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A time course study of cadmium effect on membrane permeability of single human bladder cancer cells using scanning electrochemical microscopy

Michelle S.M. Li, Fraser P. Filice, Zhifeng Ding *

Department of Chemistry, The University of Western Ontario, London, ON N6A 5B7, Canada

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ABSTRACT

Cd^{2+} is carcinogenic to both humans and experimental animals. We present quantitative time-course imaging of Cd^{2+}-induced variation in the membrane permeability of single live human bladder cancer cells (T24) to ferrocenemethanol using scanning electrochemical microscopy (SECM). High temporal resolution combined with non-invasive nature renders a time-lapse SECM depth scan, a promising method to quantitatively investigate the effectiveness, kinetics, and mechanism of metal ions based on the responses of single live cells in real time. Under unstimulated conditions, T24 cells have constant membrane permeability to ferrocenemethanol of approximately 5.0×10^{-5} m/s. When cadmium is added in-situ to T24 cells, the membrane permeability increases up to 3.5×10^{-4} m/s, allowing more flux of ferrocenemethanol to the ultramicroelectrode tip. This suggests an increased spreading between the phospholipid heads in the cytoplasmic membrane. Membrane permeability might be used as a measure to probe cell status in practical intoxication cases. The methodology reported here can be applied to many other metals and their interactions with extracellular biomolecules, leading insights into cell physiology and pathobiology.

1. Introduction

Scanning electrochemical microscopy (SECM) is a powerful analytical technique that can be used to study biological samples non-invasively through the generation or regeneration of redox mediated species [1,2]. Developed greatly by Bard et al. in 1989 [3], it has progressed from chemical based studies to being applied in biological systems [1,4–6] including: electron transfer kinetics and molecular transports [7–9], neurotransmitter releases [10,11], detecting both reactive oxygen and nitrogen species (ROS and RNS, respectively) by live cells [12–16], and the assessment of drug resistance on biological cells [14,17,18]. SECM utilizes an ultramicroelectrode (UME) of radii <25 μm biased at a potential at which a redox active species can be found in either its oxidized or reduced form. As the UME approaches the substrate, the current signal can be amplified if the UME probe approaches a conductive surface (regeneration of the non-oxidized or reduced form, positive feedback) or decreased if the UME probe approaches an insulating surface (blocked regeneration, negative feedback).

In literature, most research groups use one of two modes: constant height or constant distance. In constant height mode, the UME probe is held at a constant height and is moved along the sample surface to study the topography. However, one major drawback of this method is the convolution of the faradic processes and probe-to-sample distance effect if there is an uneven surface or a sample adhered to the substrate varying heights. The UME probe might crash to the sample, causing damage of either the sample or the electrode. In constant distance mode, the UME probe will maintain a constant distance from the substrate and move up or down accordingly to the substrate, deconvoluting the varying heights from the faradic processes.

In SECM of live cells, three types of measurements are most widely used: (1) SECM images of the cells, in which the current is measured and mapped as a function of the probe coordinates (horizontal plane) and thus revealing the topography of the adhered cell [15,16,19,20] and/or the spatial profile of extracellular redox active species [15,16,21–24]; (2) probe approach curves (PACs) which are normalized current plots vs. normalized probe-to-cell distance to quantitatively determine kinetic parameters [25,26] of a species across the cell membrane [20,27–29]; and (3) current–time curves (chronoamperometry) at specific probe-to-cell distances to study the evolutionary trend of cellular topography [24], reactivity [30], and monitoring very fast bursts of redox active species released by cells [30,31]. SECM has recently been used to investigate anti-cancer drugs or and their treatment of cancers using ROS as the probe [14]. However, the redox potentials of the ROS species are very close that make the selectivity problematic. Another study conducted by Koley and Bard has also reported a successful

* Corresponding author. Tel.: +1 519 661 2111x86161; fax: +1 519 661 3022.
E-mail address: zfding@uwo.ca (Z. Ding).
URL: http://publish.uwo.ca/~zfding (Z. Ding).

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investigation of membrane permeability of single live HeLa cells to Triton X-100 [27]. Progression into membrane permeability research [27–29,32] with SECM could widen the study of interactions between cancerous cells and potential anti-cancer drugs, and preventative measures. As the cell membrane is the first cellular barrier that anti-cancer drugs will interact with, it is important to study the membrane permeability of live cells.

Cadmium poisoning or intoxication can lead to numerous health issues, such as: cancers, immunodepression, and neurodegeneration [33,34]. Cadmium can also affect gene expression, by interacting with various transcription and translation factors [35], both of which are important for successful DNA replication and protein synthesis in healthy cells. While studies have shown that Cd intoxication leads to increased oxidative stress through increased ROS activity [36,37], which is found to be higher in cancerous cells than normal cells [38]. Here we look into the effects of Cd intoxication on membrane permeability. We may find information on this aspect similar to that of cisplatin induced cellular death of cancer cells through the formation of plasma membrane rafts to trigger the extrinsic apoptosis pathway [39–41]. We have previously developed a SECM depth scan method, where the contacting of an UME with the sample was minimized by means of approaching the UME to cells in x–z or y–z plane [14]. There is a third SECM mode recently developed by the Schuhmann group, which is a 4D shearforce-based constant-distance mode (4D SF/CD-SECM) [42]. In this mode, multiple constant-distance images can be obtained above the sample topography, where the tip-to-sample distances are controlled by the retractions of the tip based on shearforce of the SECM probe [42]. A comprehensive 4D data set can be obtained, containing the SECM tip current response as a function of *x*-, *y*-, and *z-position* of the SECM tip. Different types of 3D plots such as depth scan images can be extracted from these 4D raw data, while our method is to generate a depth image experimentally. In this report, we use this SECM depth scan mode to approach a single T24 cell, and assess the change in the permeability to the flux of ferrocenemethanol ($FcCH_2OH$), a non-toxic compound [15,21], upon Cd^{2+} addition.

2. Experimental

2.1. Materials

Cadmium chloride (anhydrous grade) and $FcCH_2OH$ (97%) were purchased from Sigma-Aldrich (Mississauga, ON) and used without further purification. Stock solution of 1 M $CdCl_2$ and 0.9 mM $FcCH_2OH$ and 0.1 M KCl (supporting electrolyte) was prepared in deionized water (18 MΩ MilliQ water, Millipore, Etobicoke, ON). All experiments were carried out at 37.0 ± 0.2 °C unless otherwise stated.

2.2. Cell culture

Human bladder carcinoma cells, T24 cells, (ATCC® HTB-4™), were purchased from American Type Culture Collection (ATCC, Manassas, VA) and maintained in McCoy's 5a medium modified with the addition of 10% fetal bovine serum (FBS). T24 cells were grown directly on uncoated T25 flasks (Becton Dickinson, Franklin Lakes, NJ), and at 80–100% confluency, cells were washed with phosphate buffer saline (PBS, pH 7.4) followed by trypsinization (0.25% trypsin, 0.03% EDTA solution) and subcultured weekly. McCoy's 5a medium modified was purchased from ATCC, while all other culture solutions and serums were purchased from Invitrogen (Burlington, ON, Canada). T24 cells were incubated at 37 °C and 5% CO_2 (Sanyo, Japan). To allow for valid comparison, all SECM live cell experiments were completed using the cells from the same passage number. All media and cell culture preparation was performed in a laminar flow hood.

Prior to the live cell SECM measurements, T24 cells were plated in a glass bottom petri dish (P50-G-0-30-F, MatTek Corporation, Ashland, MA, USA) and allowed to adhere and grow overnight. Prior to any SECM measurements, T24 cells were washed 3× with 2 mL of PBS (pH = 7.4) and refilled with 0.45 mM $FcCH_2OH$ and 0.05 M KCl in PBS (2 mL). Cadmium was added to these cells at the desired concentrations and SECM depth scans were taken every 5 min to 45 min; the addition of cadmium to the cells occurred 5 min after the first SECM scan, designated as t = 0 min.

2.3. Electrode fabrication

5 micron diameter Pt UME was fabricated by inserting a 5 micron Pt wire (Goodfellow Metals, Cambridge, UK) into a heat sealed pulled borosilicate glass capillary (o.d.: 2.00 mm, i.d.: 1.16 mm, length: 10.00 cm, Sutter Instrument, Novato, CA) using a micropipette puller (PP-83, Narishige, Japan). The sealed glass capillary with the 5 micron Pt wire was manually polished on a homemade polishing wheel with adhered alumina polishing pads (3.0, 0.3, and 0.05 μm, Buehler, ON). The ratio of the glass insulating sheath diameter surrounding the Pt wire to the Pt wire (RG) was manually polished to approximately 2 (RG = (glass insulator radius)/(Pt disk electrode radius)). The electrode tip was examined using optical microscopy and tested using cyclic voltammetry (CV).

2.4. Instrumentation

All SECM experiments were conducted on a modified Alpha-SNOM instrument (Witec, Ulm, Germany) adapted with a homemade UME holder. Using the Alpha-SNOM positioning system along with an inverted objective lens (Nikon objective, 50× lens), the UME's approach to the T24 cell can be visualized and controlled without cellular penetration. All the electrochemical experiments were performed using an electrochemical analyzer (CHI 800B, CH Instruments, Austin, TX, USA), and the signal was transported into one data acquisition channel of the Alpha-SNOM instrument microscope. In all SECM experiments described in this report, a Ag/AgCl was used as a combined auxiliary and reference electrode. The details of this SECM instrumentation and operation procedure have been described elsewhere [14].

2.5. SECM measurements

SECM measurements were obtained by mounting a petri dish containing the adhered T24 cells (in PBS solution containing $FcCH_2OH$) in the heated scanning stage of the instrument. The temperature of the heating stage (Bioscience Tools, San Diego, CA) was controlled at 37.0 ± 0.2 °C throughout the duration of the experiment to mimic physiological conditions of the cell line. Using the inverted objective lens, a single T24 cell was located and the UME was then approached in the proximity of the cell and biased at the oxidation current plateau of $FcCH_2OH$ (0.300 V vs Ag/AgCl, as determined by CV) and PACs was obtained by drawing the vertical lines above the cell. Each scan has 128 × 128 pixels with a scan scale of 60 μm in width and either 20 or 25 μm in depth. An integration time of 0.01 s was chosen for these scans.

The distance of the cell-UME probe was optimized using a T24 cell found on the petri dish. SECM depth scans were completed using this cell. Experimental PACs were studied immediately after each scan to confirm that no contact with the cell was being made. Once optimal cell-probe distance was determined, another T24 cell (on the same petri dish) was studied and the first SECM-depth scan of this cell was declared to be taken at t = 0 min.

When collecting the SECM-depth scan, the UME was first moved in the z direction to half of the depth distance away from the cell (declared the largest distance away the cell), taking scans along the x-direction (width). After the first row of points was taken in the x direction (width of the scan), the electrode returned to x = 0 point, but went down in the z direction (dependent on number of points/line and