Development and Characterization of Multi-Functional Probes for use with Scanning Electrochemical Microscopy

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Abstract
Using Scanning Electrochemical Microscopy (SECM) with electrode probes that make simultaneous current and impedance measurements is useful for analysis of biological substrates. The current and impedance measurements are both dependent upon the proximity to a surface. Experiments with the single carbon fiber electrodes show that the impedance measurements are independent of the current and applied potential. In order to detect neurotransmitter release from a cell, the electrode must be positioned very close to the cell surface. Impedance-based positioning eliminates the need for addition of biologically toxic redox mediators to position the electrode. Furthermore, it was found that multiple carbon fiber electrodes are able to explore a larger surface area than the single carbon fiber electrodes; this is useful for larger biological substrates, such as isolated taste buds. The distance-dependent impedance signal from multiple fiber electrodes was also characterized. Topographical images of biological substrates are generated by moving the electrode over the surface of the cells while monitoring the distance-dependent impedance signal. Because the current and impedance measurements are independent of each other, it is possible to combine imaging and other electrochemical techniques using the same electrode.

Introduction
The Scanning Electrochemical Microscope (SECM) can be used for simultaneous current and impedance measurements. Both the current and impedance measurements obtained with the carbon fiber electrode used with the SECM can be utilized to detect the distance between the electrode and a surface. Impedance is a measure of the resistance to current flow when a potential is applied. The counter and working electrodes detect the distance of the solution. Both the current and impedance measurements change as the electrode comes closer to a surface due to limited diffusion of molecules and ions to the surface of the electrode1. Since the impedance measurement is independent of the current measurement, it can be used to position the electrode close to the cell surface. Using the impedance measurement to position the electrode will eliminate the need to add potentially cytotoxic redox mediators or apply a potential that can generate reactive and cytotoxic intermediates. Additionally, since these measurements are independent of each other, it is possible to combine imaging with other electrochemical techniques, such as amperometry or cyclic voltammetry.

Results and Discussion
The impedance measurement is independent of the applied potential
When the potential of -1.2V is applied to the electrode, dissolved oxygen in the buffer is reduced to water. The current generated at the electrode is the result of this reduction. As the electrode approaches a surface, such as a cell, the diffusion of oxygen to the electrode surface is limited and the resulting current decreases (Figure 3A, red line). When no potential is applied at the electrode, the current does change as the electrode approaches the surface (Figure 3B, red line). The impedance measurements (Figure 3A, black lines) change as the electrode approaches the surface. This change is independent of the applied current and impedance measurements are directly compared in figure 3C.

Experimental
Carbon fiber electrodes were constructed with multiple (3 to 33) 7 µm diameter fibers (Themoel T650, Cytiva Industries) and beveled at 90° on a diamond polishing wheel (model BV-10, Sutter Instrument Company). Voltammetric measurements were conducted with a bipotentiostat in the 3-electrode mode (E1400, Cypress Systems), and potentials were reported against an Ag/AgCl reference electrode. Carbon fiber electrodes were positioned onto an isolated taste bud using a piezoelectric positioning device (Exfo-Burleigh) until the electrode just abutted the taste bud. The impedance measurements were taken by the electrode to determine the topography of the PC12 cells. Experiments were conducted in Hanks buffer at a pH of 7.2.

In order to explore a larger surface areas with biological substrates, such as taste buds, larger multiple carbon fiber electrodes can be used. The distance-dependent impedance characteristics for these multiple carbon fiber electrodes (3-33 fibers) are characterized below.

Impedance can be used to generate topographical images of the cells.
Since the impedance measurements are distance dependent, it is possible to generate a topographical image of cells (biological substrates) (Fig. 4). The advantage of impedance-based topographical imaging is that there is no need to add potentially cytotoxic redox mediators or generate reactive oxygen intermediates near the cells.

Since the electrode position can be determined by impedance measurements, it is possible to monitor neurotransmitter (NT) release from cells by setting the electrode to an oxidizing potential of +0.8V. This potential is able to oxidize the neurotransmitters; serotonin, norepinephrine, epinephrine, and dopamine; when they are present in solution. However, using the 53 µm carbon fiber electrode with a larger collection of cells, like those found in the taste bud, poses a problem with positioning the electrode next to NT-containing cells. Only 20% of cells in taste bud cells contain NT. Larger multiple carbon fiber electrodes were fabricated to assay the entire exposed surface of the cells.

Carbon fiber electrodes can be used for distance-dependent measurements by recording the impedance from the cells. The electrode can also monitor topography at different potentials, such as -1.2V, 0V, +0.8V. The significance of this technique is that the carbon fiber electrode can record simultaneous amperometric and topographic data. Also, it is beneficial to use the multiple carbon fiber electrodes for large biological substrates, such as taste buds. In the future, topographical images can be produced using multiple carbon fiber electrodes. These topographical images can be combined with other electrochemical techniques as well.

References

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