Supplementary information

Benzene layers-aligned electrochemical transformation of SWCNTs to redox-active macro-walled CNTs: Enabling oxygen interference-free monitoring of ROS release from HeLa cancer cells

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S1. 2′,7′-Dichlorodihydrofluorescein diacetate assay

After the ascorbic acid (AA) treatment, the HeLa cells were pelleted using a centrifuge at 2000 RPM, and the cell pellets were washed with PBS. Later, a working concentration of 20μM of 2′,7′-Dichlorodihydrofluorescein diacetate (H2DCFDA) mixed with PBS, and added to the cells and kept in the dark for 30 minutes [68]. After the incubation, the cells were pelleted again using a centrifuge and diluted with 2ml of PBS for ROS measurement. The H2DCFDA molecule initially enters the cell membrane. The esterases enzymes present inside the cell remove the acetate functional groups from H2DCFDA. During these processes, intermediate H2DCF is formed. The ROS present inside the cells oxidize the intermediate H2DCF, turning it into fluorescent (FL). Similarly, from the demonstrated experiment, we observed an increment in the FL counts, confirming the presence of ROS under induced conditions (Fig. S2, curve a & b).

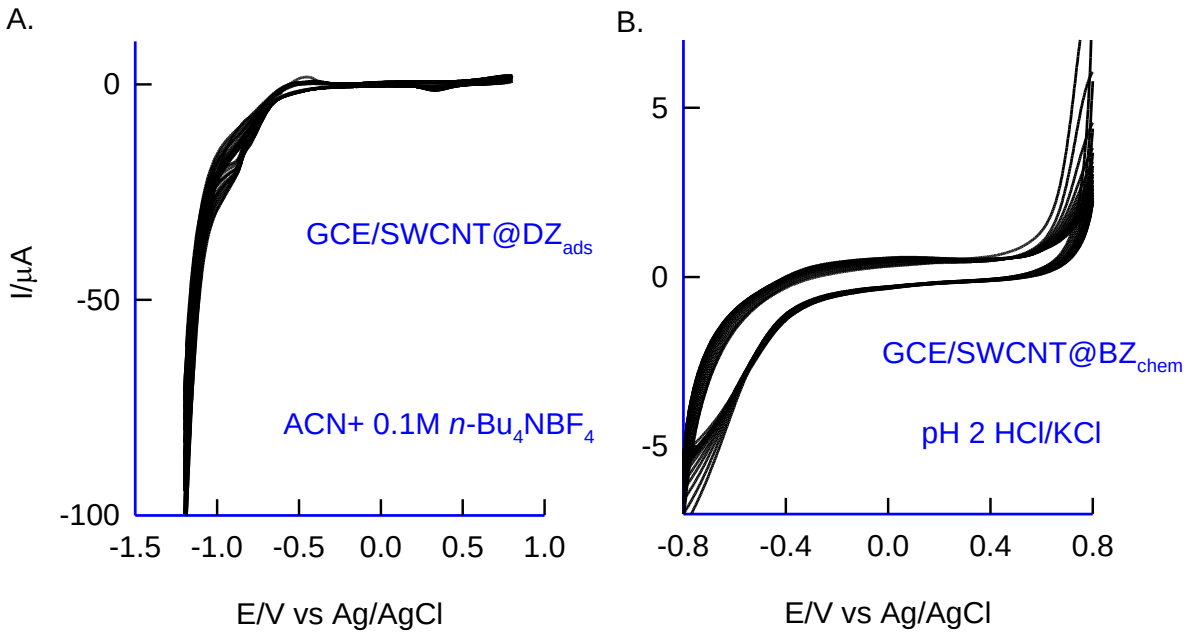

Fig. S1. Continuous CV response of (A) GCE/SWCNT@DZ_{ads} in presence of 5 mM of 4-nitrobenzenediazoammonium containing acetonitrile solution with 0.1M of n-Bu_4NBF_4 (N_2 purged) (B) CV response of GCE/SWCNT@BZ_{chem} in presence of pH 2 HCl-KCl electrolyte at the scan rate of v= 50 mVs^{-1}.

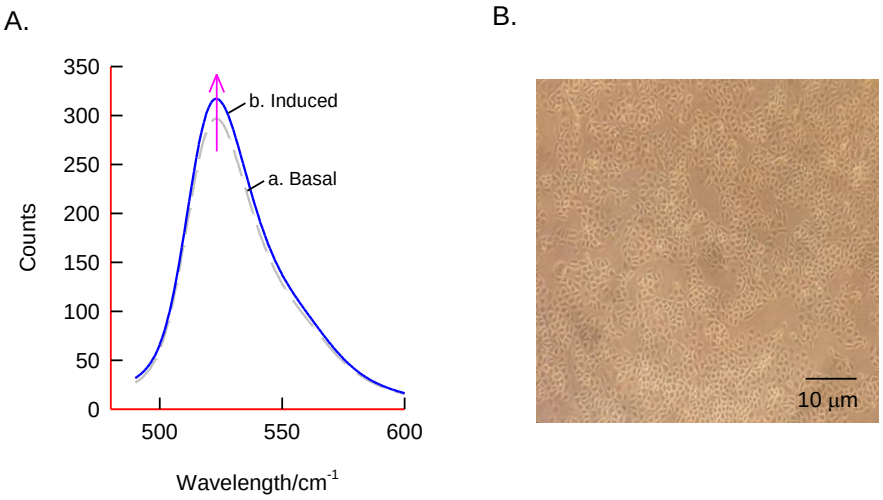

Fig. S2. (A) Fluorescence response of HeLa cells incubated for 60 mins (a) Basal and (b) Induced level with 10μM AA. (B) Microscopic image of HeLA cells.

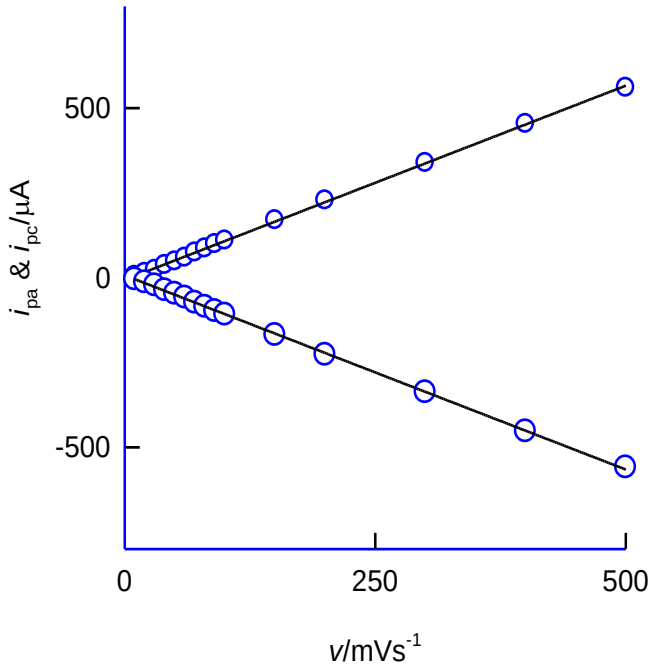

Fig. S3. The plot of peak currents (i_{pa} & i_{pc}) vs v for the scan rate effect CV responses of GCE/SWCNT@BZ-Redox in pH 2 HCl/KCl solution.

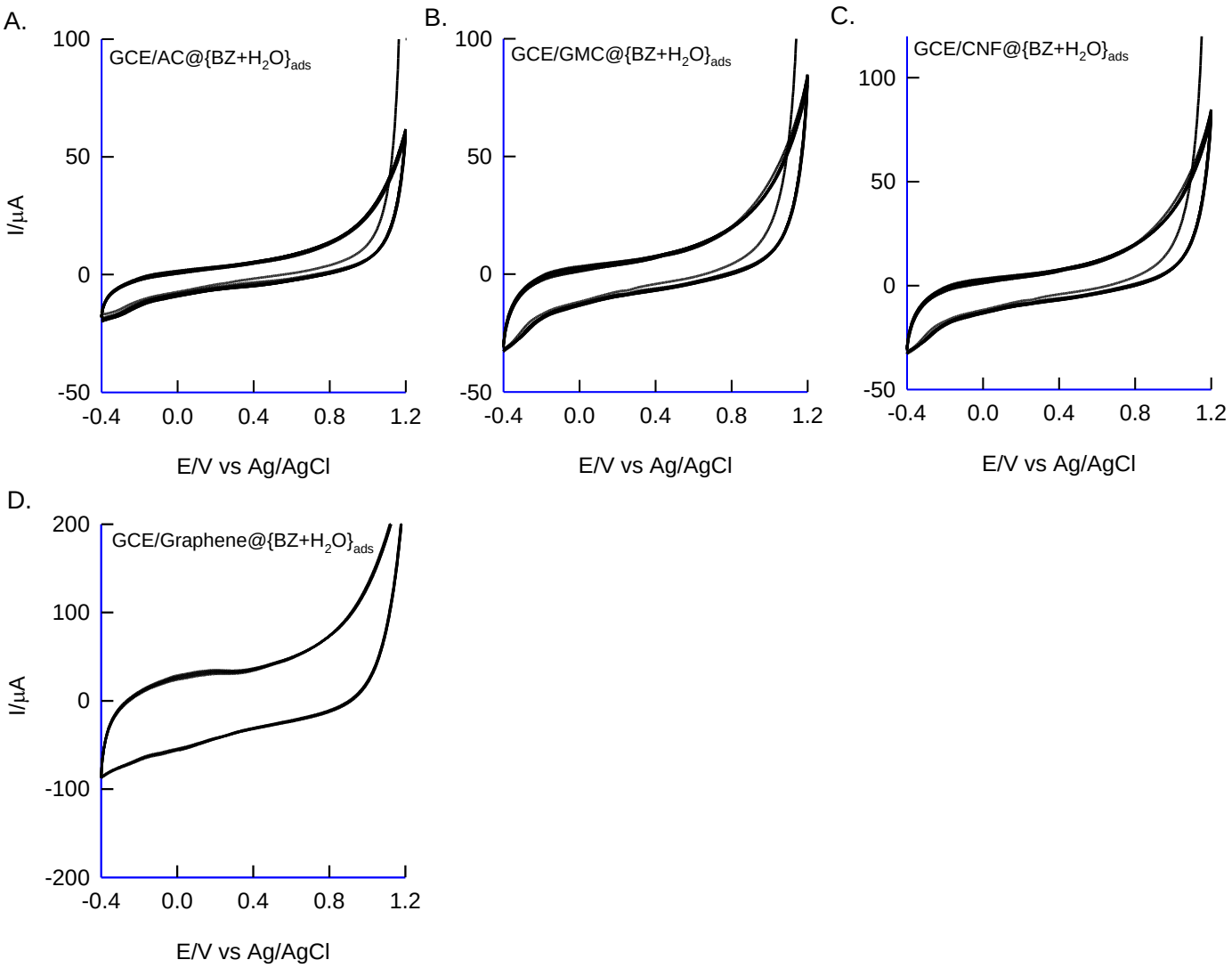

Fig. S4. Effect of carbon nanomaterial. Continuous CV responses of (A)GCE/AC@{BZ+H_2O}$_{ads}$, (B)GCE/GMC@{BZ+H_2O}$_{ads}$, (C)GCE/CNF@{BZ+H_2O}$_{ads}$ and (D) GCE/Graphene@{BZ+H_2O}$_{ads}$ in presence of pH2 HCl/KCl solution at the scan rate of v= 50 mVs^{-1}. AC-activated charcoal; GMC-graphitized mesoporous carbon; CNF- carbon nanofiber.

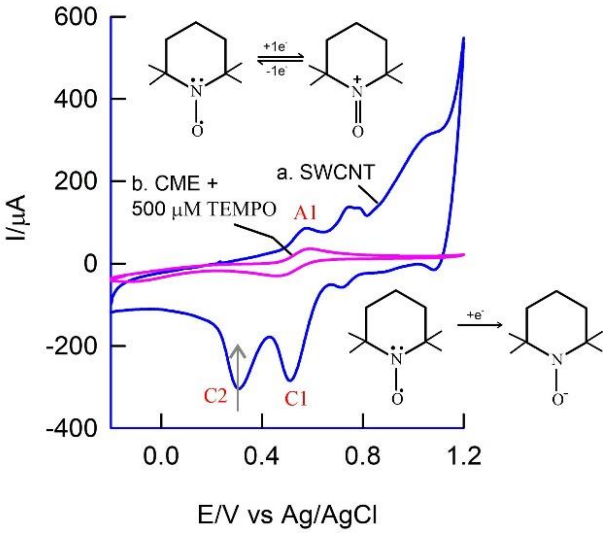

Fig. S5. CV response recorded with (a) GCE/SWCNT and (b) GCE/SWCNT@BZ-Redox in 500 μM of TEMPO containing pH 2 HCl-KCl solution (N_2 purged). Insets are typical molecular reactions of the TEMPO

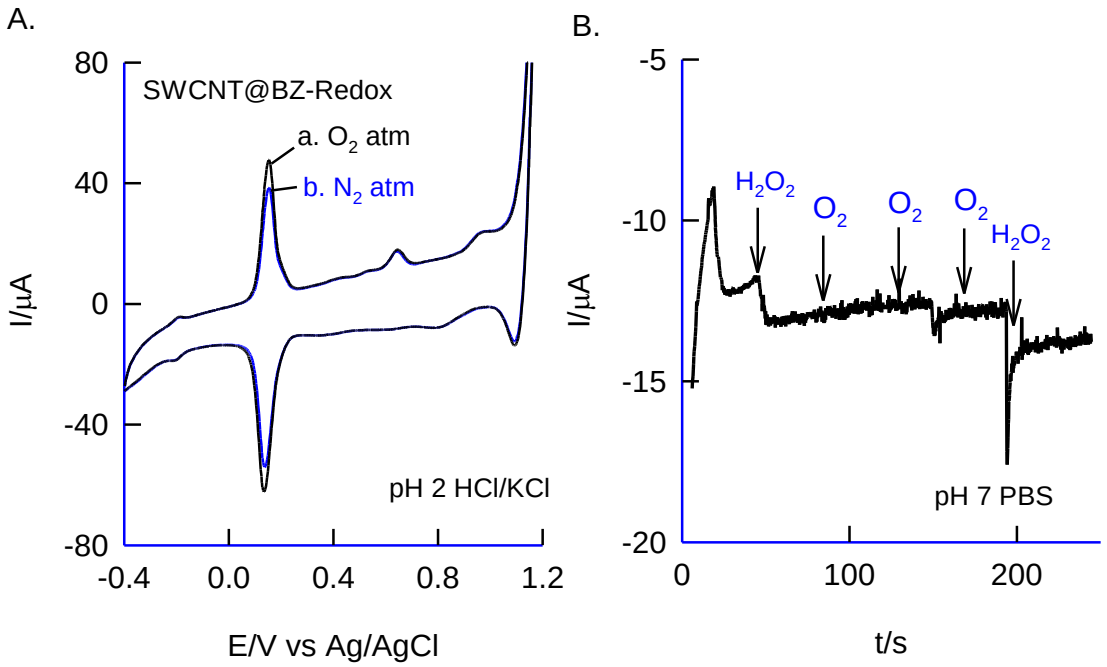

Fig. S6. Effect of dissolved oxygen. (A) CV responses of SWCNT@BZ-Redox in presence of saturated (a) O_2 atm and (b) N_2 atm in pH 2 HCl/KCl solution at v=10 mVs^{-1}. (B) Amp i-t response of the SWCNT@BZ-Redox with continuous spikes of 50µM H_2O_2 and O_2 saturated solution at E_{app}=-0.25 V vs Ag/AgCl in 0.1M PBS of pH 7

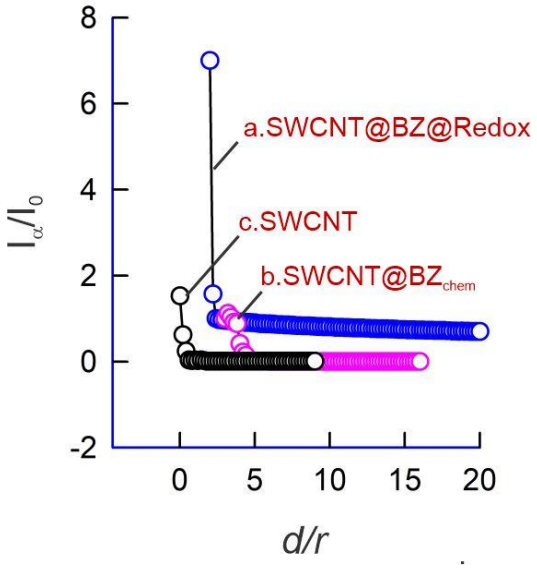

Fig. S7. SECM approach curves of (a) SWCNT@BZ-Redox, (b) SWCNT@BZ_{chem} and (c) SWCNT modified substrates with a 25µm Pt_{tip} electrode-probe in 5 mM $[Fe(CN)_6]^{3-}$ containing pH 2 HCl/KCl solution; E_{tip}=0.5V vs Ag/AgCl, E_{sub}=0.3V vs Ag/AgCl.

Table S1. Tabular column representation of various CMEs reported for electrochemical sensing applications for H_2O_2 detection in HeLa cells

S.No	Modified electrode	Soln	Linear range/ µM	LOD/µM	Sensitivity µA/ μM^{-1}	Ref	Remarks
1.	GCE/NG	PBS	0 − 3000	0.054	−	[73]	ORR interference [61]
2.	GCE/FF hydrogel-Fmoc/HRP	PBS	0.1 − 60.2	0.018	0.29	[57]	Temperature sensitivity [74]
3.	GCE/MIL-100(Cr)-B	PBS	0.5 − 3000	0.1	0.047	[58]	ORR interference [75]
4.	GCE/SGN@NCs	PBS	1 – 300	0.3	−	[59]	ORR interference [62]
5.	PB-EA	Locke's Buffer	2 –5000	1.9	-0.010	[76]	Instability in the neutral pH [77]
6.	GCE/SWCNT@BZ-Redox	PBS	2-60	0.53	1.2	This work	Highly stable, ORR interference free

NG-Nitrogen doped graphene; Fmoc-FF- N-Fluorenylmethpxycarbonyl diphenlaline; HRP- Horseradish peroxidase; PB-EA- Prussian blue-based electrode array; SWCNT-Single walled carbon nanotube; BZ- Benzene; SGN-SnO_2/Graphene nanosheet; NCs- Nanocages

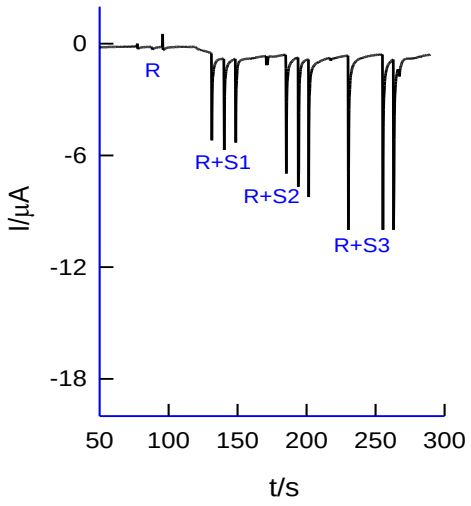

Fig. S8. BIA responses of SPE/SWCNT@BZ-Redox for the analysis of a tannery waste sample using standard addition approach at an applied potential, E_{app}= -0.25V in presence of PBS of pH 7. R= Real sample and S1-S3= Standard H_2O_2 samples of 5,10 and15µM respectively.