John Wesley Powell Student Research Conference

2013, 24th Annual JWP Conference

Apr 20th, 2:00 PM - 3:00 PM

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 resistance 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 electrode[1]. 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.

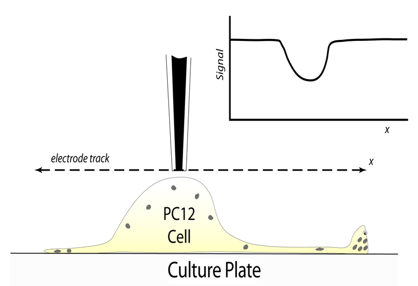

Figure 1. Current and impedance measurements are dependent upon the position of the electrode to the surface.

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-fiber electrodes (3-33 fibers) are characterized below.

Experimental

Carbon fiber electrodes were constructed with multiple (3 to 33) 7 µm diameter fibers (Thornel T650, Cytec Industries) and beveled at 90° on a diamond polishing wheel (model BV-10, Sutter Instrument Company).[2] Voltammetric measurements were conducted with a bipotentiostat in the 3-electrode mode (EI400, 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.

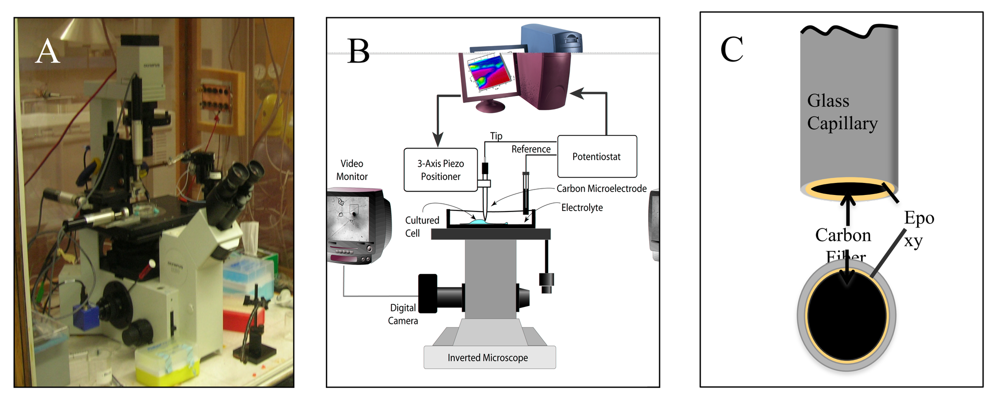

Figure 2: Scanning Electrochemical Microscope with Electrode Probes. A. Photograph of SECM instrument. B. Schematic of instrument. C. Schematic of electrode.

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.

$$4H^+ + O_2 \rightarrow 2H_2O$$

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,B, 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.

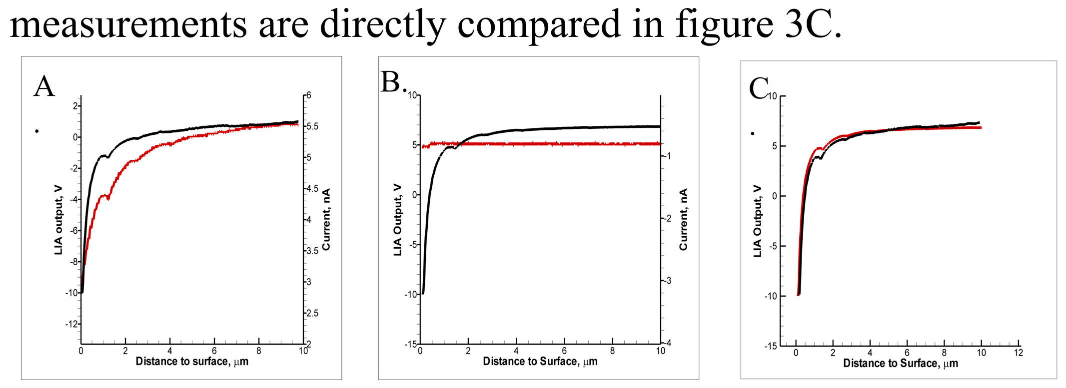

Figure 3:
A. At the reducing potential of -1.2V, both current (red) and impedance (black) change as the electrode approaches the cells. B. At 0V, the current (red) is constant because nothing is being oxidized or reduced, but the impedance (black) changes as the electrode approaches the surface. C. The impedance measurements are shown as the electrode approaches the surface (red = -1.2V, black = 0V). The impedance has a relatively constant distance dependence that is independent of the applied potential.

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.

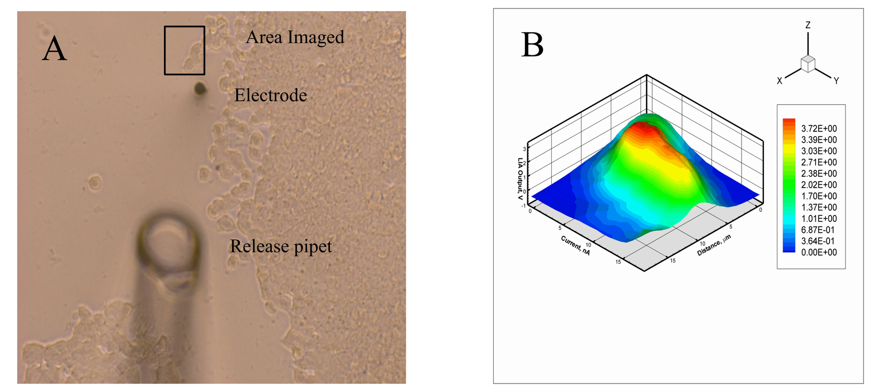

Figure 4:
A. Photograph of PC12 cells. B. Topographical image of a PC12 cell using distance-dependent impedance measurements.

A larger electrode is needed to monitor neurotransmitter release from larger biological substrates such as taste buds.

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 5µm 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 buds contain NT. Larger multiple carbon fiber electrodes were fabricated to assay the entire exposed surface of the cells.

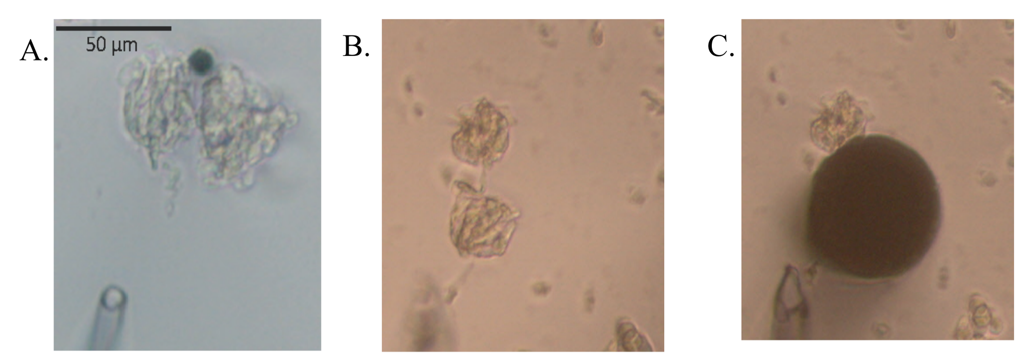

Figure 5:
A.Isolated taste buds with 5 µm single carbon fiber electrode. The electrode is very small compared to the size of the taste bud. B. Isolated taste buds. C. Same taste buds as fig. B with the 33 fibers (7µm) carbon fiber electrode. This electrode covers the entire surface of the taste bud.

Impedance-based positioning can be used with multiple carbon fiber electrodes.

In order to detect NT release from isolated taste buds, the multiple carbon fiber electrode must be positioned directly next to the taste bud. The multiple carbon fiber electrode must be positioned using impedance measurements since it cannot be visualized when they are approaching the surface. The impedance measurement is dependent on the size of the electrode. Figure 6A shows approach curves for three different types of electrodes. As the size of the electrode increases, the distance-dependent impedance measurement decreases much farther from the surface. Figure 6B-D shows the scanning electron microscope photographs of each electrode.

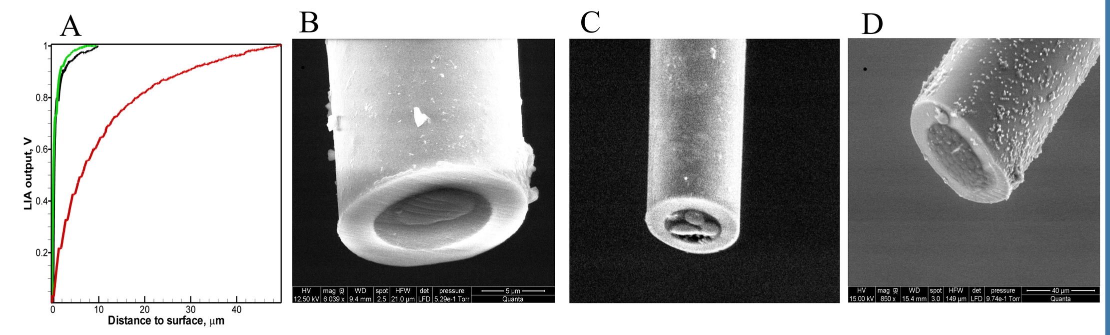

Figure 6:
A.The green line represents the 5µm single carbon fiber electrode. The black line represents the 3 carbon fiber electrode, with the diameter of each fiber being 7µm. The red line represents the 33 carbon fiber electrode, with the diameter of each fiber being 7µm. B. Single fiber electrode , 5 µm in diameter. C. Multiple carbon fiber electrode, containing 3 fibers, each about 7µm in diameter. D. Multiple carbon fiber electrode, containing 33 fibers, each about 7µm in diameter.

Conclusion

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

1. Eckhard, Kathrin and Wolfgang Schuhmann. "Alternating current techniques in scanning electrochemical microscopy (AC-SECM)." *The Royal Society of Chemistry*. (2008): 133: 1486-1497. Web. 29 Aug. 2012.
2. Kelly R.S. and Wightman R.M. "Beveled carbon fiber ultramicroelectrodes." *Analytica Chimica Acta*. (1986): 187:79-87.

Acknowledgments

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Dr. John Baur and Dr. Melinda Baur