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Mapping Cd^{2+}-induced membrane permeability changes of single live cells by means of scanning electrochemical microscopy

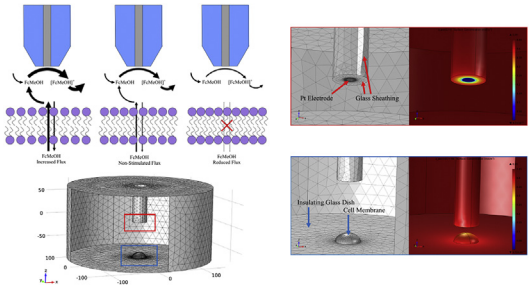
Fraser P. Filice, Michelle S.M. Li, Jeffrey D. Henderson, Zhifeng Ding*

Department of Chemistry, The University of Western Ontario, 1151 Richmond Street, London, ON N6A 5B7, Canada

HIGHLIGHTS

- Scanning electrochemical microscopy (SECM) was used to map membrane permeability of T24 cells under Cd^{2+} stress.
- Depth scan SECM imaging generating 2D current maps of live cells relative to electrode position in the x–z or y –z plane.
- Full 3D models were developed for the SECM system of live cells.
- Experimental probe approach curves to any location above the cell membrane are usable.
- The Cd^{2+} was found to activate cell apoptosis, with cells shrinking in size and the membrane permeability decreasing.

GRAPHICAL ABSTRACT



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ABSTRACT

Scanning Electrochemical Microscopy (SECM) is a powerful, non-invasive, analytical methodology that can be used to investigate live cell membrane permeability. Depth scan SECM imaging allowed for the generation of 2D current maps of live cells relative to electrode position in the x-z or y-z plane. Depending on resolution, one depth scan image can contain hundreds of probe approach curves (PACs). Individual PACs were obtained by simply extracting vertical cross-sections from the 2D image. These experimental PACs were overlaid onto theoretically generated PACs simulated at specific geometry conditions. Simulations were carried out using 3D models in COMSOL Multiphysics to determine the cell membrane permeability coefficients at different locations on the surface of the cells. Common in literature, theoretical PACs are generated using a 2D axially symmetric geometry. This saves on both compute time and memory utilization. However, due to symmetry limitations of the model, only one experimental PAC right above the cell can be matched with simulated PAC data. Full 3D models in this article were developed for the SECM system of live cells, allowing all experimental PACs over the entire cell to become usable. Cd^{2+}-induced membrane permeability changes of single human bladder (T24) cells were investigated at several positions above the cell, displaced from the central axis. The experimental T24 cells under study were incubated with Cd^{2+} in varying concentrations. It is experimentally observed that 50 and 100 μM Cd^{2+} caused a decrease in membrane permeability, which was uniform across all locations over the cell regardless of Cd^{2+} concentration. The Cd^{2+} was found to have detrimental effects on the cell, with cells shrinking in size and volume, and the membrane permeability decreasing. A

* Corresponding author.
E-mail address: zfding@uwo.ca (Z. Ding).

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mapping technique for the analysis of the cell membrane permeability under the Cd^{2+} stress is realized by the methodology presented.

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1. Introduction

Scanning electrochemical microscopy (SECM) is a powerful non-invasive analytical method in the Scanning Probe Microscopy (SPM) family. SECM operates by moving a biased ultra-microelectrode (UME) (25 μm diameter electrode or smaller) over a substrate with extreme precision [1–3]. The UME tip and substrate are submerged in an electrolyte solution containing a redox agent. An electrochemical current is monitored with reference to UME-substrate position, which is affected by topographical changes and electrochemical characteristics of the substrate as the electrode sweeps in the vicinity of the sample surface [1–5]. Developed greatly by Bard et al. since 1989 [1], SECM has been employed for chemical kinetics studies, chemical imaging, potential distributions, and microfabrications [6–8]. Recent development towards biological applications have been pursued [9–15], such as electron transfer kinetics and molecular transports [16–18], neurotransmitter releases [19–21], reactive oxygen and nitrogen species release [22–29], and the assessment of drug resistance on biological cells [28,30,31]. SECM offers great opportunities to study physiological processes at the cell membrane in real-time, and to investigate cellular properties such as membrane permeability [32–34]. As the probe makes no contact to the cells, these physiological processes remain unchanged, assuming that the selected redox mediator itself has no effect on the cell's homeostasis [32]. This is advantageous over common methods of live cell monitoring, such as continuous time-lapse fluorescence experiments, which suffer from photo bleaching that potentially alters cell homeostasis [35–37].

The UME is biased at a potential generating a steady state current in a redox active solution, as the electrochemically driven reaction progresses. Upon approach to a substrate, diffusion of new mediator towards the electrode tip is hindered (Fig. S1). This reduces the rate of reaction at the electrode tip, and consequently the reaction draws less current. In cases such as an approach to some substrates (conductors, for instance) spent mediator can be regenerated, causing an increase in current [1].

Traditionally, acquiring a SECM probe approach curves (PACs) involves a biased UME approaching the sample at a single point vertically [19,32,33]. To characterize a single sample at different locations, multiple approaches would be required, which is both time consuming and difficult. By completing multiple approaches, there is also an increased risk of crashing the electrode into the sample or substrate, due to the possible height variations of the substrate surface. An electrode crash can be damaging to the sample or the electrode itself. In our group, a different method of SECM analysis called depth scan imaging has been previously developed [28,34,38,39]. The depth scan mode passes the UME in x-axis or y-axis direction performing a horizontal line scan at that constant height. The electrode is then lowered in the z-axis by a preset depth distance and another line scan is carried out. This action is repeated until the desired depth is reached. The repeated horizontal sampling at various approaching heights to the sample produces a two-dimensional image of the current feedback in the x-z or y-z plane in real-time. The depth scan imaging method allows the distance to the sample to be more easily gauged during the scan, removing the limitation of conventional SECM approach methods. One depth image can also provide hundreds of PACs, limited by the user defined image width and resolution. By simply selecting vertical cross-sections from the image, experimental PAC data can be extracted, producing a plot of current vs. the distance to the substrate. Notice that the Schuhmann group developed a 4D shearforce-based constant-distance mode (4D SF/CD-SECM) [40], where multiple constant-distance images can be obtained above the sample topography, a comprehensive 4D data set 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 generates a depth image experimentally.

Extracted experimental PACs are then compared to simulated PACs to obtain quantitative analysis of sample traits, such as the reaction kinetic and physical properties [3,4,17]. Conventionally, simulations of this system are completed using a 2D axially symmetric model geometry in finite element analysis software, such as COMSOL Multiphysics. At the time of computation, the 2D model is duplicated in a radial fashion about the axis of symmetry creating a pseudo three-dimensional (3D) model for computation. This symmetric model design saves a great deal of computational time and system resources. However, the requirement that a model be symmetric is a severe limitation. The PACs to a substrate of non-uniform geometry produced in each SECM depth scan image are not all usable for comparison to the conventional theoretical PACs. Only the PAC directly above the center of the symmetric sample can be matched with simulated PAC data. By expanding to a 3D model, off axis PAC analysis of single live cells becomes possible, as seen in our very recent development of a full 3D simulation model for SECM of an interdigitated array of gold electrodes [38].

The use of 3D models for characterization of SECM data, can allow for even broader use of SECM than what was possible before. The primary issue with performing 3D simulations previously has been the heavy demand on system resources. Long compute times, large amounts of memory, and heavy CPU utilization have previously required powerful computers to execute. The simulation geometry, meshing and physics have been optimized to allow for such simulations to be computed in reasonable time using consumer grade hardware.

With SECM, monitoring cell membrane permeability is commonly achieved by comparing experimental results to simulated PACs of known permeability coefficients [33]. The shape of a PAC is dependent on the ability of a mediator to pass through the membrane. At close proximity of the UME to the cell, diffusion is limited similar to an approach towards an ideal insulating substrate. As the UME approaches the membrane, the flux of the redox mediator across the membrane is detected as an additional faradaic current. The redox mediator used in this study is ferrocenemethanol, which is commonly employed for biological electrochemistry as it is non-toxic to many cells lines [30,34,41,42]. Ferrocenemethanol is able to diffuse across the cell membrane to the cell interior, and back out. As the electrode approaches closer to the cell membrane, more rapid flux of ferrocenemethanol occurs through the cell membrane toward the electrode tip. The rate of diffusion across the membrane is dependent on the membrane permeability coefficient. This enables matching of experimental and simulated PACs for quantification of membrane permeability.

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