Supporting Information

Polyaniline Wrapped Aminated Graphene Composite on Nickel Foam as Three-Dimensional Electrodes for Enzymatic Microfuel Cell

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Characterisation of GO, NH_2-G, PAni and NH_2-G/PAni by AFM, FTIR, Raman and XRD:

Polyaniline wrapped aminated graphene (NH_2-G/PAni) was synthesized through *in-situ* aniline polymerisation on the surface of aminated graphene sheets in the acidic condition.[1] The characterization was carried out using AFM, FTIR, Raman spectroscopy and XRD. AFM image in Figure S1a demonstrated the size variation in GO nanosheets. The lateral dimension of nanosheets was found to vary between 1 to 4 μm with uniform thickness in the range of 1 nm. In FTIR spectra (Figure S1b), peaks identified at 1628 and 1723 cm^{-1} in GO corresponded to C=O bonds of carbonyl and carboxylic acid, respectively, while peak detected at 3434 cm^{-1} was identified as hydrogen bonds present in O-H group. Further, the peaks observed at 655 cm^{-1} and 1224 cm^{-1} corresponded to the epoxy group and C-O bonds, respectively. After amine functionalization, the significant decay of 3434, 1723, and 1628 cm^{-1} peaks indicated the successful reduction and surface functionalization of GO.[2] The N-H plane stretching of

aminated graphene was observed at 1580 cm^{-1}, while the N-H bond stretching was observed at 3300−3500 cm^{-1}. Further the peak observed at 1020-1250 cm^{-1} arose from stretching mode of C−N bond present in aromatic amines.[3] In FTIR spectra of PAni, peaks detected at 1481 cm^{-1} and 1293 cm^{-1} corresponded to the quinoid ring distortion with the vibration of C=C bond and benzoid ring deformation with the stretching of C-N bond, respectively. Raman spectroscopy carried out on NH_2-G sample showed peaks at 1344 and 1590 cm^{-1} attributed to disordered phase (D) and graphitic (G) phase of carbon, respectively (FigureS1c). The intensity ratio between D band and G band also confirmed the disordered and graphitic nature of carbon. The intensity of G band is lower than D band in NH_2-G indicated the increase in disordering of graphite with the amine functionalization.[4] In the case of PAni, peaks observed at 1160, 1172 and 1504 cm^{-1} are assigned to aniline ring vibration due to C-H bond stretching, C-H and N-H bending vibrations, respectively. The bipolaronic vibrations of quinoid ring were identified by the peaks at 572, 638, 720 and 812 cm^{-1}. The polaronic vibration of amine was observed at 512 cm^{-1}. Further, the peak at 406 cm^{-1} corresponded to the polaronic torsion in C-N-C bond.[5] For NH_2-G/PAni, the peak identified at 1590 cm^{-1} were broader as compared to NH_2-G curve, which confirmed the effective unification of polyaniline with the aminated graphene.[6] XRD peaks observed at 11.1° of (001) plane confirmed the formation of GO with the interlayer spacing of ~0.79 nm (Figure S1d). After the solvothermal treatment of GO with ethylene glycol and aqueous ammonia, the peak was observed at 2θ ~ 23.6° with the interlayer *d*-spacing of ~0.37 nm confirmed the formation of NH_2-G. The peak broadening occurred due to loose attachment of aminated graphene. The XRD peak of PAni was observed at 2θ ~ 23.4°. The interlayer spacing was calculated to be ~0.35 nm. The slight shifting of peak for NH_2-G/PAni composite confirmed the wrapping of PAni over graphene with the interlayer spacing of ~0.39 nm.[1]

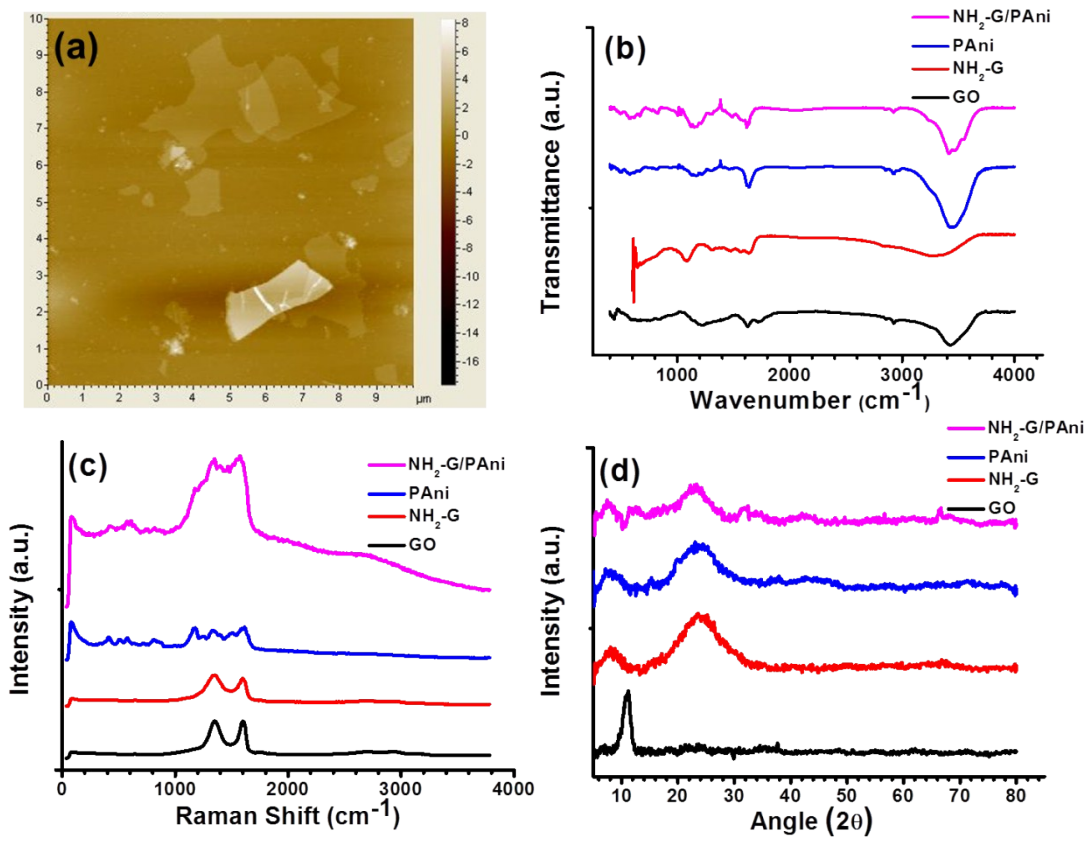

Figure S1: FTIR, Raman and XRD

Figure S1: (a) AFM (b) FTIR (c) Raman and (d) XRD pattern of GO, NH_2-G, PAni and NH_2-G/PAni composite.

Morphological Characterization: FESEM images of NH_2-G/PAni-2 and NH_2-G/PAni-3 composites are shown in Figure S2. From the images, it is evident as PAni content was increased as in the case of NH_2-G/PAni-3, the composite morphology appeared more like PAni i.e., more grainy in nature and less of flake like structure as seen for NH_2-G/PAni-2.

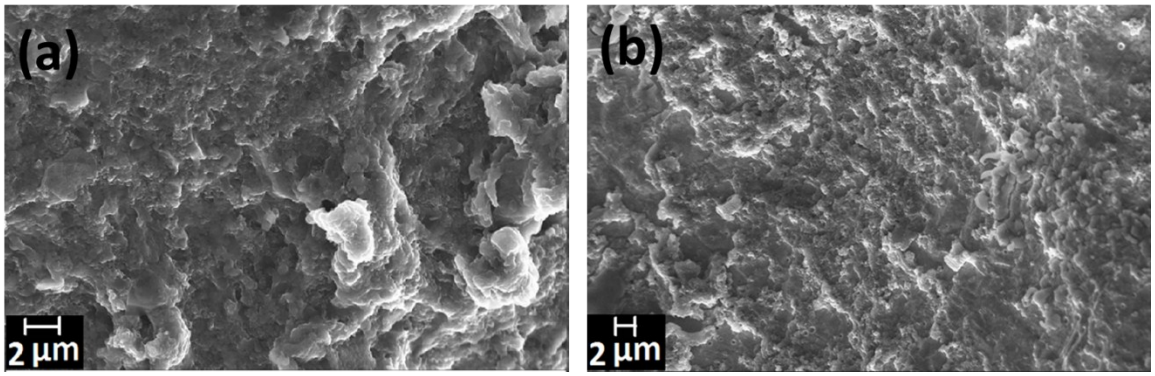

Figure S2: FESEM images of (a) NH_2-G/PAni-2 and (b) NH_2-G/PAni-3 composites.

Cyclic Voltammogram Studies: CV performed on bare and amine functionalized Ni foam after attachment of enzyme using cross-linker molecule is shown in Figure S3. The scan was performed in the voltage range -0.2 to +0.6 V vs Ag/AgCl as reference electrode and platinum wire as counter electrode at 10 mV s^{-1}.

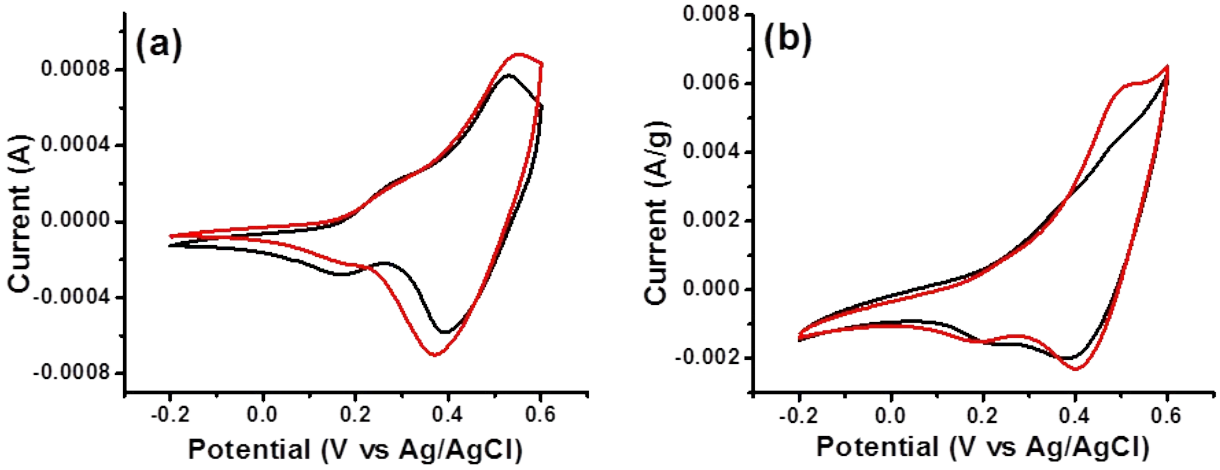

Figure S3: Cyclic voltammogram for PEGDGE:GOx (1:2) immobilized on (a) bare and (b) amine functionalized Ni foam in absence (black) and presence (red) of glucose at 10 mVs^{-1}.

Doping and Stability of GOx Enzyme on Electrode Surface:

Immobilization of enzyme was analyzed by measuring the FITC-labelled glucose oxidase enzyme present on the electrodes. For this purpose, electrodes were immobilized in FITC-labelled GOx solution for 24 hours and washed thrice with PBS solution. UV-Visible absorption spectra was recorded for FITC-labelled GOx solution, the resultant solution after electrode immobilization. **Figure S4** showed the absorption curve obtained for amine functionalized Ni foam and NH_2-G/PAni-1 electrodes that were prepared using third protocol. From **Figure S4a**, it is clear that enzyme attachment is very poor onto amine functionalized Ni foam electrode as more than 85% enzyme has been estimated in the discarded solution.

However, in the case of amine functionalized NH_2-G/PAni-1 shown in **Figure S4b**, almost 68 % enzymes are retained onto the electrode and they are intact after subsequent washes. **Table S1** detailed of the amount of enzyme present on the electrode surface of Ni and NH_2-G/PAni-1 for the three protocols adopted. The results clearly indicate that enzyme attachment is better on the composite material and best results are obtained for amine functionalized NH_2-G/PAni-1.

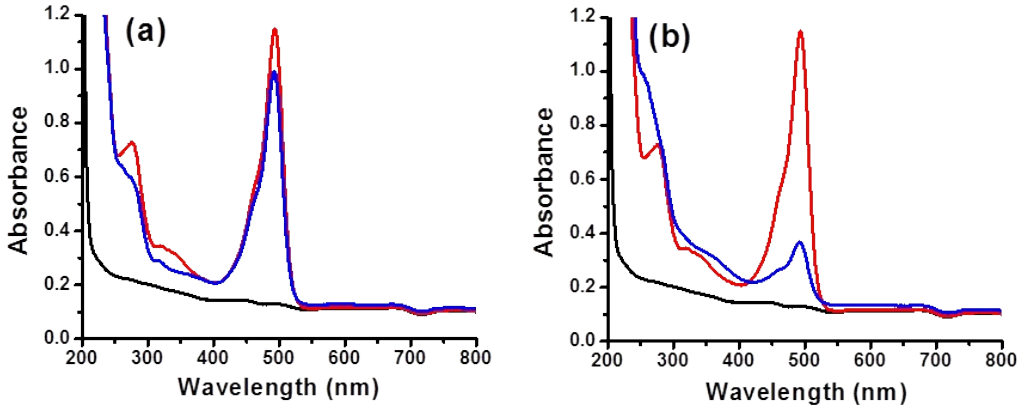

Figure S4: Absorption curve obtained for amine functionalized (a) Ni foam and (b) NH_2-G/PAni-1 electrodes that prepared using third protocol. Legend: Red- FITC labelled GOx-PEGDGE in PBS solution, Blue- after washing and Black – PBS solution.

Table S1: Estimation of amount of GOx washed off after immobilization using three different protocol for amine functionalized (a) Ni foam and (b) NH_2-G/PAni-1 electrodes.

Sample	**Wash 1 (%)**
Ni Proto-1	83.40
Ni Proto-2	91.79
Ni Proto-3	86.28
NH_2-G/PAni-1 Proto-1	58.99
NH_2-G/PAni-1 Proto-2	53.33
NH_2-G/PAni-1 Proto-3	32.06

To visualize the immobilization of enzymes on various electrodes surfaces, we have carried out fluorescence imaging. We have used FITC labelled GOx enzyme for immobilization to monitor them under fluorescence microscope. In **Figure S5** fluorescence image captured from various electrodes are shown. The green fluorescent signal is very weak from Ni, PAni and NH_2-G based electrodes (see **Figure S5a-c** respectively) and good from NH_2-G/Pani-1 composite coated electrode (see **Figure S5d-f)**. The best results are obtained for amine functionalized NH_2-G/Pani-1 coated electrode utilizing PEGDGE cross-linker molecule as it shows uniform green fluorescence speckled over the surface of the electrode. Hence, it can be concluded that immobilization utilizing PEGDE as cross-linker is most effective in chemically bonding the enzyme onto the electrode surface.

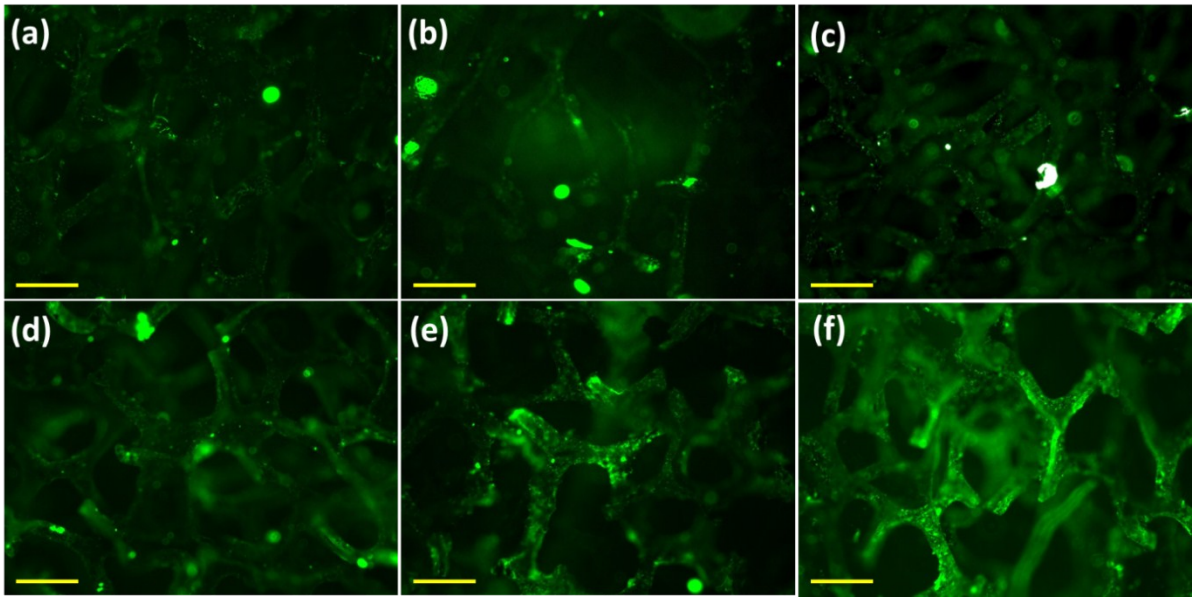

Figure S5: Fluorescence image obtained for FITC labelled GOx-PEGDGE for (a) Ni foam, (b) PAni and (c) NH_2-G and on NH_2-G/Pani-1 electrode after following three protocols (d-f) respectively (scale -100 μm).

For checking the stability of enzymes on the electrode surface, we have performed cyclic voltammogram on the electrode, stored the same for six weeks at 4 ºC in PBS solution and then performed the cyclic voltammogram again. The change in current was less than 9% from the

initial run as seen in **Figure S6**. This results indicate that enzymes were quite stable and enzyme activity losses due to storage and several runs was quite small and negligible.

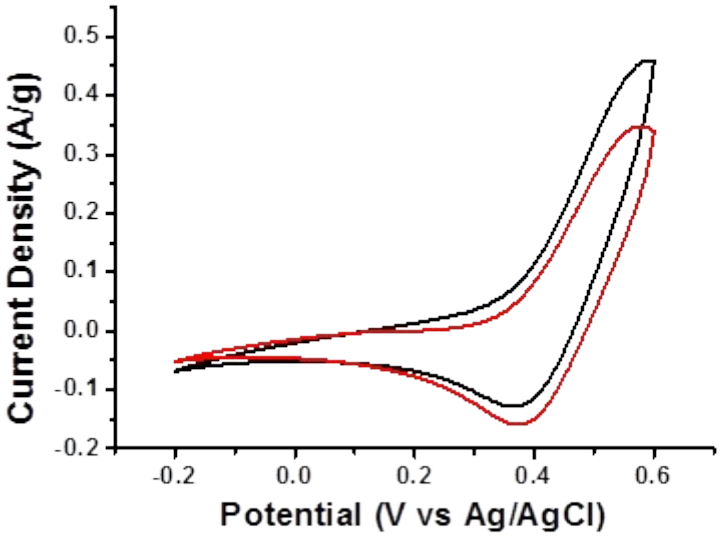

Figure S6: Cyclic voltammogram on NH_2-G/Pani-1 electrode before (black) and after (red) storage for 6 weeks in PBS solution.

References

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