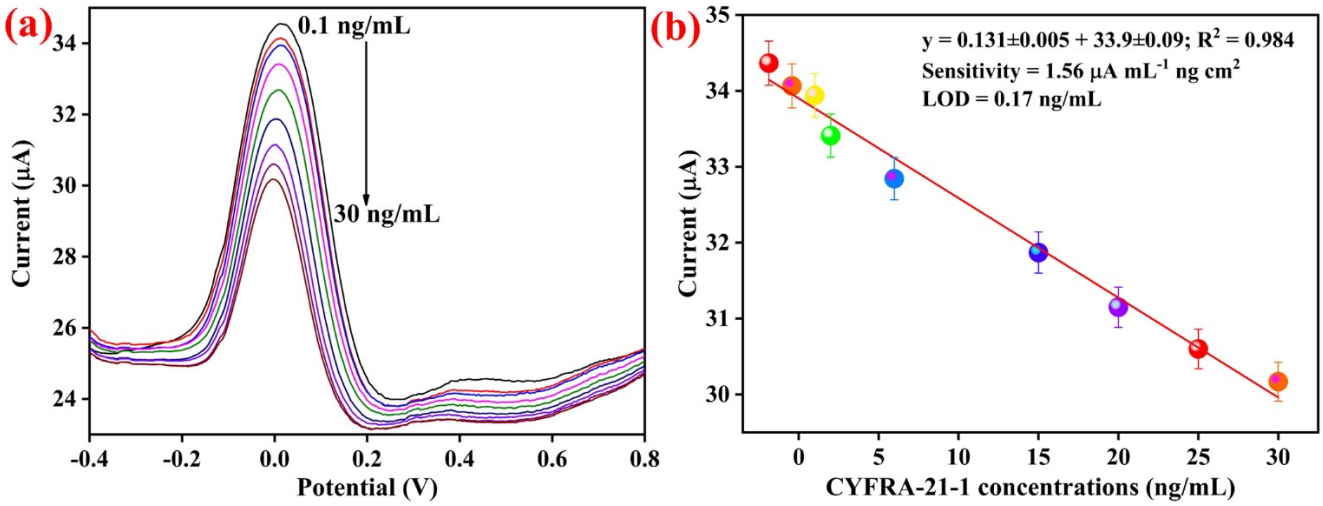


Figure S1: (a) DPV responses of the fabricated BSA/anti-CYFRA-21-1/SPE in 0.2 M PBS with the additions of CYFRA-21-1 concentrations ranging from 0.1 to 30 ng/mL; and (b) The calibration graph was made between the CYFRA-21-1 concentrations and peak current.

Electrochemical Sensing Studies of TP53 using DPV technique: -

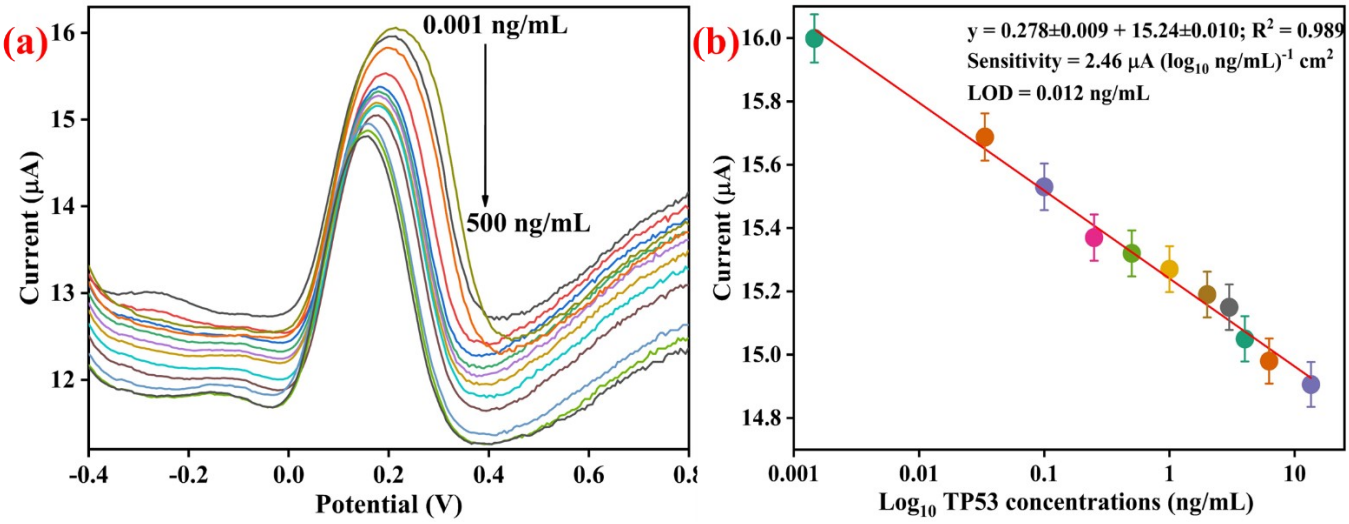


Figure S2: (a) DPV responses of the fabricated BSA/anti-TP53/SPE in 0.2 M PBS with the additions of TP53 concentrations ranging from 0.001 to 500 ng/mL; and (b) The calibration graph was made between the TP53 concentrations and peak current.

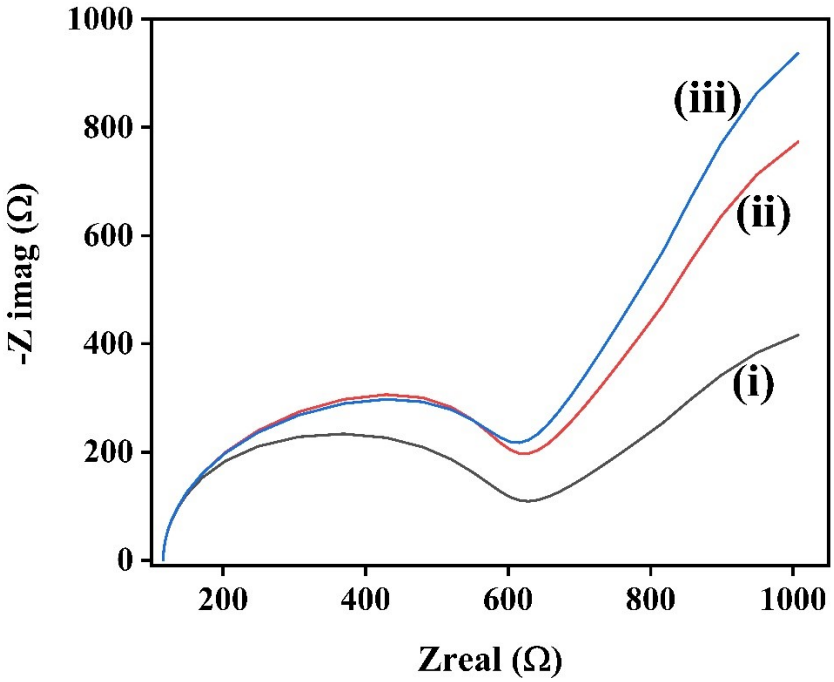


Figure S3: EIS curves of (i) SPE, (ii) anti-CYFRA21-1/SPE (iii) BSA/anti-CYFRA21-1/SPE.

Table S1: EIS parameters

Electrodes	Rs (Ω)	Rct (Ω)	CPE (µF)	Zw (mMho)
Bare SPE	116	456	6.08	2.04
anti-CYFRA-21-1/SPE	113	578	6.06	1.10
BSA/anti-CYFRA-21-1/SPE	113	550	6.00	906 µMho

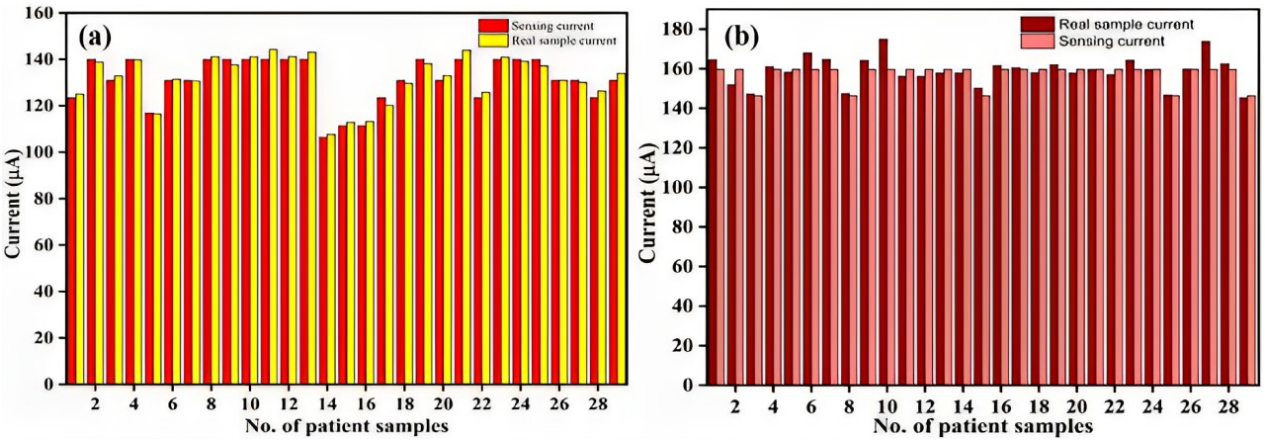


Figure S4: Comparison of current responses of the patient samples obtained from evaluation of ELISA results and the standard samples using the fabricated (a) BSA/anti-CYFRA21-1/SPE and (b) BSA/anti-TP53/SPE immunoelectrodes.