Electronic Supplementary Material (ESI)

Cu Single-Atom Catalysts-based Flexible Hydrogen Peroxide Electrochemical Sensor with Oxygen Resistance for Monitoring ROS Bursts

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1. Experimental Procedures

1.1 Reagents and Instrumentation

Sodium hydroxide (NaOH), potassium dihydrogen phosphate (KH_2PO_4), copper nitrate ($Cu(NO_3)_2$), hydrofluoric acid (HF), hydrogen peroxide (H_2O_2) were purchased from Sinopharm Chemical Reagent Co. LTD. Hexaazatriphenylenehexa cabonitrile (HAT-CN_6) was purchased from Macklin. Glucose (Glu), glucose oxidase (GOx), uric acid (UA) and Nafion (5 wt%) were obtained from Sigma-Aldrich. Polydimethylsiloxane (PDMS) was purchased from Dow Coming. Chemicals were of at least analytical reagent and were used as received. All aqueous solutions were prepared with ultrapure water (18.2 MΩ cm^{-1}).

Electrochemical detections were performed by using a conventional three-electrode system. Bare or modified glassy carbon electrodes (GCE), standard Ag/AgCl (3 M KCl) and a platinum wire were served as the working electrode, reference electrode and counter electrode, respectively. The electrochemical measurements were performed on a computer-controlled electrochemical analyzer (CHI 1030, Shanghai Chenhua Instruments, Co., China). The morphology of the samples was taken with a Hitachi H7650 transmission electron microscope (TEM) and a Hitachi SU-8010 scanning electron microscope (SEM). High-angle annular dark-field scanning transmission electron microscope (HAADF-STEM) images and spherical aberration correction images were performed by FEI Themis z at 200 kV. High-resolution transmission electron microscopy (HR-TEM) images and their corresponding energy-dispersive X-ray spectroscopy face-swept elemental distributions (mappings) were conducted with a JE 2100F from Japan Electronics Company (JEOL). Powder X-ray diffraction (XRD) was performed by a Shimadzu XRD-6100 using Cu Kα radiation source (λ = 1.5406 Å). X-ray photoelectron spectra (XPS) were obtained by Thermo Fisher's EscaLab using Al^+ radiation at 3.1 × 10^{-8} Pa at room temperature.

1.2 Electrochemical Testing

The electrochemical tests were carried out at CHI 1030 electrochemical workstation (Shanghai Chenhua Instruments) in 50 mM PBS (pH 7.4) buffer system. Flexible stretchable electrodes or glassy carbon electrodes modified with materials were used as working electrodes, Ag/AgCl electrodes were used as reference

electrodes, and platinum wires were used as counter electrodes. For electrochemical experiments, the H_2O_2 and other substances added to the reaction system were prepared or diluted with PBS (pH 7.4).

1.3 Culture of Human Umbilical Vein Endothelial Cells

The lyophilized cell suspension is thawed by shaking rapidly in a 37°C water bath. After that add 5 mL of culture medium and mix well. All cell suspensions were then added to the cell culture dishes and incubated at 37 °C in a humidified incubator containing 5% CO_2. The culture medium was changed the next day to remove residual DMSO and to check cell density. Thereafter, the medium was changed every 2 to 3 days, and when the cell density reached more than 80%, the cells were treated with trypsin and passaged.

Preparation of endothelial cell medium: Endothelial cell medium consists of 1 % penicillin and streptomycin (double antibodies) + 1 % endothelial cell growth factor + 5 % fetal bovine serum + 93 % endothelial cell basal medium. After preparation, the medium was stored in the refrigerator at 4°C and used for two weeks.

1.4 Electrochemical Experiments on Cells

The Cu SACs/PDMS electrodes were autoclaved at 120°C for 30 min, then removed on an ultra-clean table and coated with 250 μg/mL PDL solution for 5 min. Cells were first passaged (cell density of approximately 107 /mL), and then the sterilized PDL-coated Cu SACs/PDMS were placed in the newly passaged in the cell culture dish. The electrodes were removed after 24h of incubation and the surfaces were washed twice with 37°C PBS solution. Immediately afterwards, the electrode with cells incubated was fixed on the stretching device and conducted with copper wires for electrochemical experiments. The ambient temperature of the test and buffer solutions were maintained at about 37°C.

2. Supplementary Figures

2.1 Characterization of CN

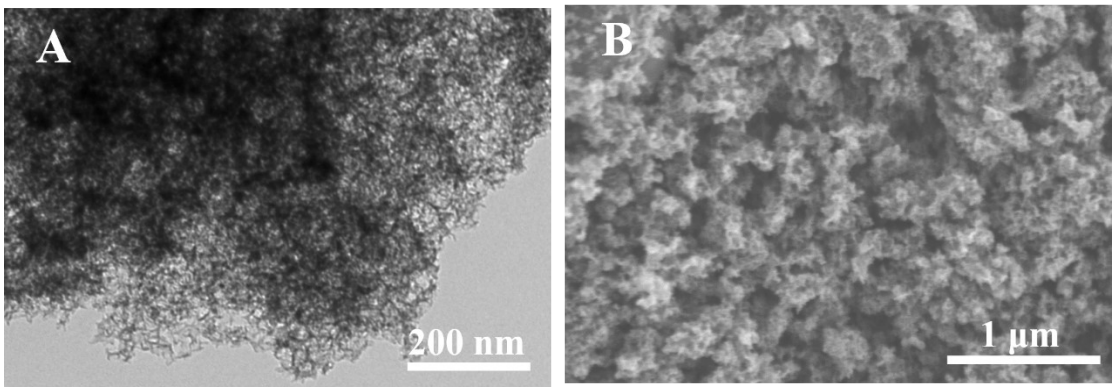

Figure S1. (A) TEM image of CN. (B) SEM image of CN.

2.2 Electrochemical Performance Analysis of CN for H_2O_2

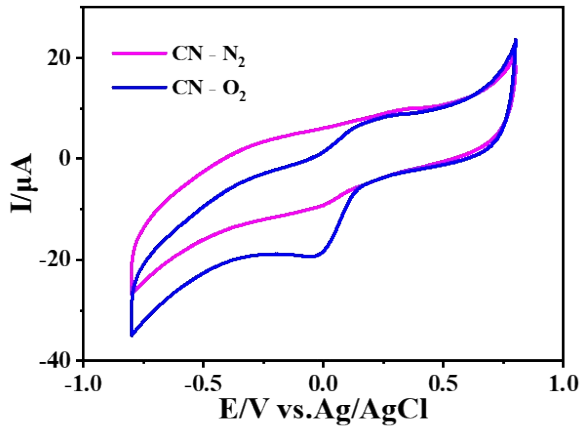

Figure S2. Cyclic voltammetry response of CN to O_2.

2.3 Characterization of Cu SACs/PDMS Electrodes

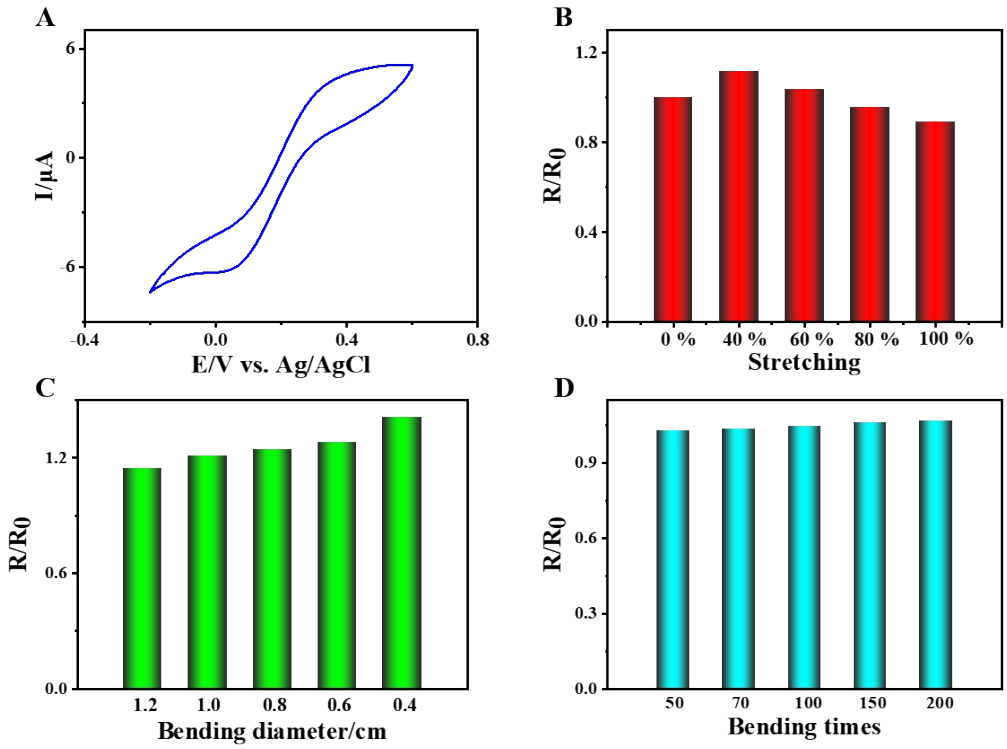

Figure S3. (A) Cyclic voltammetry response of Cu SACs/PDMS electrode in 50 mM $K[Fe(CN)_4]$ solution. (B) Relative changes of resistance of Cu SACs/PDMS electrode at different tensile levels. (C) Ohmic resistance of Cu SACs/PDMS electrode at different bending diameters. (D) Relative changes of ohm resistance of Cu SACs/PDMS electrode with different bending times at 0.8 cm bending diameter.

2.4 Schematic Diagram of Electrode Preparation

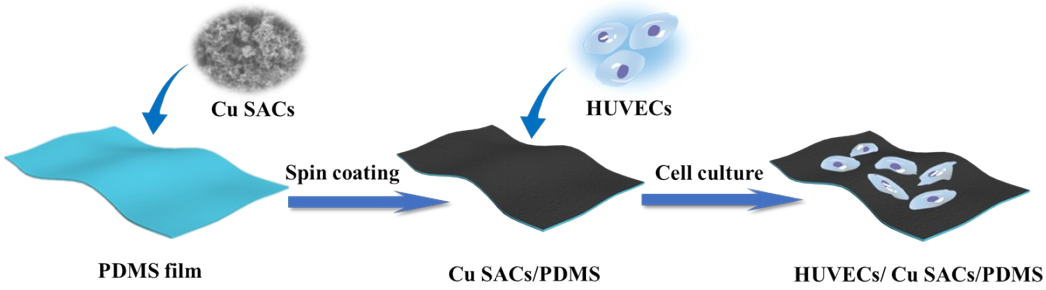

Figure S4. Flexible stretchable electrodes fabrication for the culture of human umbilical vein endothelial cells (HUVECs).

2.5 Cultured Cells with or without Electrodes

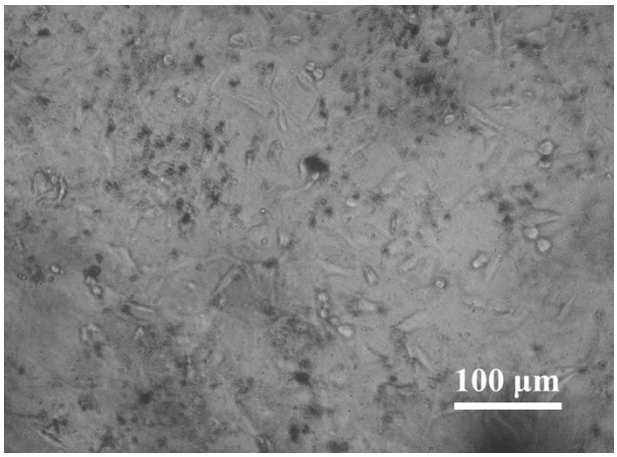

Figure S5. Morphology of HUVECs cultured for 24 h on Cu SACs/PDMS electrodes.

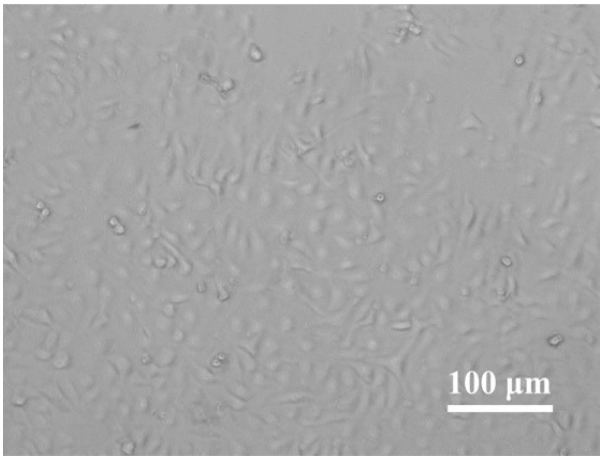

Figure S6. Morphology of HUVECs cultured for 24 h in petri dishes without electrodes.

2.6 GOx/Cu SACs/PDMS for Electrochemical Detection of Glu

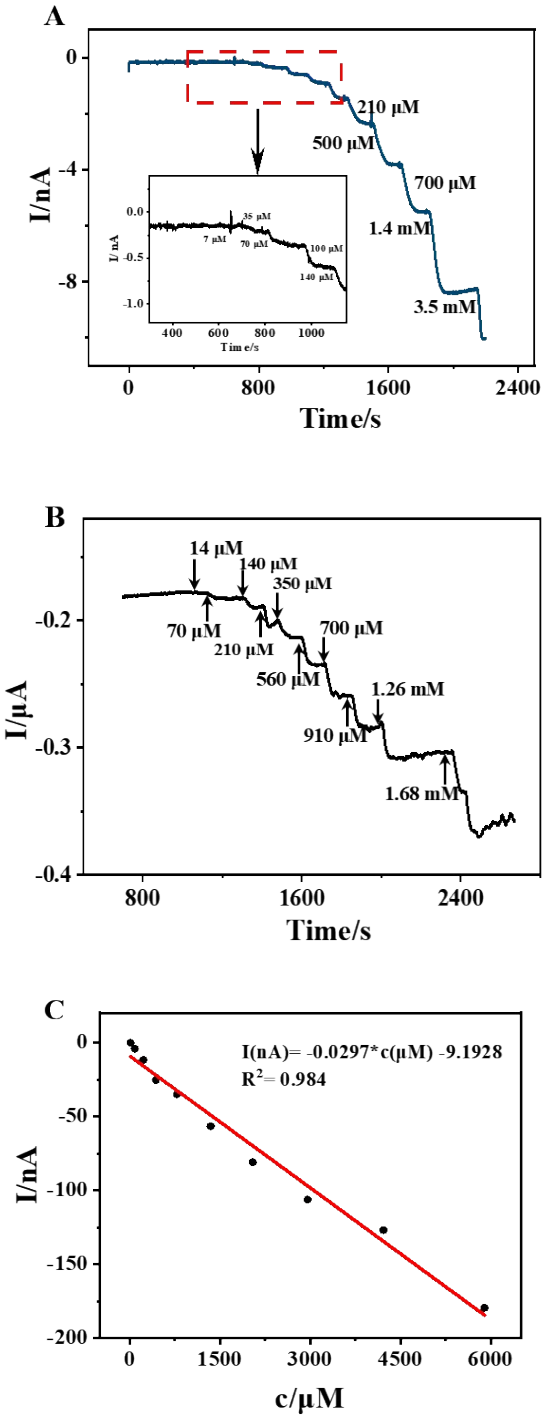

Figure S7. (A) Amperometric response of prepared GOx/Cu SACs/PDMS electrode at 0.20 V with an increasing concentration of H_2O_2. (B) Amperometric response of prepared GOx/Cu SACs/PDMS electrode at 0.20 V (vs. Ag/AgCl) with an increasing concentration of Glu. (C) The calibration curves of peak current intensities to the concentrations of Glu. I(nA) =-0.0297*[H_2O_2] (μM)-9.1928, R^2=0.984.