Supplementary Information

Designing of unique bioreceptor and fabrication of efficient genosensing platform for Neonatal Sepsis detection

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1. Chemicals and Reagents

Graphene oxide (GO), bovine serum albumin (BSA) and 1-(3-(dimethylamino)-propyl)-3-ethylcarbodiimide hydrochloride (EDC) ($C_8H_{17}N_3$) were purchased from Sigma-Aldrich. E coli K1 stain was purchased from National Centre for Microbial Resources (NCMR), Pune, India. Luria Bertini (L. B.) agar, L. B. broth, agarose and ethidium bromide ($C_{21}H_2OBrN_3$) were procured from Himedia Laboratories Pvt. Ltd., India. Ammonia, hydrogen peroxide, and acetonitrile were procured from Sisco Research Laboratories Pvt. Ltd., India. N-hydroxysuccinimide (NHS) was purchased from Fisher Scientific. Sodium dihydrogen phosphate dihydrate ($NaH_2PO_4.2H_2O$), disodium hydrogen phosphate dihydrate ($Na_2HPO_4.2H_2O$), potassium hexacyanoferrate (II) trihydrate [$K_4(Fe(CN)_6).3H_2O$)], potassium hexacyano ferrate (III) [$K_3(Fe(CN)_6)$] and sodium chloride (NaCl), Calcium chloride ($CaCl_2$), Potassium chloride (KCl), and Magnesium sulphate ($MgSO_4$) were procured from Merck Life Science Pvt. Ltd. Phosphate buffer saline (PBS) solutions of different pH were prepared using $NaH_2PO_4.2H_2O$ (0.2 mol L^{-1}) and $Na_2HPO_4.2H_2O$ (0.2 mol L^{-1}) in ultrapure water and stored at 4°C for desired experiments.

2. Instrumentation

For the amplification of *fimA* gene, Polymerase Chain Reaction (PCR) was performed by Thermal cycler (Eppendorf, Mastercycler thermal gradient). Amplified product by PCR was observed by Gel Doc imaging system (Genesys G Box Chemi XRQ). X-ray diffraction (Brucker, D8 Discover) was used to investigate the phase purity and crystallinity of GO with Cu-Kα radiation (λ=1.5406 A°). Morphological properties of the nanomaterial and fabricated electrodes were observed through scanning electron microscopy (SEM; JEOL JSM 6610LV, Japan). The Fourier transform infrared spectrometer (FT-IR; Shimadzu IR Affinity 1S) was used to investigate the functionalities of GO and amide bond formation after immobilization of probe DNA onto GO/ITO electrode. All the electrochemical studies were performed by Autolab Potentiosat Galvanostat (AUT204, The Netherlands). The conventional three electrodes system, consisting of modified ITO coated glass electrode as working electrode, platinum electrode as counter electrode and Ag/AgCl electrode as reference electrode were used. Freshly prepared 0.2 M phosphate buffer solution (PBS; 0.9% NaCl) of pH 7.0 was used as an electrolyte containing 5 mM of Ferro-Ferri cyanide $[Fe(CN)6]^{3-/4-}$ as a redox species.

Figure S1: PCR amplification of *fimA* gene. Lane 1: DNA ladder; Lane 2: positive control; Lane 3: negative control; Lane 4: PCR product of *fimA* gene (447 bp).

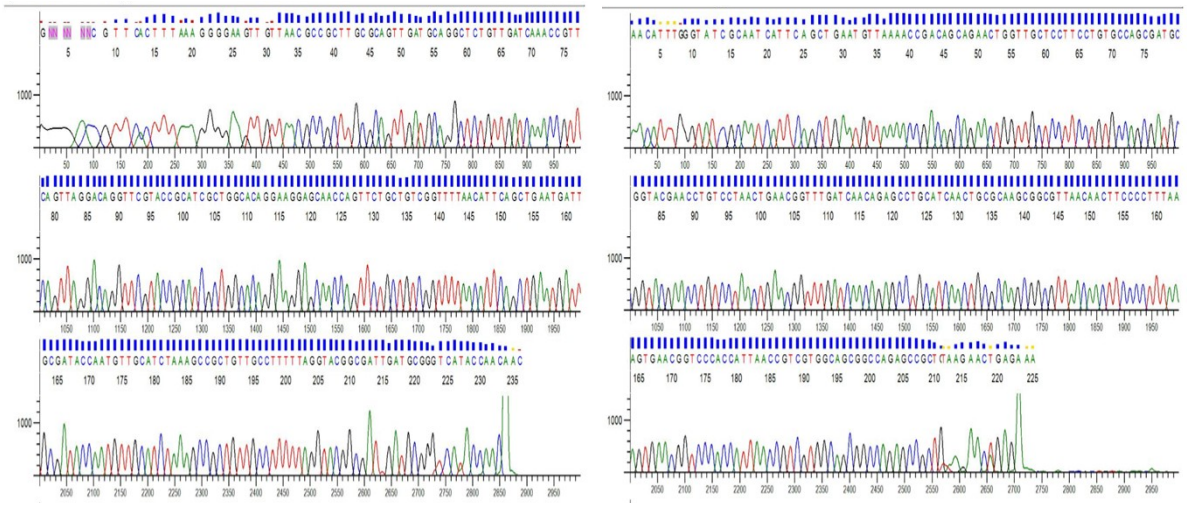


Figure S2: Sanger sequences of the *fimA* gene amplified using designed (a) forward and (b) reverse primer.

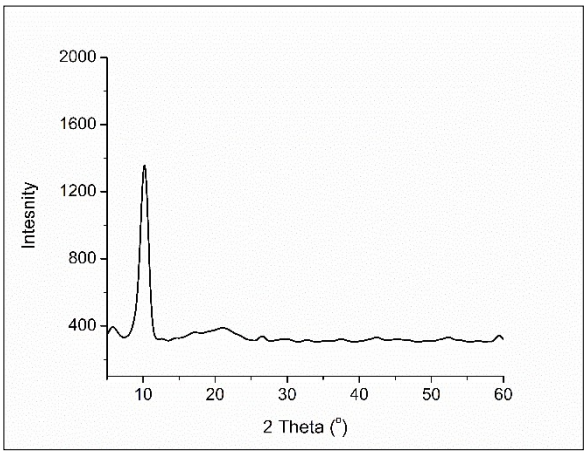


Figure S3: X-ray diffraction pattern of GO.

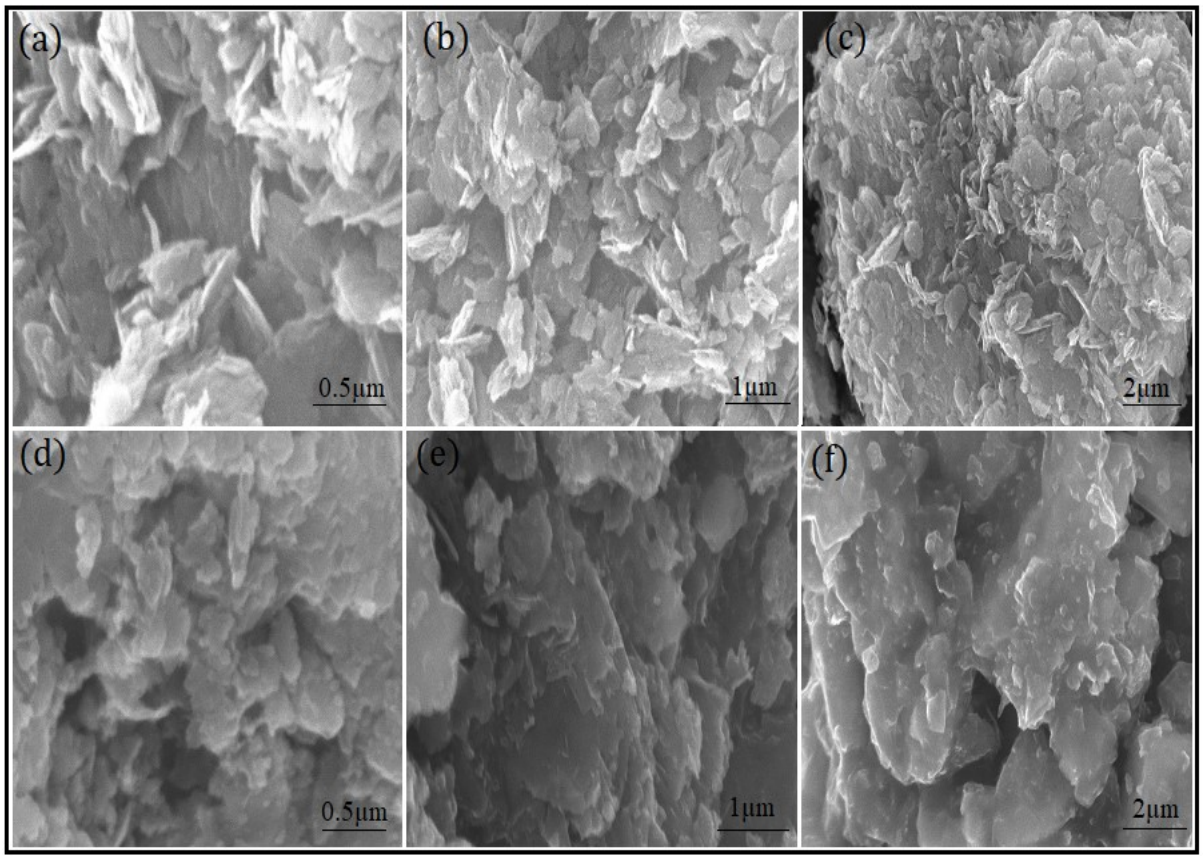
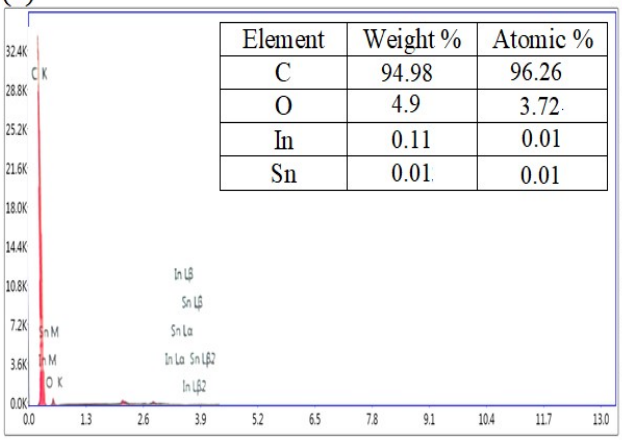


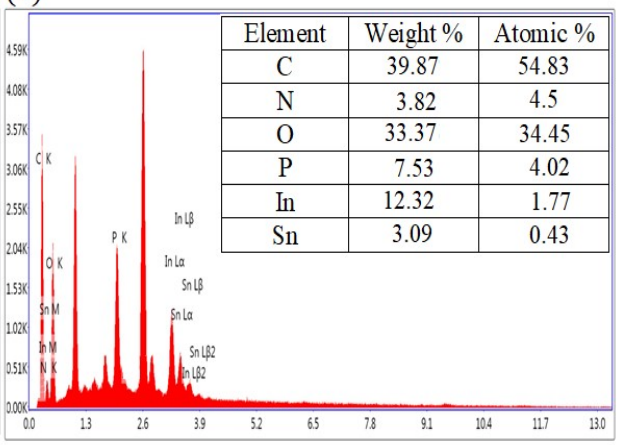
Figure S4: SEM images of GO/ITO (a, b, c) and pDNA/GO/ITO at (d, e, f) at different scale bars.

(a)



Element	Weight %	Atomic %
C	94.98	96.26
O	4.9	3.72
In	0.11	0.01
Sn	0.01	0.01

(b)



Element	Weight %	Atomic %
C	39.87	54.83
N	3.82	4.5
O	33.37	34.45
P	7.53	4.02
In	12.32	1.77
Sn	3.09	0.43

Figure S5. EDX of (a) GO/ITO, and (b) pDNA/GO/ITO

To investigate the elemental analysis of fabricated electrodes, before and after immobilization of pDNA molecules, Energy-dispersive X-ray spectroscopy (EDX) studies have been conducted. From the obtained results (Shown in **Figure S5a**), the weight % (wt %) of carbon, oxygen, indium and tin elements onto the GO/ITO electrode are 94.98%, 4.9%, 0.11% and 0.01% respectively**.** However, after the immobilization of pDNA onto the GO/ITO electrodes, wt % of carbon, oxygen, nitrogen, phosphorus, indium and tin are 39.87%, 33.37%, 3.82%, 7.53% 12.32% and 3.09% respectively (Shown in **Figure S5b**). The presence of phosphorous and nitrogen elements indicate the successful immobilization of pDNA biomolecules onto the GO/ITO electrode.

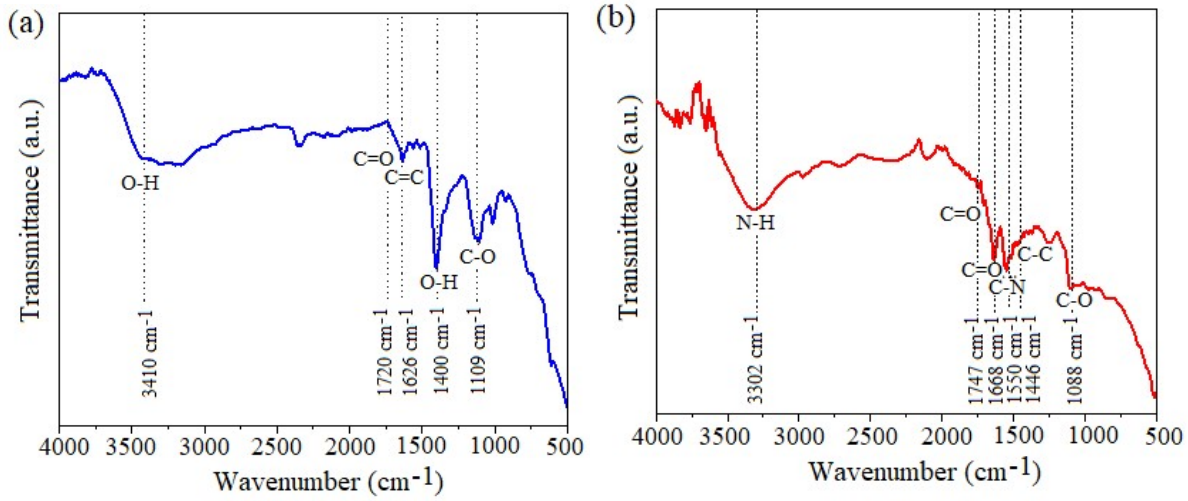


Figure S6: FT-IR spectra of (a) GO/ITO and (b) pDNA/GO/ITO electrodes.

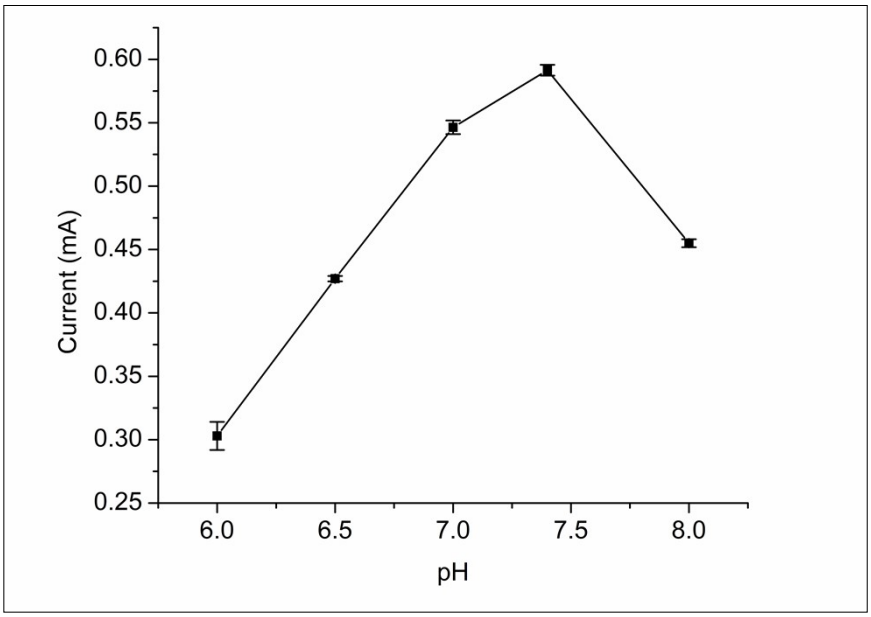


Figure S7. Current response generated by the electrode at different electrolytic pH ranging from 6.0 to 8.0.

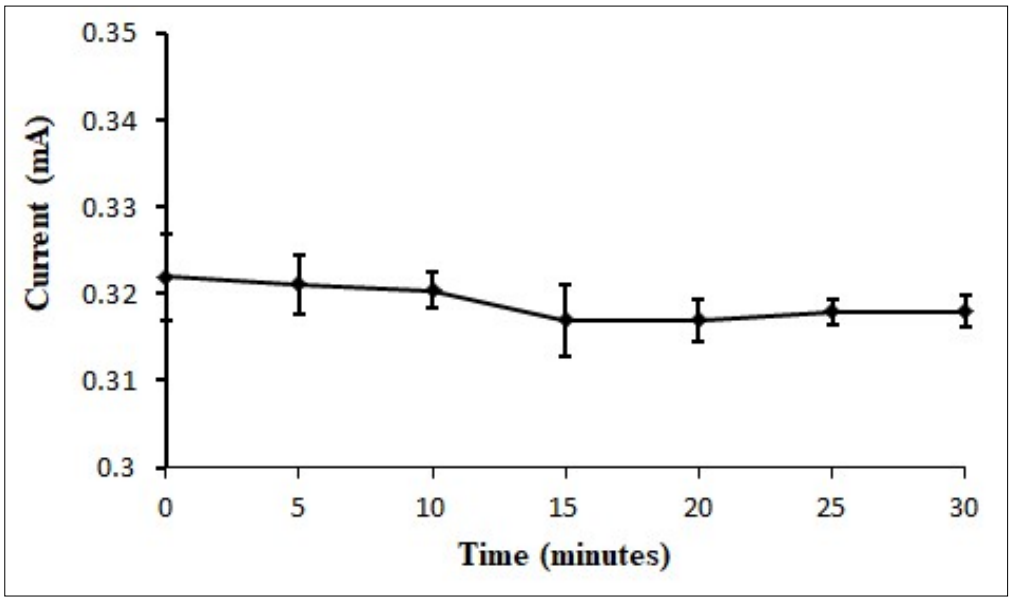


Figure S8. Incubation time studies for binding of tDNA with BSA/pDNA/GO/ITO electrode.

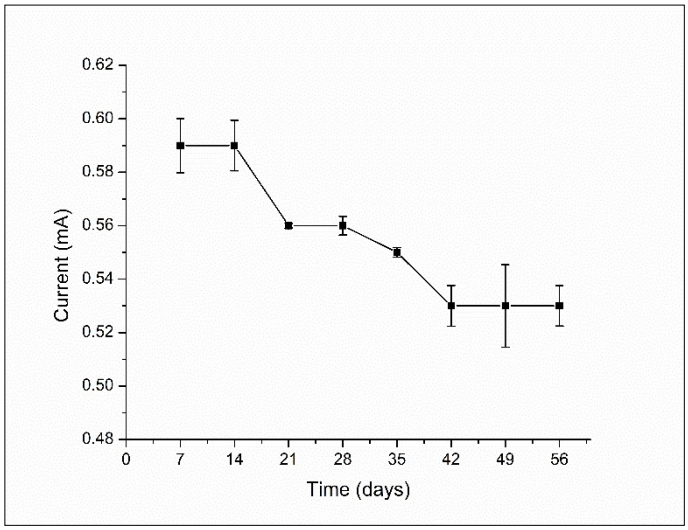


Fig. S9 Shelf life study of BSA/pDNA/GO/ITO electrode.

Equations:

$Ipa_{(BSA/pDNA/GO/ITO)}$ = (0.076 ± 0.0008) mA $(mV\ s^{-1})^{-1/2}$ × [scan rate $(mV\ s^{-1})$]$^{1/2}$ + (0.083 ± 0.006) mA, R^2 = 0.99 ……...(S1)

$Ipc_{(BSA/pDNA/GO/ITO)}$ = -(0.054 ± 0.0005) mA $(mV\ s^{-1})^{-1/2}$ × [scan rate $(mV\ s^{-1})$]$^{1/2}$ -(0.108 ± 0.005) mA, R^2 = 0.99 ……...(S2)

$\Delta Ep_{(BSA/pDNA/GO/ITO)}$ = (0.017 ± 0.0004) V $(mV\ s^{-1})^{-1/2}$ × [scan rate $(mV\ s^{-1})$]$^{1/2}$ + (0.19 ± 0.004) V, R^2 = 0.98 ……...(S3)

$Ip = (2.69\times10^5)\ n^{3/2}AD^{1/2}C\upsilon^{1/2}$……...(S4)

$Ip = -[0.0286 \pm 0.001\ mA\ [M]^{-1} \times [\text{concentration of tDNA (M)}] + [0.518 \pm 0.011]\ mA$, $R^2 = 0.99$ ….....(S5)