Monitoring oxidative stress in BT20 and 4T1 cancer cells with chronoamperometry

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INTRODUCTION

Oxidative stress (OS) can cause chronic inflammation, resulting in most chronic diseases including cancer, diabetes, cardiovascular and other diseases.[1] It is evident that tumor cells release more hydrogen peroxide (H_2O_2), a product of OS as compared to normal cells.[2] The combination of H_2O_2's transient nature and matrix effects make monitoring this molecule a challenge in biological samples.[3] BT-20 and 4T1 cancer cells are investigated by the selective and quantitative measurement of H_2O_2.

Prussian Blue Zinc oxide carbon nantoubes (PB/ZnO/COOH-MWNTs) sensor is useful for studying OS of cells. BT-20 cancer cells are related to primary human breast cancer. They are also a host of many transfection studies.[4] Since 4T1 cancer cells mimic human breast cancer, this cell line is important for stage IV human breast cancer studies. The standard addition methods (SAM) with chronoamperometry (CA) shows a good linear response in (1.0-21.0) μM range (R^2 = 0.9932) for monitoring H_2O_2 concentration as an OS markers for rapid quantification that offers advantages over standard enzyme linked immunosorbent assays (ELISA). [3]

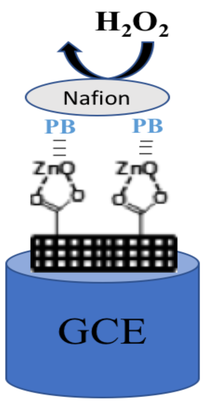


Figure 1: PB/ZnO/COOH-MWNTs sensor to monitor H_2O_2

EXPERIMENTAL

After synthesizing ZnO using a reflux process, equal masses of refluxed ZnO and COOH-MWNTs (4.0 mg each) were vortex mixed in 1.0 mL absolute anhydrous ethyl alcohol (AAEA) solvent to prepare ZnO/COOH-MWNTs electrocatalyst composite. Then, the mixture was sonicated for 1 hr. After drying the composite at 80^0 C, 4.0 mg of ZnO/COOH-MWNTs and 2.0 mg of PB were added to 1.0 mL of phosphate buffer solution (PBS) at pH 6.6 for attachment and stirred for 5 hrs using magnetic stirrer in small glass vials. Glassy carbon electrodes (GCEs) were polished using a 1.0-μm diameter Al_2O_3 slurry, and then further polished using a 0.05-μm diameter Al_2O_3 slurry to obtain a mirror like finish. They were then cleaned by sonication in a 1:1 volume mixture of concentrated HNO_3:H_2O for 5 min. A 5-μL colloidal aliquot of PB/ZnO/COOH-MWNTs nanocomposite was drop-casted onto a 3 mm diam. GCE. After drying for 20 min, a 10-μL aliquot of 2% Nafion™ was dropped on top to cap the composite and dried in an oven at 80^0 C for an additional 12 min to obtain the Nafion™/PB/ZnO/COOH-MWNTs/GCE sensor for further study. [3]

RESULTS

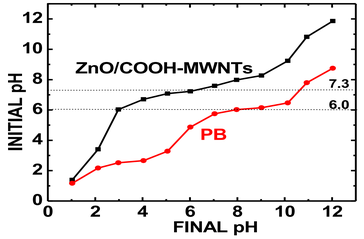


Figure 2: PZC of PB and ZnO/COOH-MWNTs

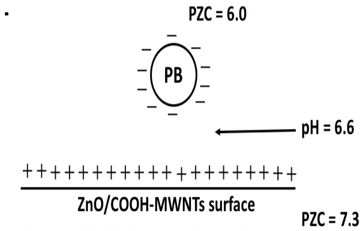
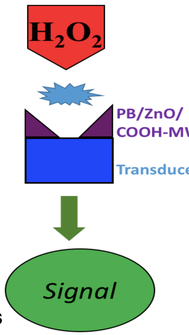


Figure 3: Scheme for electrostatic attraction



Cheaper bio-sensor for rapid analysis of oxidative stress in cells

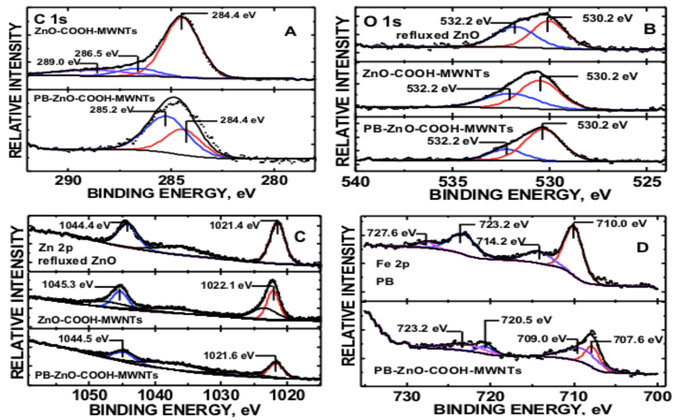


Figure 4: XPS analysis of PB/ZnO/COOH-MWNTs

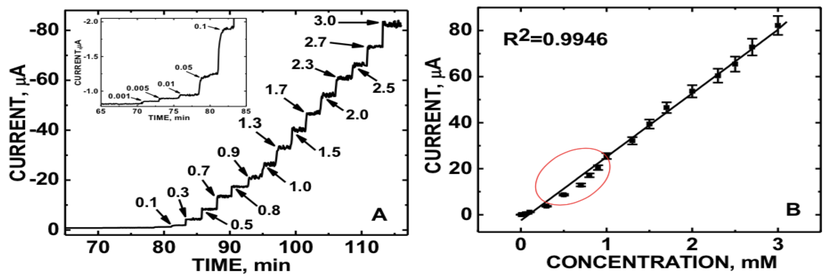


Figure 5: H_2O_2 under CA at pH 7.0 and its calibration curve

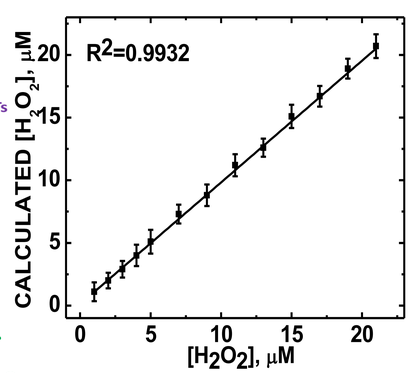


Figure 6: SAM CA validation control Plot at (1.0-21.0) μM H_2O_2 range using PB/ZnO/COOH-MWNTs

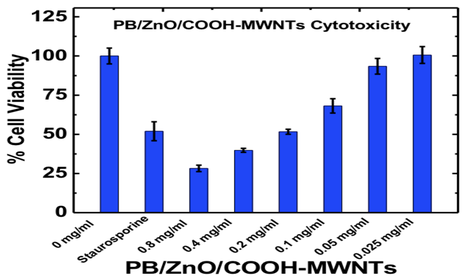


Figure 7: Cytotoxicity results of sesnor

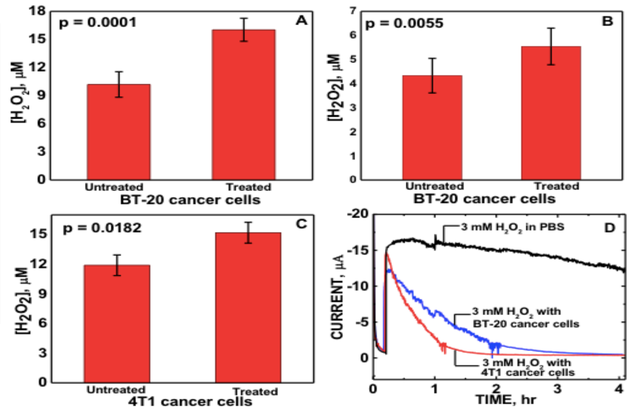


Figure 8: Comparison of H_2O_2 in cancer cells (A, C, D) using our sensor and (B) ELISA

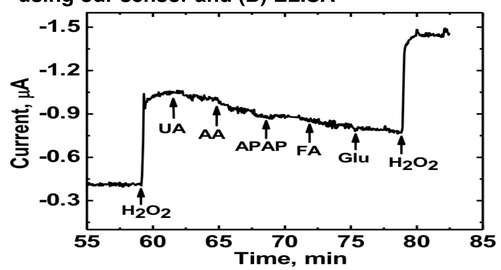


Figure 9: Selectivity study of H_2O_2 using CA

CONCLUSIONS

- Fe in PB was minimized to low content (~0.04 atom%) to avoid reactive oxygen species artifacts via Fenton reactions.
- PB/ZnO/COOH-MWNTs detects H_2O_2 in the range of 1 μm to 3 mM under CA with 0.0 V at pH = 7.0.
- Excellent linearity within the assay range (1.0-21.0) μM H_2O_2 under SAM CA was applied to study oxidative stress of H_2O_2 in breast cancer cell lines.
- Selectivity of PB/ZnO/COOH-MWNTs for H_2O_2 is pH dependent.
- SAM CA was successfully applied in BT-20 and 4T1 cancer cells for studying oxidative stress.

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