Electronic Supplementary Material (ESI)

Au Nanoflowers Film-based Stretchable Biosensor for In-situ Recording Superoxide Anion Release in Cell Mechanotransduction

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1. Experimental Procedures

1.1 Reagents and Instrumentation.

Ferrocenemethanol ($FcCH_2OH$), hydroxylamine hydrochloride ($NH_2OH{\cdot}HCl$) and chloroauric acid tetrahydrate ($HAuCl_4{\cdot}4H_2O$), Zymosan A, polydimethylsiloxane (PDMS), and L-Cysteine etc. were purchased from Sigma. L-Cysteine solution was freshly prepared and deoxygenated by bubbling pure nitrogen gas for at least 40 min prior to use. Bovine erythrocyte copper-zinc SOD (Cu_2Zn_2SOD, EC 1.15.1.1), xanthine oxidase (XOD, EC 1.1.3.22, bovine milksource), xanthine, uricacid (UA), H_2O_2, L-ascorbicacid (AA), dopamine hydrochloride (DA), 5-hydroxytryptamine (5-HT) and diphenylene iodonium (DPI) were purchased from Sigma. The $O_2^{\cdot-}$ solutions were prepared in O_2 saturated phosphate buffered solution (PBS) containing 0.002 unit of XOD and xanthine. The purity of other chemicals was at least analytical reagent and used directly. Electrochemical data was obtained by CHI 1030 electrochemical analyzer (Shanghai, China). A three-electrode glass cell equipped with a saturated Ag/AgCl (3 M KCl) reference electrode, a platinum wire counter electrode, and a working electrode was used. A combined energy dispersive X-rayspectroscopy (EDX) and elements mapping analysis system attached to the SEM were used for elemental analysis. X-ray-diffraction (XRD) was tested on a Bruker D8 advance powder diffractometer with Au radiation. X-ray Photoelectron Spectroscopy (XPS) were measured on a VG Micro-tech ESCA 2000.

1.2 Human Umbilical Vein Endothelial Cells (HUVECs) Culture.

HUVECs were cultured in a humidifi ed incubator (37℃, 95% air, 5% CO_2) and the culture medium (430167) contain 1% streptomycin and penicillin, 15% fetal bovine serum and 84% H-DMEM. In testing of cells, HUVECs were cultivated on SOD/Cys/Au SE at a density of ~1×10^6 cell/cm^2. HUVECs were kept in the incubator for 1 day to allow cells adhesion and these loosely bounded HUVECs were removed by washing.

1.3 Electrochemical Measurements.

All electrochemical measurements were conducted on an electrochemical workstation (Shanghai, Chenhua, CHI1000C) with a three-electrode system at 298 K. Ag/AgCl electrode was used as the reference electrode (RE). Pt was used as the counter electrode (CE). All the electrochemical experiment was carried out in a solution made of 0.05 M phosphate buffer (PBS) solution. Additionally, for study both the mechanical stability of strechable electroed and cell mechanotransduction, the bare strechable electrode/ HUVECs cultured strechable electrode were fixed on a stretching device, and conducted electricity through copper wire to achieve electrochemical signal monitoring.

2. Supplementary Figures

2.1 Characterization of Stretchable Au Film Electrode.

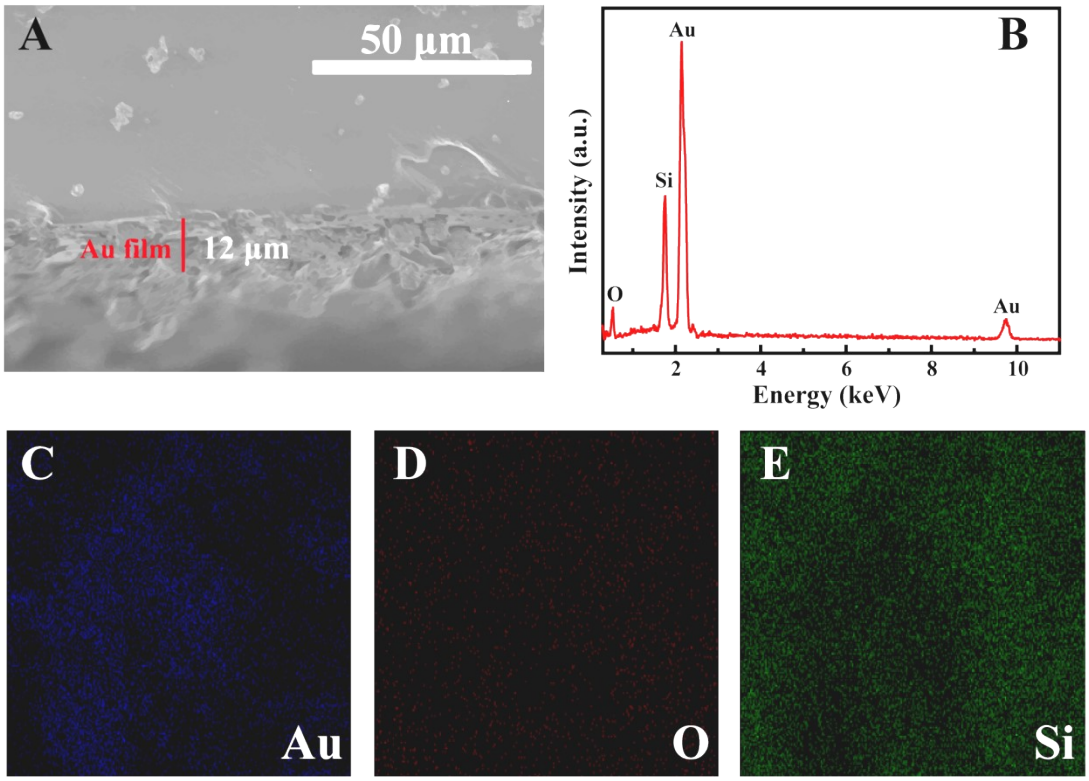

Fig. S1 (A) Cross-sectional view and (B) EDX analysis of stretchable Au film. The corresponding elemental mapping images of (C) Au, (D) O, and (E) Si.

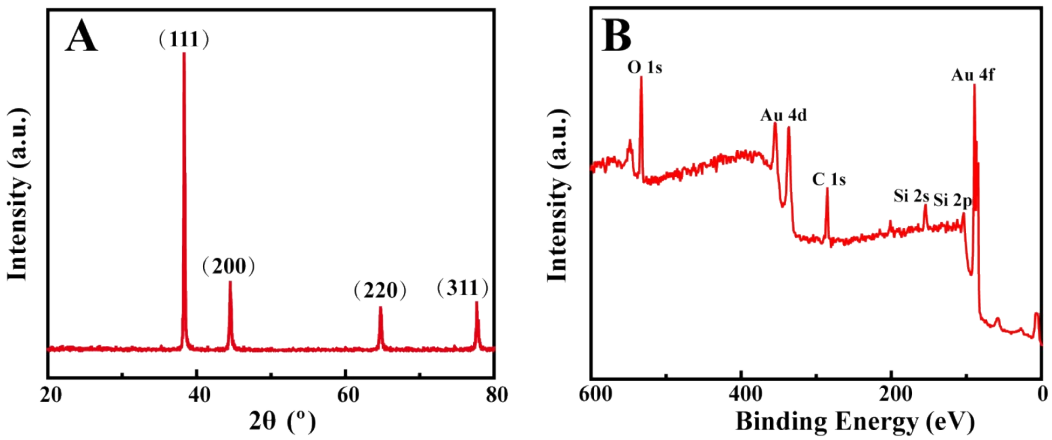

Fig. S2 (A) XRD pattern of the stretchable Au film electrode. (B) XPS spectra of the stretchable Au film electrode.

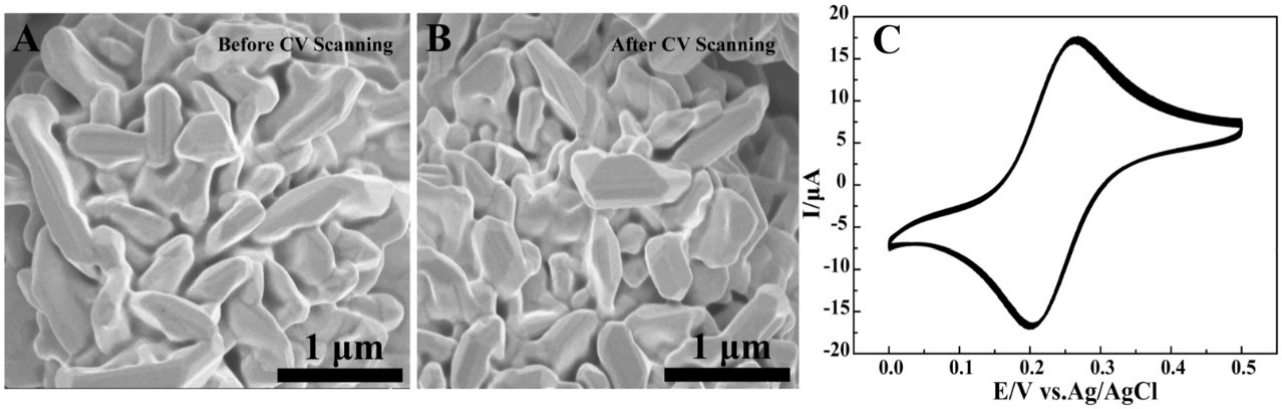

Fig. S3 SEM images of stretchable Au film electrode before (A) and after (B) the 50-cycle cyclic voltammetry (CV) scanning. (C) Continuous 50-cycle CV responses of stretchable Au film electrode in 1 mM $FcCH_2OH$ solution.

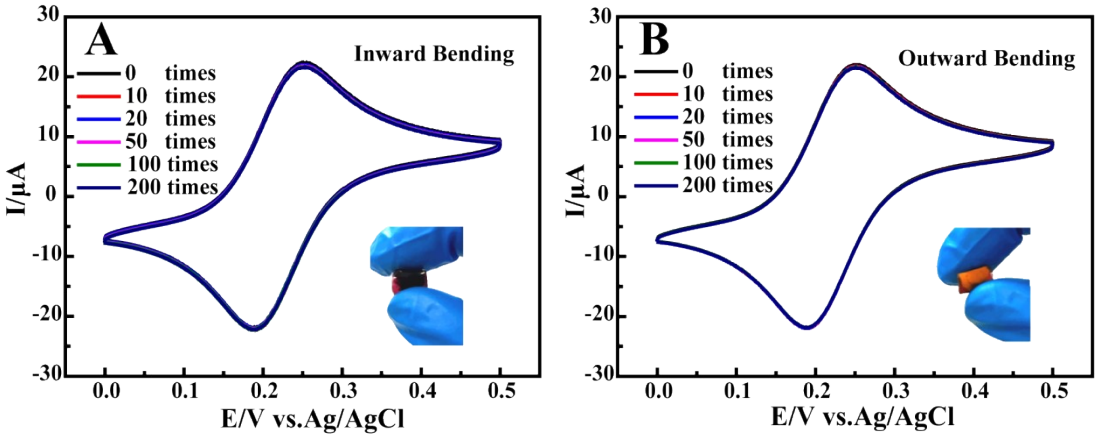

Fig. S4 Voltammetric responses of Au film electrode in 1 mM $FcCH_2OH$ solution with the Au film bent inside (A) and outside (B) for different times with a bending radius of 0.5 cm.

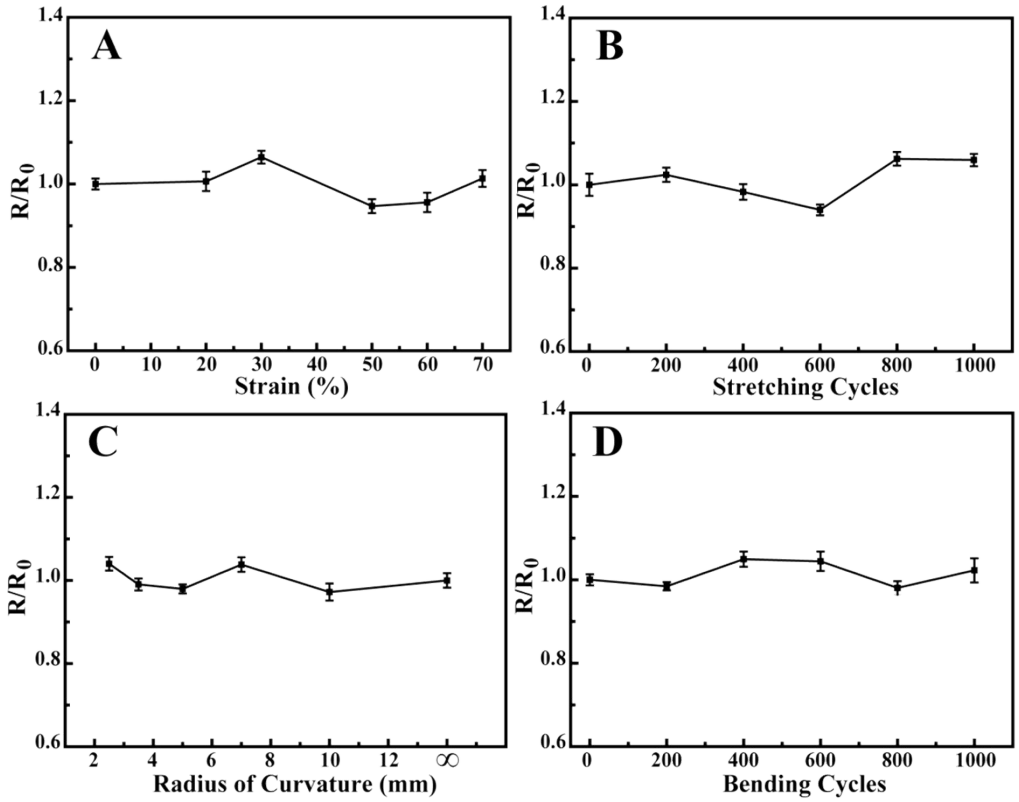

Fig. S5 (A) Relative variations of electrical resistance as a function of tensile strains of increasing intensities. (B) Relative variations of the ohmic resistance of a same Au film electrode along the number of stretching cycles involving a strain of 50%. (C) Relative variations of electrical resistance as a function of bending strains. (D) Relative variations of the ohmic resistance of a same Au film electrode along the number of bending cycles at a radius of 0.5 cm. Error bars represent the standard deviation of quintuple replicates.

2.2 Comparison of the Au Film and Au Film-Based Biosensors Designed by Different Strategy.

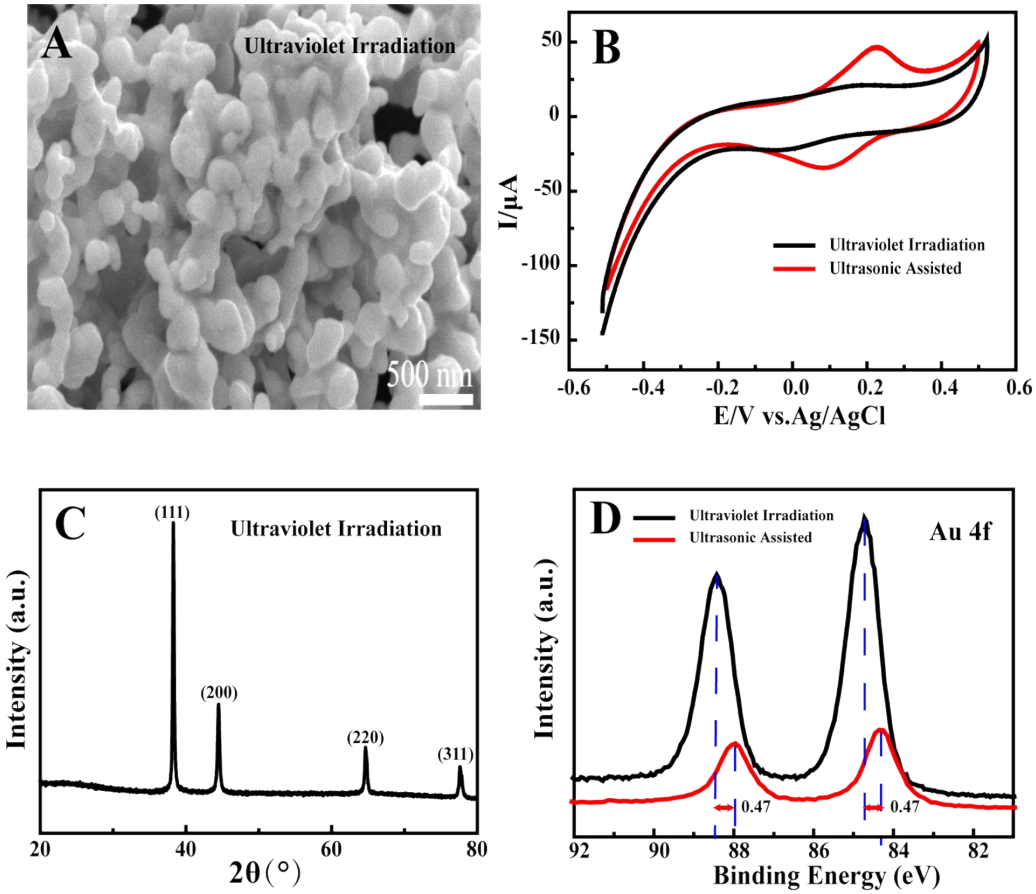

Fig. S6 Morphology, physical and electrochemical behavior characterization comparisons of the nanostructured Au film fabricated by ultrasonication-assistant and ultraviolet irradiation approach, respectively. (A) SEM image of Au film under ultraviolet irradiation. (B) CVs of SOD/Cys/Au SEs fabricated with different approach in 50 mM PBS at 100 mV/s. (C) XRD pattern of Au film under ultraviolet irradiation. (D) XPS spectra of Au film under different methods.

2.3 Electrochemical Behaviors and Amperometric Detection of $O_2^{\cdot-}$ on SOD/Cys/Au SE.

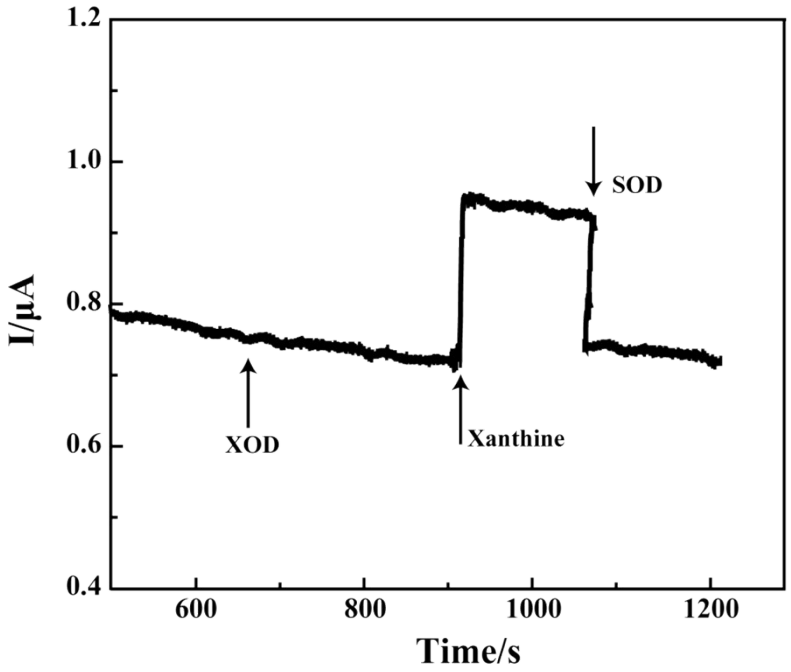

Fig. S7 Typical current-time response of SOD/Cys/Au SE in O_2 saturated solution upon the addition of 0.002 unit of XOD, 100 nM xanthine and 6 μM SOD, sequentially. The SOD/Cys/Au SE was polarized at 0.25V in the solution gently stirred with a magnetic stirrer.

3. Supplementary Tables

3.1 Element Characterization of SOD/Cys/Au SE.

Table S1. Element composition of Cys/Au (left) and SOD/Cys/Au SE film (right).

Element	Wt %	At %
N K	2.52	24.42
O K	0.61	5.16
S K	1.03	4.37
Cu K	0.00	0.00
Zn K	0.00	0.00
Au L	95.84	66.05

Element	Wt %	At %
N K	2.94	19.91
O K	5.27	31.29
S K	0.90	2.65
Cu K	0.77	1.15
Zn K	1.60	2.32
Au L	88.53	42.68

3.2 Comparison of SOD/Cys/Au SE with Previously Reported Studies.

Table S2. Electrochemical rate constant of SOD at SOD/Cys/Au SE surface and analytical performance of the present $O_2^{\cdot-}$ stretchable biosensor, compared with those previously reported.

Sensing Interfaces	k_s (s^{-1})	Applied potentials (mV)	Sensitivity (nA $\mu M^{-1}cm^{-2}$)	Linearity range (μM)	Detection limit (nM)	Reference
SOD/Cys/Au SE	35.2	250	2400	0.2-2	50	This Work
GC/NTA SOD	24.3	200 -300	263.4 86.4	0.1-250 0.1-250	21 45	1
Au/Cys/ SOD	1.2	300 -200	24000 22000	0.013-0.13 0.013-0.13	5 6	2
GNP/Cys/ SOD		210		0.08-64		3
PVA/SOD	2.1	-150		0.2-1.6	100	4
AuSN/SOD	10.4	250 -300	24.5 18.3	0.2-220 0.5-180	100 300	5
AuRN/SOD	8.1	250 -300	18.9 11.7	0.35-168 0.73-131	200 400	5
AuPN/SOD	6.7	250 -300	22.3 14.9	0.26-207 0.61-147	100 400	6
Au/Cys/sol-gel/SOD	3.4	100 200		0.05-0.4 0.05-0.4		7

4. References

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