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Supporting Information

<u>S1. Experimental Method</u>

S1.1 Preparation of *f*-MWCNT ink and electrode fabrication



Fig.S1. Fabrication of *f*-MWCNT-PF nanocomposite electrode: (a) MWCNT in ethanol (before sonication), (b) *f*-MWCNT ink after sonication for 10 hrs, (c) *f*-MWCNT film made on glass slide by doctor's blade method, (d & e) flexible MWCNT-PF nanocomposite developed after thermal transfer technique, and (f) *f*-MWCNT-PF nanocomposite bioelectrode at glass plate.

Oxidation of *p*-MWCNTs was performed by stirring 50 mg of *p*-MWCNTs with a mixture of H_2SO_4 and HNO_3 3:1 (v/v) in a round-bottomed flask at 60°C for 6 h. Oxidized MWCNTs (*f*-MWCNTs) were repeatedly washed with double-distilled water until the wash solution/water reached a neutral pH. It was followed by oven drying at 120°C for 24 hrs. The amount of surface carboxylic functions formed at MWCNT was determined by the Boehm titration method (1) using sodium bicarbonate and sodium hydroxide bases.

The *f*-MWCNTs- ink was prepared by placing 50 mg of *f*-MWCNTs in a glass bottle (15 ml), and 1.0 ml of ethanol was added at intervals of one hour while continuously sonicating the mixture for 110 hrs. Exposure of CNTs to intense ultrasonic waves could mechanically damage the conductive structure of CNTs due to stress and heat (2, 3) therefore, the sonication is done at low power ('100 watts) in an ice-bath so that the temperature of the bath does not rise above 40°C and do not affect the CNT structure adversely. It also prevents ethanol evaporation. The resulting stable, uniform ink containing f-MWCNTs dispersed in ethanol was manually transferred to a glass slide (6 x 2 cm²) using the doctor's blade method. The film was dried after keeping it overnight at room temperature so that all of the ethanol was evaporated. A piece of PF (6 x 2 cm²) was casted over a MWCNT film spread on the glass plate and heated on the hot plate at constant temperature (65°C) to form a nanocomposite film. Finally, the f-MWCNT-PF was cut into small pieces using a sharp scissor and cleaned by sonication in ethanol and water for 5 min, in turn, and then dried in an oven for further use. The small pieces of nanocomposite film were employed for various studies and directly integrated with wire to be used as working electrodes. The steps of fabrication of f-MWCNT-PF nanocomposite electrode are shown in Fig.S1.

S1.2 Potentiometric Titration study for the determination of carboxylic acid functional groups at *f*-MWCNT (powder) surface and *f*-MWCNT-PF nanocomposite film

The amount of carboxylic acid group generated at *p*-MWCNT surface by chemical oxidation was determined by Boehm titration (1). The *f*-MWCNTs powder (0.2 mg) and pieces of *f*-MWCNT-PF nanocomposite film containing the same amount of *f*-MWCNT were taken in different test tubes containing 10.0 mL Na₂CO₃ (0.04N) solutions. Each mixture was agitated for 30min and kept overnight for 18 hrs to reach the equilibrium state. The resulting mixtures were titrated with 0.04N HCl using a pH meter (Hanna HI5522-01). A blank experiment (without MWCNTs) was performed as above. All steps were executed at room temperature in triplicate. The amount of oxygen functional group generated was calculated from first derivative plot.



Fig. S1.2. Titration curves and their first derivative of *f*-MWCNT (a & b), *f*-MWCNT-PF nanocomposite film (c & d) with 0.04 N Na₂CO₃ against 0.04 N HCl.

Potentiometric titration curves for *f*-MWCNT and *f*-MWCNT-PF nanocomposite film are shown in Fig.S1.2a & c respectively. Since the theoretical equivalence point was not clear from the potentiometric titration curves, therefore, corresponding first derivative plots were obtained as represented in Fig. S1.2 (b & d). The potentiometric quantification of surface carboxylic groups attained in *f*-MWCNT and *f*-MWCNT-PF nanocomposite film are 1.2949 mmol/g of MWCNT and 1.1466 mmol/g respectively.

S2. GOx immobilization on f-MWCNT-PF nanocomposite

EDC-NHS chemistry was used to immobilize GOx at the surface of the *f*-MWCNT-PF nanocomposite with carboxylic functional groups to form a GOx-*f*-MWCNT-PF nanocomposite electrode. In short, a section of the *f*-MWCNT-PF nanocomposite surface was

activated by treating it with 0.1 M NaOH solution for 90 s, followed by a reaction with EDC (400 mM) and NHS (100 mM) solution at pH 7.0 with continuous stirring for 2 h at room temperature. A semi-stable amine-reactive NHS-ester was obtained, and excess EDC was washed off from the *f*-MWCNT-PF nanocomposite with cold distilled water. The activated CNTs were further treated with GOx solution (1mg/ml) containing sodium phosphate buffer (0.1M) at pH 7.4 with continuous stirring for an additional 2 h at room temperature. Finally, the material was washed with phosphate buffer containing 0.5% BSA to remove excess GOx until no oxidase activity was detected in the washing buffer solutions. The supernatant solution was used for protein analysis. Washed nanocomposite films were directly used for the determination of enzyme activity and stability studies.



S3. EDAX Images of MWCNTs and *f*-MWCNT-PF nanocomposite

Fig. S3. EDX graph of (a) *p*-MWCNT, (b) *f*-MWCNT, and (c) *f*-MWCNT-PF nanocomposite. Inset showing the wt% and atomic% of the different elements present in the samples.

References:

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