Supporting Information (SI)

Bioinspired porous and electroactive reduced graphene oxide hydrogel based biosensing platform for efficient detection of Tumor Necrosis Factor-α

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S1. Chemicals and reagents

Graphene oxide nano-powder, L-cysteine (L-cys), sodium dihydrogen phosphate monohydrate [$NaH_2PO_4.H_2O$], disodium hydrogen orthophosphate [Na_2HPO_4], sodium chloride [NaCl], sodium hydroxide (NaOH), potassium ferricyanide ($K_3[Fe(CN)_6]$), and potassium ferrocyanide $K_4[Fe(CN)_6].3H_2O$ were obtained from Sisco research laboratories, India. Bovine serum albumin (BSA), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC), and N-hydroxy-succinimide (NHS) were sourced from Sigma Aldrich, India. Human Tumor Necrosis Factor-α (TNF-α) recombinant protein (catalog # RTNFAI) and TNF- α monoclonal antibody (catalog # 7124-MSM5-P1) were bought from Thermo Fischer Scientific. Artificial saliva (CAS:7732-18-5) was sourced from Nanochemazome. The commercial screen-printed electrodes (ItalSens Gold SPE W1-3) were obtained from PalmSens. All the chemicals used were of high analytical quality and were used as supplied without any additional modification. Phosphate-buffered saline (PBS) solutions of various pH were made using $Na_2HPO_4.H_2O$ and NaH_2PO_4 in deionized water having a resistivity of 18 MΩ cm. Saline (0.9% NaCl) was added to the buffer solutions to maintain the osmolarity. All the dilutions of antigen and antibody were made in PBS of pH 7.0 and kept at 4 °C till further use.

S2. Instrumentation and characterizations

The successful reduction of GO to rGO and the formation of the L-cys_rGO hydrogel were validated using multiple characterization techniques. To investigate the surface modification, such as functional groups and various bonds present in L-cys_rGO hydrogel, an Agilent Cary 630 Fourier transform infrared spectrometer (FTIR) was utilized. Brunauer-Emmett Teller (BET) analyzer was used to calculate the porosity parameters using the Quantachrome instrument. The morphology of L-cys_rGO hydrogel was studied by Field emission scanning electron microscopy (Thermo Fischer Apreo S Low vac FESEM and Zeiss Gemini 300

FESEM) at an operating 20 kV potential. To analyze the crystallinity and structure of the hydrogels, TEM analysis was done using High-resolution transmission electron microscopy HR-TEM, JEOL JEM-2200 FS, which was operated at 200 kV. The samples for TEM analysis were prepared by dropping L-cys_rGO hydrogel powder solution on a carbon copper grid. Electrochemical analysis were done using an Autolab (PGSTAT204) electrochemical analyzer from Metrohm, India. Gold screen printed electrodes (gSPE) were used in the study, which has a three-electrode system with a circular working electrode of gold (4 mm diameter), a silver pseudo-reference electrode, and a counter electrode also made of gold. Electrochemical studies of the electrodes were done in PBS with $[Fe(CN)_6]^{3-/4-}$as the redox coupler.

Table S1: Tabular representation of peak current values of different electrodes in CV and DPV response curves

Electrode	**Peak current (µA)**	
	CV	**DPV**
Bare gSPE	78.61	92.13
L-cys_rGO hydrogel/gSPE	91.34	110.06
anti-TNF-α/L-cys_rGO hydrogel/gSPE	87.68	83.63
BSA/anti-TNF-α/L-cys_rGO hydrogel/gSPE	77.88	65.10

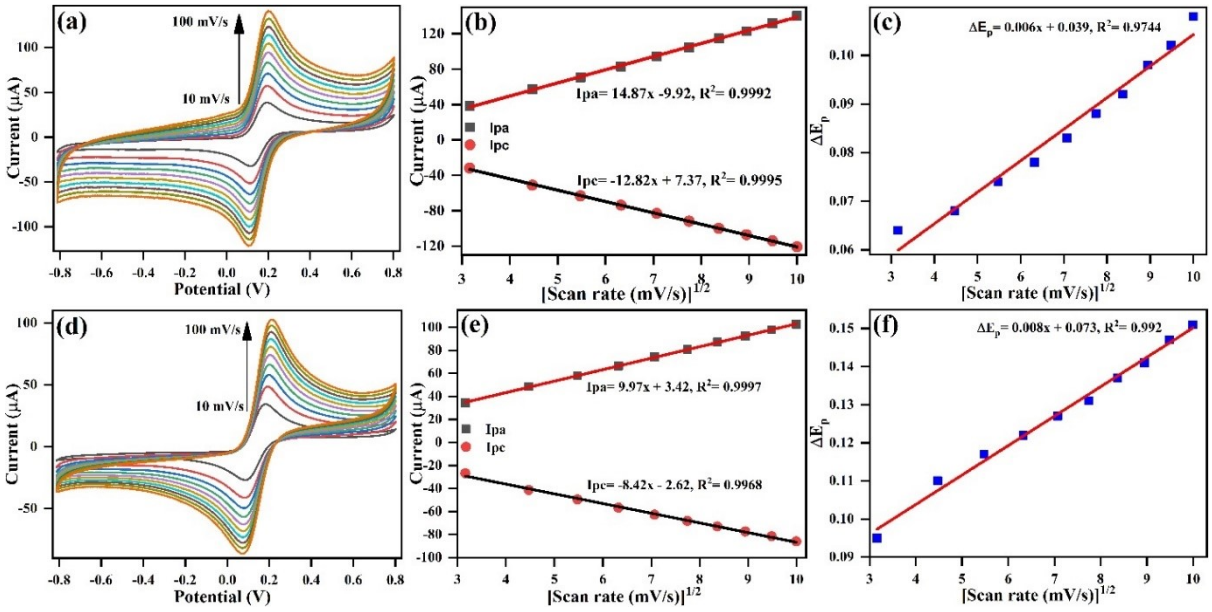

Fig. S1: Scan rate studies of (a) L-cys_rGO hydrogel/gSPE electrodes and (d) BSA/anti-TNF-α/L-cys_rGO hydrogel/gSPE electrode in PBS. I_{pa} and I_{pc} peak current curves of (b) L-cys_rGO hydrogel/gSPE and (e) BSA/anti-TNF-α/L-cys_rGO hydrogel/gSPE immunosensor, respectively, against the square root of the scan rates. Peak potential changes (ΔE_p) versus square root of the scan rates for (c) L-cys_rGO hydrogel/gSPE and (f) BSA/anti-TNF-α/L-cys_rGO hydrogel/gSPE electrode, respectively.

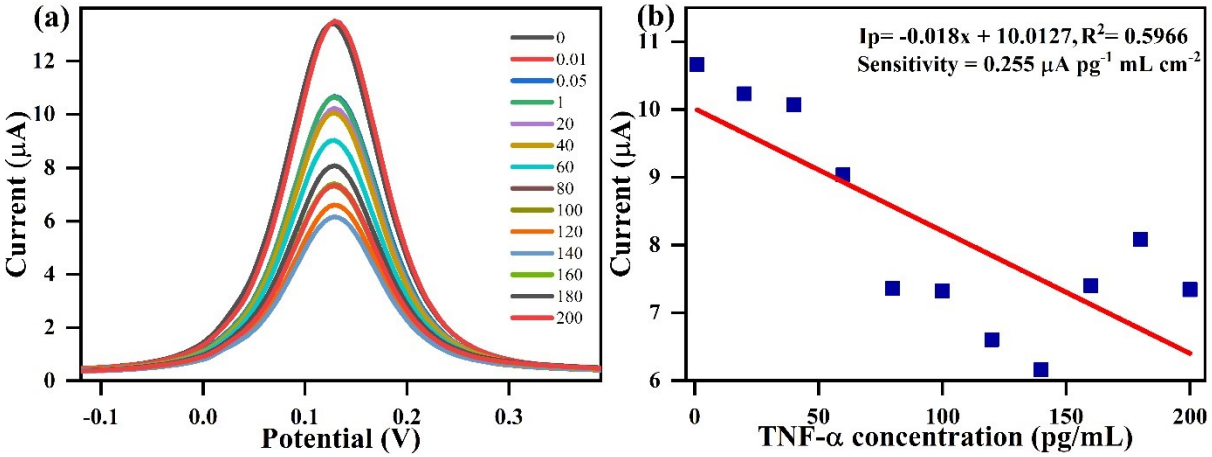

Fig. S2 Control study depicting (a) the electrochemical response study of the rGO/gSPE against TNF-α. (b) A linear correlation plot of the rGO/gSPE sensor demonstrates the relationship between peak currents and TNF-α concentrations.

Table. S2: A spiked sample analysis of the BSA/anti-TNF-α/L-cys_rGO hydrogel/gSPE immunosensor utilizing distinct TNF-α biomarker concentrations in artificial saliva sample

TNF-α concentrations	**Peak current (µA) recorded for standard TNF-α**	**Peak current (µA) recorded for artificial saliva spiked TNF-α**	**RSD %**	**Recovery %**
0	77.04	73.07	3.74	94.85
0.01	67.95	68.17	0.23	100.32
0.05	61.98	64.18	2.47	103.55
1	59.34	57.84	1.81	97.47
20	54.19	53.72	0.62	99.13
40	50.74	50.16	0.81	98.86
60	48.13	46.89	1.85	97.42
80	46.86	44.23	4.08	94.39
100	43.94	41.64	3.80	94.77
120	42.05	39.59	4.26	94.15
140	37.75	37.59	0.30	99.58
160	36.62	35.88	1.44	97.98
180	35.22	34.7	1.05	98.52
200	33.49	33.8	0.65	100.92