Electronic Supplementary Information (ESI)

In-situ molecular detection of ischemic cells by enhanced protein direct electron transfer on a unique horseradish peroxidase-Au nanoparticles-polyaniline nanowires biofilm

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Experimental Section

Materials

Au NPs with size around 20 nm protected with sodium citrate were purchased from Sigma-Aldrich. Aniline was purified by distillation under vacuum before use and stored at 4 ^{o}C when not in use. HRP (isoelectric point of 7.2) was purchased from Sigma. All other chemicals were of analytical grade and used as received.

Apparatus and characterizations

The structure of samples was examined by field-emission scanning electron microscopy (FESEM, JSM-6700F). The structure of protein was examined by Fourier transform infrared spectroscopy (FTIR,

Bruker EQUINOX 55 DuroscopeTM). Electrochemical measurements were performed using a CHI 660C electrochemical workstation (CH Instruments Inc.). The measurements were based on a three-electrode system with the prepared electrode as the working electrode, a platinum coil as the auxiliary electrode, and a saturated calomel electrode (SCE) as the reference electrode. For cyclic voltammogram (CV) testings in 0.01 M PBS (pH 7.0) solution, if not specified, all solutions were deoxygenated by bubbling highly pure nitrogen for at least 15 min and maintained under nitrogen atmosphere during the measurements. Electrochemical impedance spectroscopy (EIS) measurements were performed at an open potential in the frequency range from 0.1 to 100,000 Hz. During the selectivity testings, dopamine (DA, 0.1 mM), ascorbic acid (AA, 0.1 mM), O_2 (saturated), and $NaNO_2$ (0.1 mM) were applied and the responses of interferences were observed relative to 0.1 mM H_2O_2.

Electrodes preparations

GCEs were polished subsequently with alumina powders with sizes of 1.0, 0.3 and 0.05 μm on a polishing cloth to a mirror-like finish. Then they were sonicated in absolute ethanol and water for 2 min, respectively, followed by drying with nitrogen. PANi nanowires were grown on cleaned GCEs (surface area of 0.071 cm^2) by a two-step galvanostatic technique in 1.0 M $HClO_4$ containing 0.1 M aniline. It was started by applying a constant current of 0.08 mA cm^{-2} for 20 min followed by 0.02 mA cm^{-2} for 90 min. The electrodes were washed with distilled water. Au NPs coating was prepared by immersing PANi nanowires on GCEs in Au NPs solution for 24 hours at 4 ^oC and then washed with distilled water. HRP immobilization was prepared by putting Au NP-PANi electrodes in 0.01 M phosphate buffered saline (PBS, pH 6.5) containing 10 mg ml^{-1} HRP overnight at 4 ^oC. The electrodes were rinsed with 0.01 M PBS to remove unabsorbed HRP, and then dried with nitrogen.

Cell culture and measurements

Rat smooth muscle cells (SMCs) were grown in Dulbecco's Modified Eagle's Medium (DMEM) (PAA Laboratories, Pasching, Austria) supplemented with 10% heat inactivated fetal bovine serum (FBS) (PAA laboratories) and 50 U mL^{-1} penicillin/streptomycin (Gibco). The cells were grown in a

humidified incubator at 37 ^{o}C with 5.0% CO_2. Ischemia was induced as reported by clamping the gas flow cannula to cell culture wells while keeping them in nitrogen protected environment for 30 min.[1] The SMCs were harvested by trypsinization and centrifuged to obtain a cell-packed pellet (~1 × 10^6 cells) for the electrochemical experiments performed in 2 mL 0.01 M PBS (pH 7.0). The measurements were carried out carefully to avoid any damage to the cell pellet.

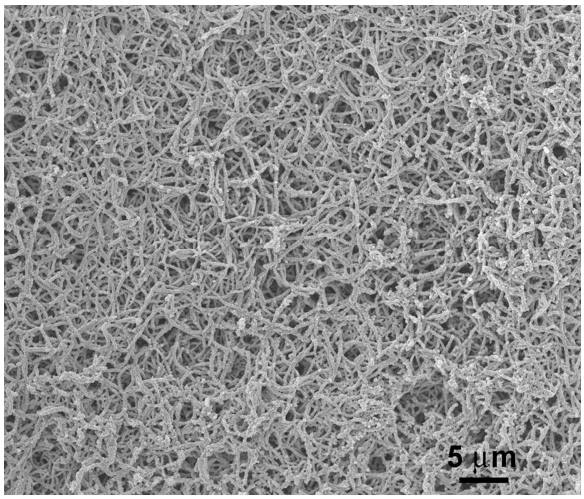

Fig. S1 Low magnification SEM image of the HPR-Au NP-PANi biofilm.

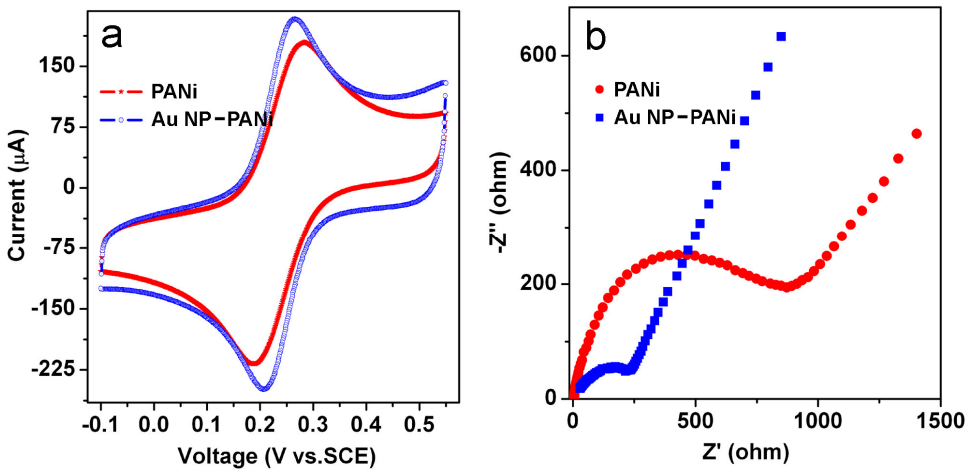

Fig. S2 CVs (a) and EIS (b) of 10 mM $[Fe(CN)]_6^{3-/4-}$-1 M KCl at different electrodes. Scan rate: 50 mV s^{-1}.

The electrochemical properties of Au NP-PANi nanowires were characterized by CV and EIS. The electroactive surface area[2] for Au NP-PANi nanowires is 16.5×10^{-2} cm^2 (Figure S2a), which is larger

than that of PANi nanowires (13.6×10^{-2} cm^2) by 1.3 times. The value of charge transfer resistance calculated from EIS (Figure S2b) in terms of Randle equivalent circuit[2] is 65.8 and 17.2 Ω cm^2 for PANi nanowires and Au NP-PANi nanowires, respectively. The electrochemical characterizations demonstrate that the Au NP-PANi nanowires have large electroactive surface area and low charge transfer resistance.

Reference

1 J. Slezak, N. Tribulova, J. Pristacova, B. Uhrik, T. Thomas, N. Khaper, N. Kaul and P. K. Singal, *Am. J. Pathol.*, 1995, **147**, 772.

2 C. X. Guo, F. P. Hu, C. M. Li and P. K. Shen, *Biosens. Bioelectron.*, 2008, **24**, 819.