Supplementary information

Glass electrospray for in situ detection of living cells by mass spectrometry

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Experimental methods

Chemical and reagents

Glutathione (GSH), PBS, Dulbecco's Modified Eagle's Medium (DMEM), and fetal bovine serums (FBS) were provided by Sangon Biotechnology Co., LTD. (Shanghai, China). 2',7'-dichlorodihydrofuorescein diacetate (DCFH-DA) and Calcein AM were obtained from Solarbio (Beijing, China). All other chemical reagents were of analytical grade and were used directly without further purification. Methanol (CH_3OH) of HPLC grade was purchased from Fisher Chemical (CA, USA). Ultrapure water (Mill-Q, Millipore, 18.2 MΩ) was used in all experiments. The equilateral triangular glass slide (vertex angle: 60 °C, side length: 20 mm; thickness: 0.2 mm) was customized by Xinhe Technology Development Company.

Sample preparation

The standard samples of GSH were respectively dissolved into 1 mM solution by the solvent of water and methanol (1:1, V/V).

Glass slide preparation

The customized equilateral triangular glass slide was located on the bottom of the petri dish for inoculating and culturing living cells. Thereafter, the triangular glass slide with living cells adherent to was taken out and dropped with the mixed solvent (methanol: water = 1:1, V/V). The high voltage from MS was applied to the mixed solvent for MS detection. The angle between the glass tip and the MS inlet was 10°.

Cell culture

Human cervical adenocarcinoma epithelial cells (HeLa) and human non-small cell lung cancer cells (A549 cells) were cultured with regular growth media consisting of high glucose DMEM and Ham's F-12K, respectively. The cell growth media were supplemented with 10% FBS, 100 μ/mL penicillin, and 100 mg/mL streptomycin and cultured at 37 °C in a 5% CO_2 humidified environment. The cells were cultured and grown on the ultrathin glass slides.

ROS probe detection

A Rosup (2 μL) that stimulates ROS production in cells was added and incubated for 30 minutes. Subsequently, DCFH-DA was introduced and incubated for an additional 30 minutes. The green

fluorescence intensity of DCF and the MS signal intensity of DCF produced by the cells were measured separately to construct a standard curve for ROS quantification.

Cell imaging

For live and dead cell staining assay, HeLa cells were seeded on a glass slide and washed with PBS, followed by staining with Calcein AM (10 μM). After 20 minutes of incubation, the medium was removed and the cells were washed several times with PBS. Cell fluorescence imaging was analyzed using a confocal laser scanning microscope (CLSM).

Mass spectrometry

MS analysis was performed using an LTQ XL linear ion trap mass spectrometer (Thermo Fisher Scientific). All MS results were obtained and processed using the Xcalibur or Microcal Origin (version 8.0) software. To form the spray droplets, a high voltage was adjusted in the range of 1.0-4.0 kV. The operational

parameters were as follows: full-scan negative (-) and positive (+) ion spectra were obtained over the m/z range from 80 to 400; the temperature of the MS inlet capillary was 275 °C; the capillary voltage and tube lens voltage was set to -26 V and -67 V in negative mode and the capillary voltage and tube lens voltage was set to 23 V and 75 V in positive mode. The maximum ion injection time was 100 ms. The identification of cell metabolites and standard samples was confirmed through collision-induced dissociation (CID) in LTQ mass spectrometry using the default parameters.

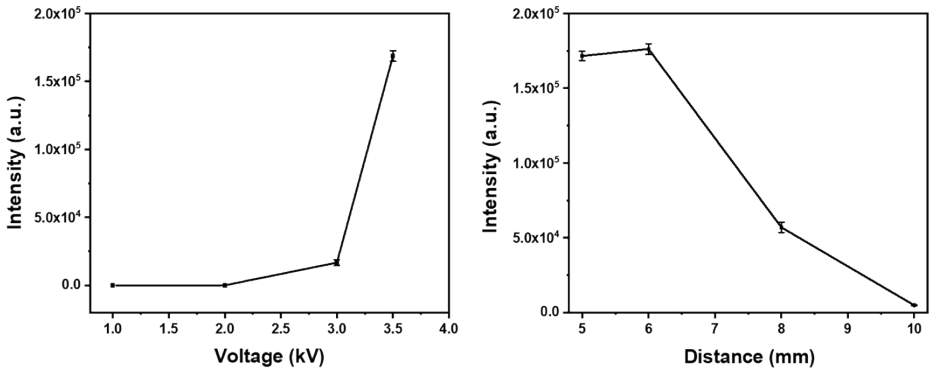

Fig. S1 The optimizations of distances between the glass tip and MS inlet and the high voltage.

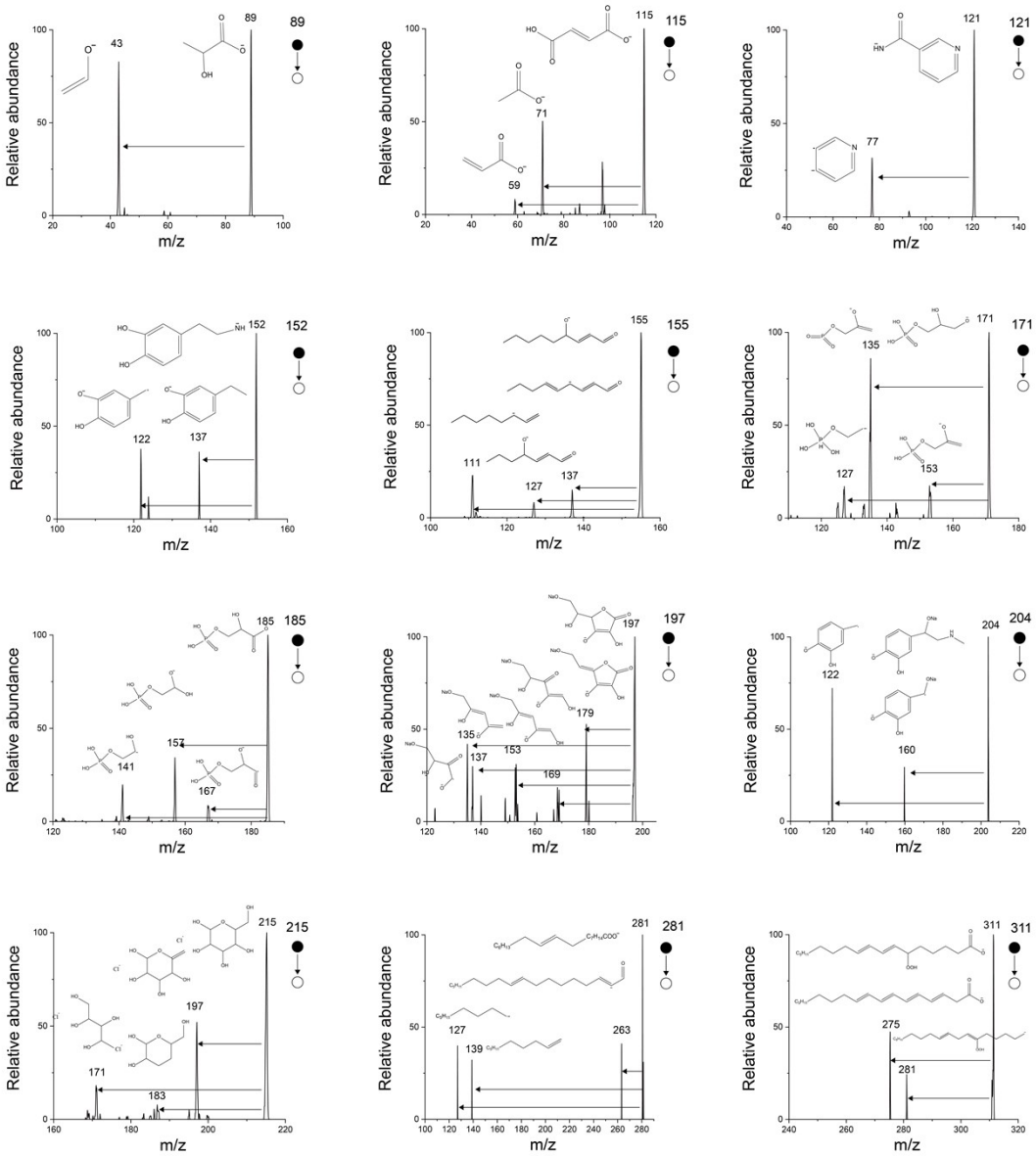

Fig. S2 MS^2 CID of metabolites in A549 cells in negative mode. Structures are simply rationalizations of the observed fragmentation patterns.

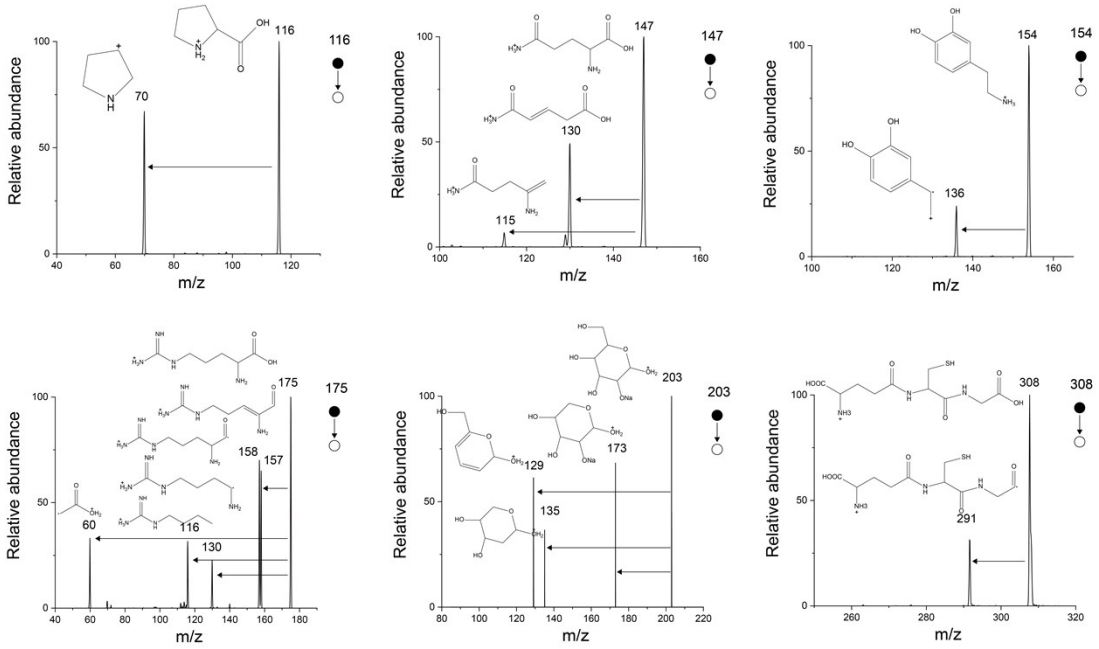

Fig. S3 MS^2 CID of metabolites in A549 cells in positive mode. Structures are simply rationalizations of the observed fragmentation patterns.

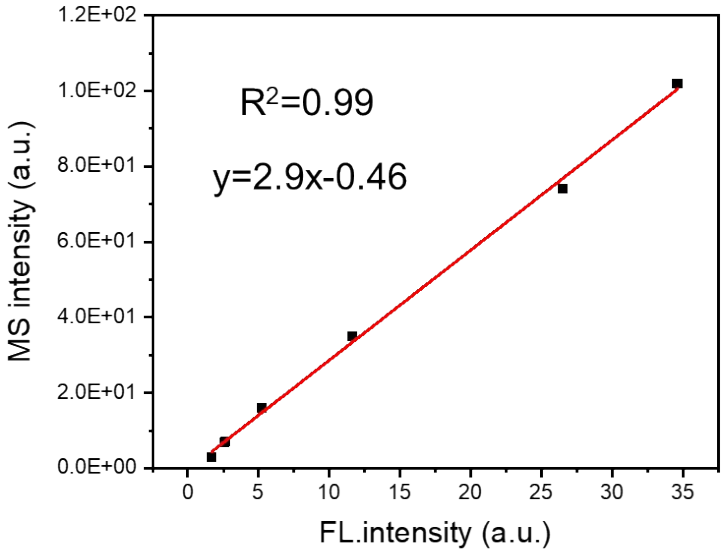

Fig. S4 The linearity between the average FL intensities of DCF (at Ex/Em 488/525 nm) and MS signals (at m/z 399) for evaluating levels of the intracellular ROS.

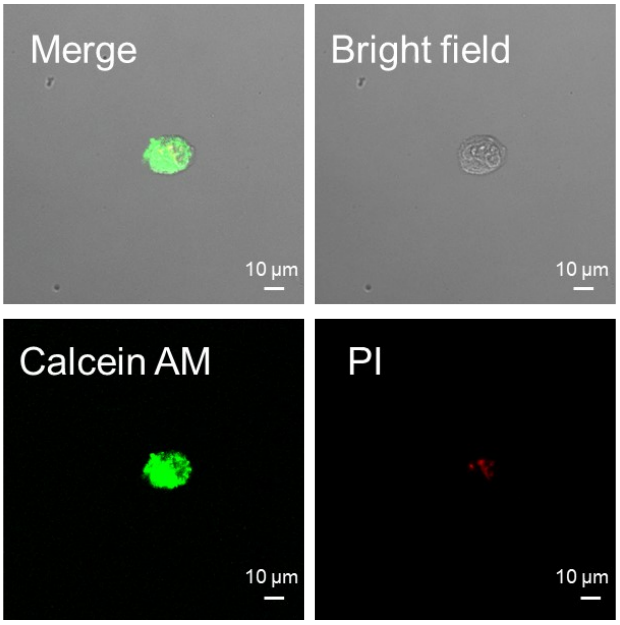

Fig. S5 The live and dead staining of released cells after the glass electrospray.

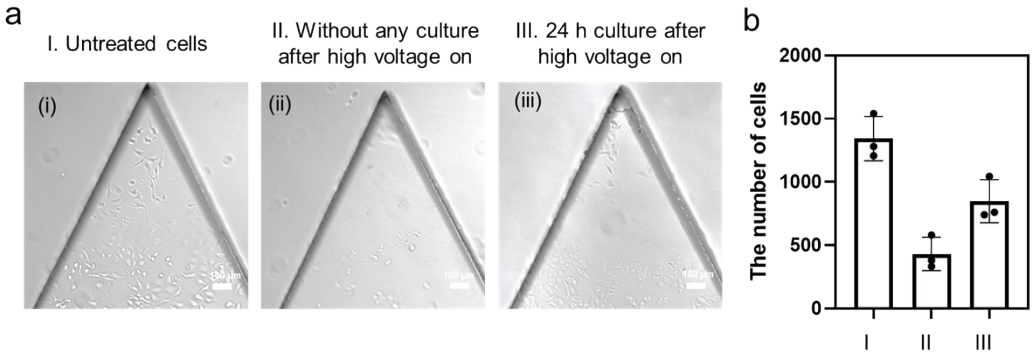

Fig. S6 (a) The confocal images of HeLa cells on the glass slide. (i) Untreated cells. (ii) Without any culture after applying high voltage. (iii) After applying high voltage on, cells were cultured for 24 h. (b) The number of cells on the various parts of the glass slide after different treatments (n=3).

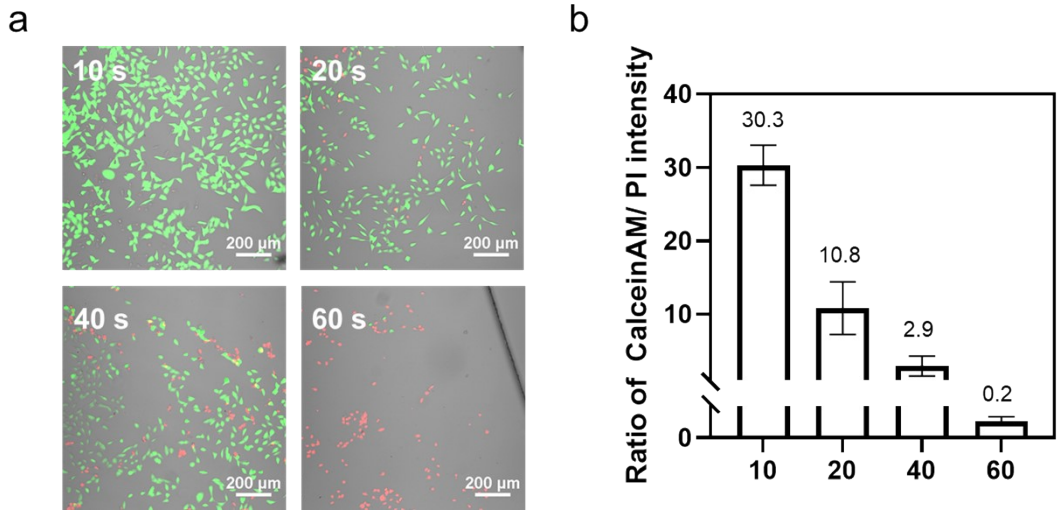

Fig. S7 (a) The live and dead stains of the residual HeLa cells after different times of glass electrospray. (b) The ratios of Calcein AM/PI intensity of the residual cells after different times of the glass electrospray.