Electronic Supplementary Information (ESI) for Chemical Communications

Real-Time Monitoring of Cell Viability by Its Nanoscale Height Change with Oxygen as Endogenous Indicator

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Experimental details

Chemicals and materials. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and Annexin V-FITC apoptosis detection kit were purchased from Sigma (St. Louis, MO, USA). Paclitaxel was acquired from Taiji Pharmaceutical Limited (Sichuan, China). 0.01 M pH 7.4 phosphate-buffered saline (PBS) containing 136.7 mM NaCl, 2.7 mM KCl, 87 mM Na_2HPO_4, and 14 mM KH_2PO_4 was sterilized before use. All aqueous solutions were prepared using ≥18 MΩ ultrapure water purified with a Millipore Milli-Q system. All other reagents were of at least reagent grade quality.

Pt wire with a diameter of 50 μm (purity 99.9%, hard) was provided by Alfa Aesar (Ward Hill, MA, USA). Borosilicate glasses with a length of 100 mm, an outer diameter of 1 mm and an inner diameter of 500 μm were purchased from Sutter Instrument Company (Novata, CA, USA). The quartz capillaries were obtained from Yongnian Optic Fiber Plant (Hebei, China) with an outer diameter of 375 μm and an inner diameter of 100 μm.

Apparatus. A Sutter P-2000 laser puller was used for the fabrication of nanoelectrodes. The subsequent polishing procedure was accomplished by a microforge MF-900 (Marishige, Tokyo, Japan). The Pt tip

image was obtained with S-4800 scanning electron microscope (SEM) (Hitachi, Japan). Brightfield and fluorescence images were taken by virtue of TE2000-U inverted fluorescence microscope (Nikon, Japan) and Leica TCS SP5 laser scanning confocal microscope (Germany). Cyclic voltammetry (CV) was performed with a three-electrode system by using a CHI 660 electrochemical analyzer (USA) equipped with a Faraday cage. A two-electrode setup comprising a 0.25-mm Ag/AgCl reference electrode and a Pt nanodisk electrode as working electrode was employed for scanning electrochemical microscopic (SECM) measurements on CHI 900 scanning electrochemical microscope (Austin, TX, USA), which was mounted on the horizontal stage of an inverted biological microscope set on a rigid aluminum platform. According to user's manual for CHI900B SECM, the *z*-piezo can travel 85 μm with a resolution down to a nanometer.

Fabrication of Pt nanodisk electrodes. Fig. S1 represents the fabrication procedure of Pt nanodisk electrodes. A 25-mm-length platinum wire was cut and inserted into a 15-mm-length quartz capillary with the polyimide layer burned off, so that about 5 mm of platinum wire protruded from both ends. Then, the capillary was inserted into a 100-mm-length borosilicate glass tube. The resulting glass/quartz/wire assembly was placed inside the laser heating chamber so that the laser beam was focused on the center of the quartz capillary. To avoid any pulling force on the glass capillary, two plastic tubes were used to hold the sleighs of the puller tightly.

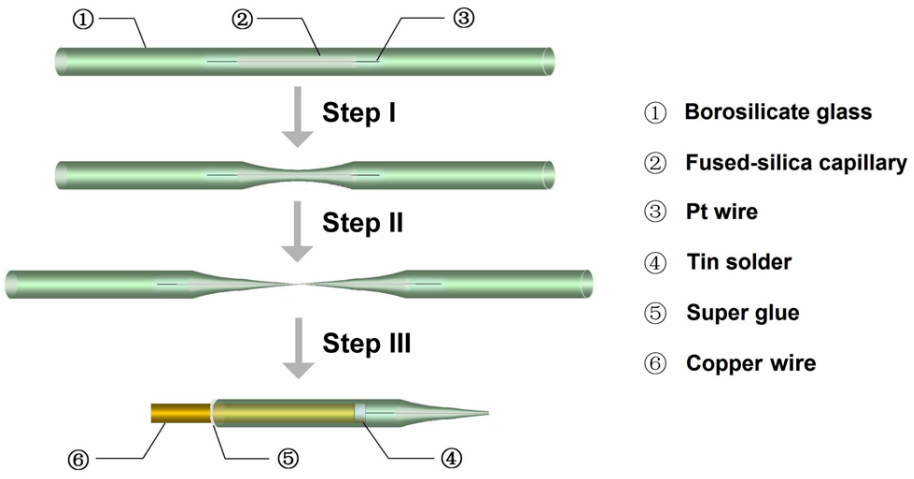

Fig. S1 Schematic diagram of the fabrication procedure of nanoelectrode tip. Steps I, II and III represent the process of fusing, pulling, and polishing and connecting, respectively.

In Step I, a program which consisted of only one line with the following parameter set was loaded, "heat: 750, filament: 5, velocity: 10, delay: 128, pull: 0". After heating for 10 s, the program of the laser puller was interrupted for a period of 20 s to allow the assembly to cool down and prevent overheating of the laser. This procedure was thrice repeated in order to assure an optimal sealing of the Pt wire inside the quartz capillary. After borasilicate glass was rotated by 180°, thrice repeated heating was carried out again for uniform heating. In Step II, two plastic tubes were firstly removed, and the second program "heat: 800, filament: 1, velocity: 130, delay: 124, pull: 220" was started to obtain two Pt nanowires sealed tightly in the capillary. Afterwards, Step III was performed for polishing and electrical connection. Polishing step was performed in a drop of water by slowly lowering down the tip-holder onto a polishing plate using manual micropositioning elements. Electrical contact to the Pt wire was established with a copper wire and a piece of tin solder. Finally, super glue was used to seal the opening end. Prior to use, a pretreatment was needed for the activation of Pt nanodisk electrode, by cyclic voltammetric sweeping between −0.2 and +1.2 V in 0.5 M H_2SO_4 solution for 30 min. After each SECM experiment, the electrode was immersed in 30% nitric acid for 30 min to eliminate the possible contamination of the electrode.

Cell culture and preparation. The BGC cell line was kindly provided by Affiliated Zhongda Hospital of Southeast University, Nanjing, China. BGC cells were cultured in a flask in RPMI 1640 medium (GIBCO) supplemented with 10% fetal calf serum (FCS, Sigma), penicillin (100 μg mL^{-1}), and streptomycin (100 μg mL^{-1}) at 37 °C in a humidified atmosphere containing 5% CO_2. 1.0×10^5 cells were then transferred into a polystyrene petri dish (Corning Incorporated, NY, USA) to culture for another 24 h, after which the BGC cells could attach to the bottom with a surface density of 5.0×10^3 cells cm^{-2} approximately.

SECM procedure. Prior to each SECM experiment, the adherent cells were twice washed with 37 °C PBS. The petri dish was filled with 4 mL PBS and mounted on the horizontal stage of SECM. The precipitation of calcium leaking from the cells was neglectable due to the very low amount of calcium

relative to the volume of testing solution. Firstly, the tip current (i_T) for reduction of dissolved oxygen at –0.4 V was recorded as a function of the tip position when the tip was scanned down to the petri dish vertically by a motor-driven XYZ stage. After stopped at 60% of the steady-state current, the sensing tip was retracted to a 14-μm separation from the petri dish (the average height of cells was 12 μm). Then, with the help of optical microscopy, single-line scan was operated laterally above the cell surface to get the profile of i_T versus the displacement of the tip along *x* axis. The optimal tip height (*h*) was obtained by gradually approaching the tip along *z* axis to the cell (every 100 nm), until a peak appeared on the single-line scan curve with the signal-to-noise ratio larger than 10. The SECM images were then recorded by continuously scanning over the BGC cells at a scan rate of 25 μm s^{-1} in the constant-height mode. The scan range was 60 μm × 60 μm. The time required to obtain one whole SECM image was about 5 min.

Fluorescent measurements of cell apoptosis. Annexin V-FITC apoptosis detection kit is used to observe early apoptosis of cells. A series of adherent BGC cells were firstly exposed to 25 °C PBS for 0, 1, 2, 3, 4, 5 and 6 h, respectively, and then the BGE cells were stained by Annexin V-FITC apoptosis detection kit on the bottom of the petri dish. After reaction for 5 min in the dark, BGC cells covered with a slide were imaged by laser scanning confocal microscopy.

MTT assay. BGC cells (1.0×10^5) in 200 μL of either medium alone or medium containing drugs at various concentrations were added to each well of a 96-well plate. The plate was incubated at 37 °C in a humidified atmosphere of 5% CO_2 for three days. MTT (20 μL, 5 mg mL^{-1}) was then added to each well. After the plate was incubated for a further 4 h, sodium dodecyl sulfate (150 μL, 0.52 M) was added to each well to solubilize the formazan dye. After 1 h the absorbance of the control and drug-treated wells was measured by using Hitachi/Roche System Cobas 6000 (Tokyo, Japan) at 490 nm. The cytotoxicity of the drug was calculated as follows:[1]

$$\text{Cytotoxicity (\%)} = 1 - \frac{\text{absorbance of drug-treated well}}{\text{absorbance of control well}} \times 100$$

Characterization of Pt nanodisk electrodes

Pt nanodisk electrodes were characterized by SEM and CV. Fig. S2a shows an abrupt decrease of the tip diameter in the heating zone. The stretched-out tip with the shape of symmetrical taper allowed its wide application in SECM measurements. SEM at higher magnification clearly identified a Pt nanodisk with 400 nm radius in the insulating glass sheath (Fig. S2b).

The ideal sigmoidal voltammograms of as-prepared Pt nanodisk electrodes in 0.1 M KCl containing 1 mM $K_3Fe(CN)_6$ were shown in Fig. S2c. The disk sizes could be obtained from the steady-state currents and following equation[2]

$$i_{T,\infty}=4nFDcr$$

where n is electron number transferred per molecule, F is the Faraday's constant, D and c are the diffusion coefficient and bulk concentration of the electroactive species, respectively, and r is the electrode radius. The effective radius of the prepared Pt nanodisk electrodes could be down to 5 nm.

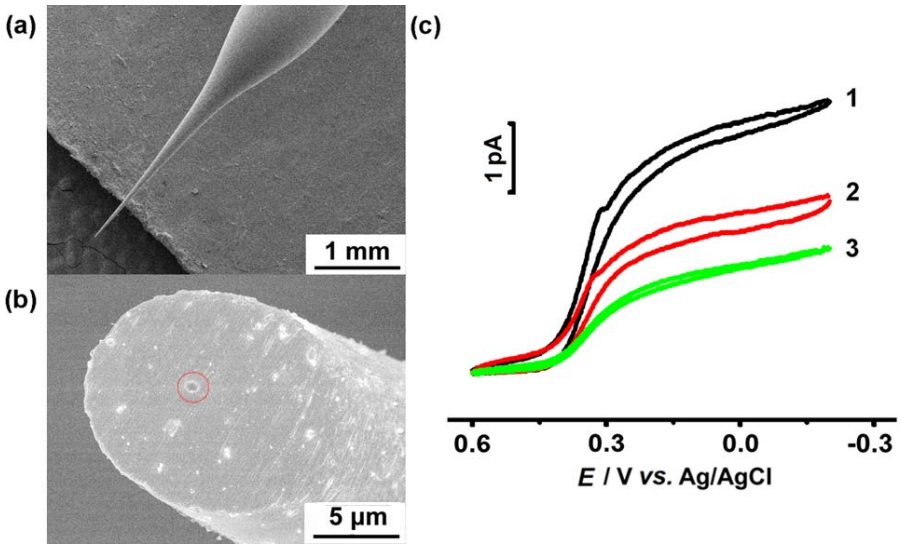

Fig. S2 SEM images of (a) taper-shaped nanoelectrode tip and (b) the front end of a tip, and (c) cyclic voltammograms of 1 mM $K_3Fe(CN)_6$ in 0.1 M KCl as supporting electrolyte at Pt nanodisk electrodes with different radii of (1) 32 nm, (2) 12 nm, (3) 5 nm. Scan rate, 50 mV s^{-1}.

Response of Pt nanodisk electrode to oxygen reduction

The Pt nanodisk electrode showed sensitive response to oxygen reduction with a maximum reduction current at -0.4 V vs. Ag/AgCl.

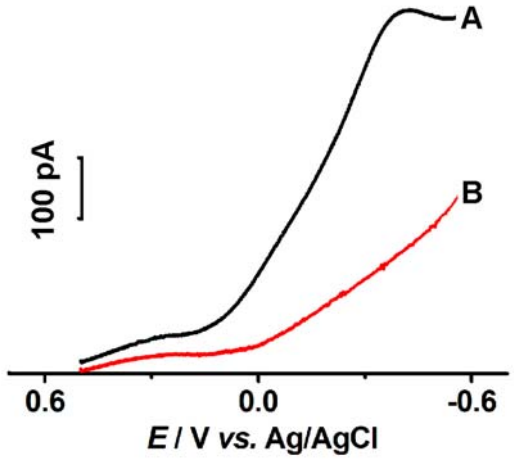

Fig. S3 Cathodic sweep curves of Pt nanodisk electrode with a radius of 400 nm in (A) air-saturated PBS and (B) PBS deaerated with nitrogen for 5 min at 30 mV s^{-1}.

Approach curve to the center of BGC cell surface using $Fe(CN)_6^{3-}$ as the indicator

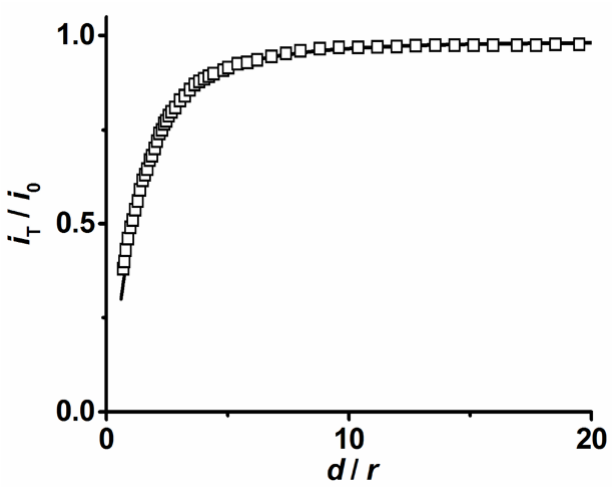

Fig. S4 Plot of normalized current (i_T/i_0) *vs.* normalized distance (d/r) at a 400-nm-radius Pt-disk tip approaching to the center of BGC cell in PBS containing 0.1 mM $K_3Fe(CN)_6$. Potential, 0.1 V *vs.* Ag/AgCl; i_0, steady-state reduction current measured in bulk solution; d, tip-cell distance; r, tip radius. The result shows that the experimental approach curve to BGC cell (grids) fits the theory curve to a planar surface for RG $\geqslant$ 10 (solid line).

Single BGC cell imaged by brightfield microscope

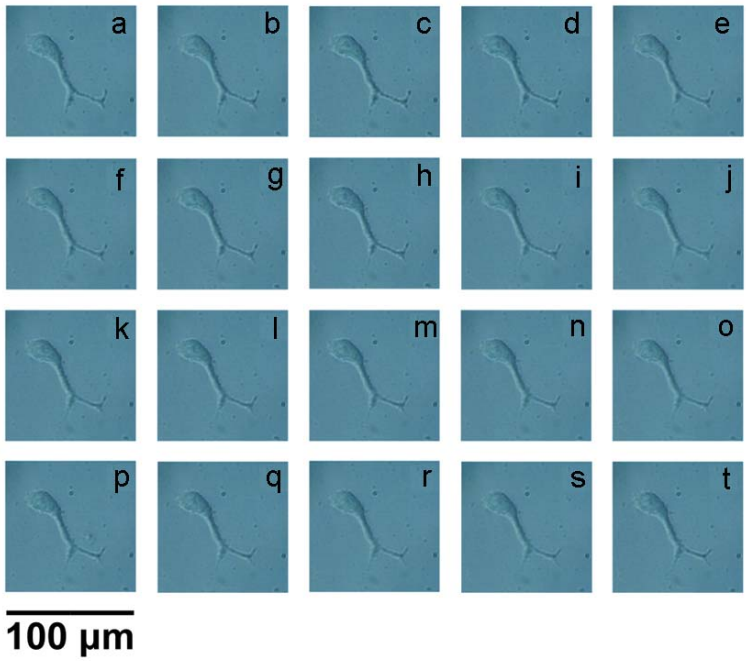

Fig. S5 Sequential brightfield images of single BGC cell taken by inverted fluorescence microscope in 25 °C PBS at every 7.5 min from (a) 0 to (t) 142.5 min.

Examination of tip drift

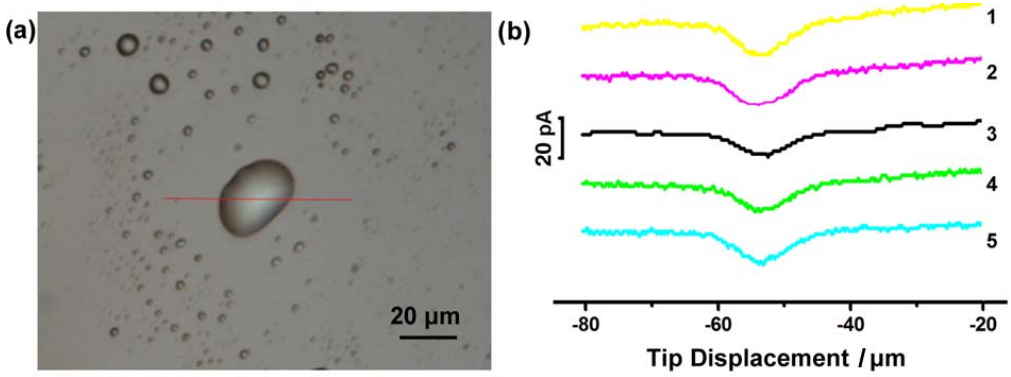

Fig. S6 (a) Optical image of the PDMS protrusion on a petri dish. (b) Consecutive constant-height SECM scan curves along the red line at (1) 0, (2) 0.5, (3) 1, (4) 1.5, and (5) 2 h. SECM measurements were performed in PBS solution with a 400-nm-radius Pt tip at −0.4 V *vs.* Ag/AgCl with a scan rate of 25 µm s^{-1}. The steady-state current in bulk solution was 171 pA.

Fluorescent images characterizing early apoptosis of BGC cells

Once the membrane phospholipids phosphatidylserine translocates from the inner face of the plasma membrane to the cell surface during early apoptosis, it can be detected by staining with a fluorescent FITC conjugate of Annexin V, while propidine iodide can not penetrate the intact cell membrane, but can penetrate cell membrane and stain the karyon for late apoptotic cells or dead cells.

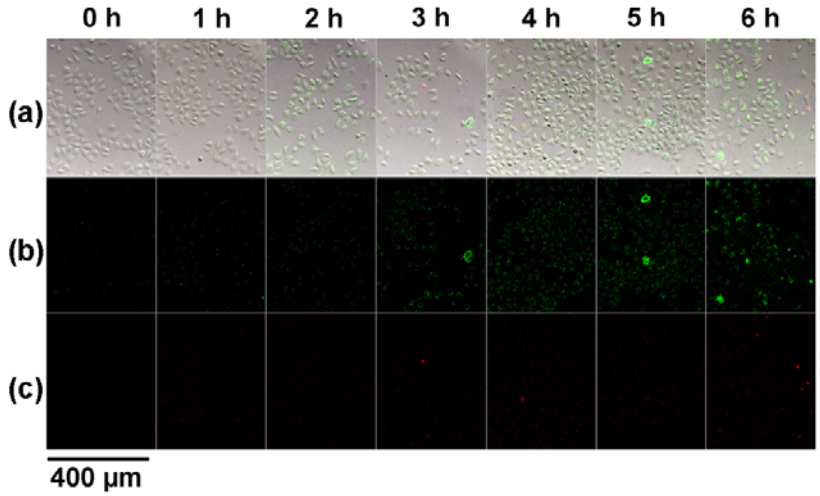

Fig. S7 (a) Brightfield, (b) green channel and (c) red channel confocal fluorescent images of BGC cells exposed to 25 °C PBS from 0 to 6 h, stained with Annexin V-FITC apoptosis detection kit.

Effect of paclitaxel on cell height change

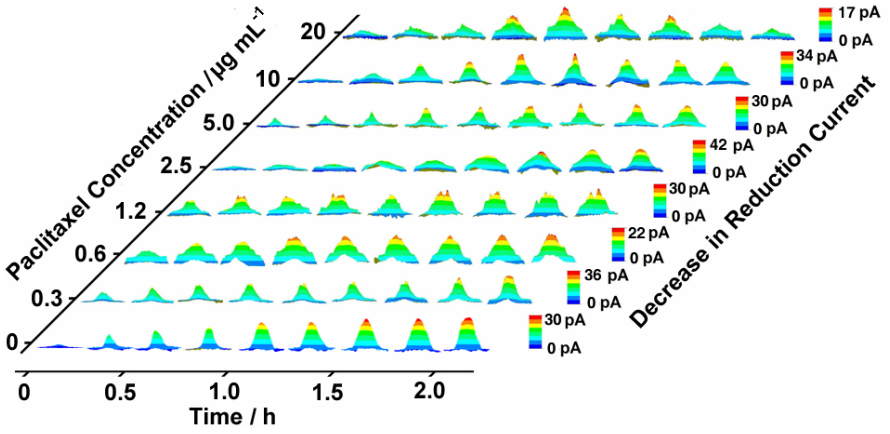

Fig. S8 3D SECM images of BGC cells treated with paclitaxel at concentrations from 0 to 20 μg mL^{-1} in 25 °C PBS. Each line shows the sequential images obtained with a 400-nm-radius Pt tip at every 15 min in 2 h. Conditions: tip potential, −0.4 V *vs.* Ag/AgCl; scan rate, 25 μm s^{-1}; rest time, 2 min. The steady-state currents in bulk solution were 169 ± 2 pA for all the measuements.

Drug sensitivity test by MTT assay

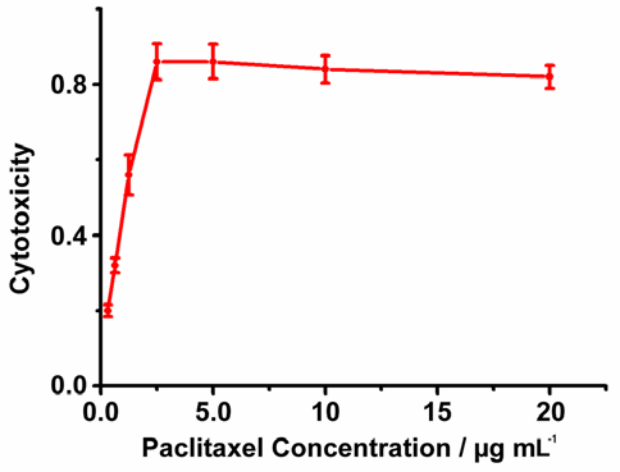

Fig. S9 Cytotoxicity curve obtained by MTT assay for 72 h exposure of BGC cells to paclitaxel.

References

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