Supplementary Material (ESI) for Analyst

Imaging and Detection of Morphological Changes of Single Cells before and after Secretion Using Scanning Electrochemical Microscopy

Wei Wang, Yue Xiong, Fu-Ying Du, Wei-Hua Huang, Wen-Zhan Wu, Zong-Li Wang, Jie-Ke Cheng* and Yi-Fu Yang*

Department of Chemistry, Wuhan University, Wuhan, 430072, P. R. China

*Corresponding Authors:
Prof. Jie K. Cheng
Tel: +86-27-68762291
Fax: +86-27-68754067
Email: jkcheng@whu.edu.cn

Prof. Yi F. Yang
Tel: +86-27-87218624
Fax: +86-27-68754067
Email: yang-y-f1@vip.sina.com

SUPPORTING INFORMATION

EXPERIMENTAL SECTION

Cell culture and Solutions

Human umbilical vein endothelial cells isolated from human umbilical cord vein were used for the SECM imaging of cell morphology and detection of nitric oxide release. The method of isolating cells is referred to the work of Jaffe in 1973[1]. Cells were cultured in collagen coated culture flasks (25 cm^2, Coring, NY) and maintained in medium RPMI-1640 medium (Sigma, St. Louis, MO) supplemented with 12% FBS, 100 U/mL penicillin, 100 μg/mL streptomycin and were placed in incubator with 5% CO_2 at 37°C.

For cell morphology imaging and nitric oxide release experiments, cell suspension of 5 mL (3-5×10^6/mL) was firstly centrifuged at 800 rpm for 10 min. The supernatant was discarded and the cells were re-suspended in 10 mL culture medium. These cells were seeded on polylysine (Sigma) coated with a cover glass (Deckglaser, Germany). After seeding, cells were put into the incubator for 24 hours to let them recover and adhere tightly to the glass. Then, cells were ready for use in electrochemical measurements.

Cyclic voltammetry experiments were carried out in 20 mM $K_3Fe(CN)_6$ and 1.0 M KCl . SECM mediator was prepared at a concentration of 1.0 mM $Ru(NH_3)_6Cl_3$ (Aldrich) in Hank's balanced salt solution (HBSS) and 10 mM HEPES (Sigma) and adjusted to pH 7.4 with 1 M NaOH. All reagents were used as received from commercial sources. HUVECs were stimulated with 3 mM L-arginine (Sigma) prepared in Hank's balanced salt solution and 10 mM HEPES. Aqueous solutions were prepared in 18 MΩ•cm deionized water (Beijing, Epoch).

Electrodes Construction

The CFMDE for SECM probe prepared from carbon fibers (diameter 5-7 μm Goodfellow Co., Oxford, UK). The pulled glass capillary was utilized throughout the course of this work. After connecting the carbon fiber with a copper wire, all the CFMDE were made by pulling the copper wire on to the glass capillary but did not let the carbon fiber protrude the tapered end of the glass capillary. Epoxy was then drawn into the tip of the electrode via capillary action. The electrode tip was polished by 5 different processes to obtain a flat disk tip surface after curing at 80°C for 5 h. The microscope image of the CFMDE was performed on an inverted microscope (Axiovert 200M, Zeiss Germany). Prior to use in the SECM, electrodes were tested using steady-state cyclic voltammetry in 20 mM $K_3Fe(CN)_6$ and 0.5 M KCl, All data were recorded using a 3 M Ag/AgCl reference electrode. For nitric oxide release, a carbon fiber micro-disk electrode (dia 5-7 μm) modified with single-walled carbon nanotubes (SWNTs) and Nafion was used as the microsensor.

The epoxy resin-sealed CFMDE is clean, well-proportioned and without any air bubbles, because low curing temperature is controlled to avoid any unexpected results occurred, and the tip surface has been refreshed every time before SECM imaging.

Cell Imaging and Nitric Oxide (NO) release

For SECM imaging, the electrochemical chamber with the cells was mounted on a *x,y,z*-positioning stage driven by computer controlled stepper-motors having a nominal resolution of 1 nm per micro-step (Exfo Burleigh, Victor, NY), and a bipotentiostat was used to control the tip potential and measure the tip current. The constant height mode was used to image the cell morphology. The tip current (i_T) was recorded as a function of the tip position when tip was scanned laterally in a horizontal (*x*-*y*) plane above the cell surface. Probe approach curves were recorded by holding the electrode at a fixed x,y-position and monitoring changes in the steady-state current of $[Ru(NH_3)_6]^{3+}$ reduction at -400 mV vs Ag/AgCl when microelectrode tip travels, at a velocity of 8 μm/s. Before SECM imaging, line scans across a single HUVEC at different tip potentials were performed at the optimal scan rate of 5 μm/s. SECM images of bare cover slip and cells adhered cover glass were recorded at a scan rate of 5 μm/s. Positioning and data acquisition were controlled with a PC via an interface module and SECM software. Calculation of cell morphology was according to the equation reported by Bard[2]:

$$I_T^{Ins}(L) = \frac{1}{0.15 + 1.5385/L + 0.58\exp(-1.14/L) + 0.0908\exp[(L-6.3)/(1.017L)]}$$

For NO release, the electrochemical chamber was mounted on the stage of an inverted microscope (Axiovert 200M, Zeiss Germany) equipped with a digital camera (Axiocam HRC 3900×3090 Zeiss Germany). NO release from single cells was real-time detected by the EPC10 patch clamp amplifier (Heka Elektronik, Germany) at a constant potential of 800 mV vs Ag/AgCl. A carbon fiber micro-disk electrode (dia 5-7 μm) modified with single-walled carbon nanotubes (SWNTs) and Nafion was used as the microsensor. The production of NO was stimulated by injecting 3 mM L-arg towards the cell. The

amperometric signals and the time-course of the NO release events were detected and stored by a computer, shown as in Fig s1. Control experiment was carried out by incubating the cell with Nω-nitro-L-arginine methyl ester hydrochloride (L-NAME, Sigma), the incubation of cell with L-NAME significantly decreased the sensor's signal.

Figure s1:

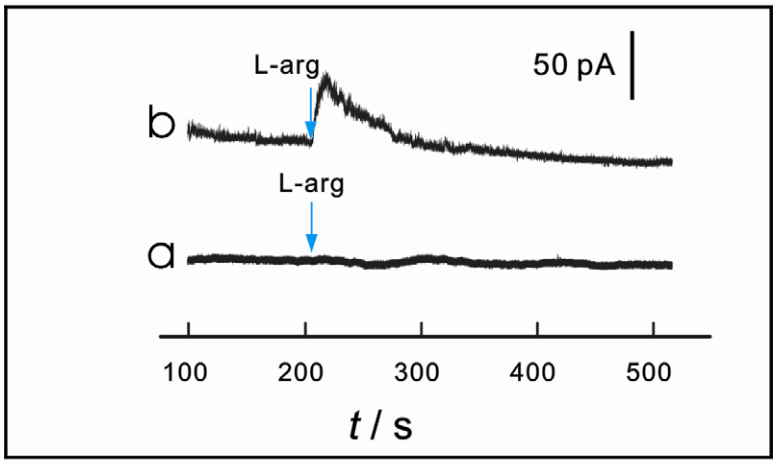

Figure s1. Measurement of NO released from a single isolated HUVEC evoked by L-arg. The arrow indicates the addition of L-arg. The curve a) the control experiment in the absence of HUVEC and, b) detection of NO released from a single isolated HUVEC stimulated by L-arg. The arrow indicates the addition of L-arg. The sensor placed at a fixed distance (generally 1 μm) away from the surface of a single isolated cell.

REFERENCES

(1) E. A. Jaffe, R. L. Nachman, C. G. Becker, C. R. Minick, *J. Clin. Invest.*, 1973, **52**, 2745.

(2) J. Kwak, A. J. Bard, Anal. Chem., 1989, 61, 1221.