

ELECTROCHEMICAL BIOSENSOR FOR POINT OF CARE CANCER AND DISEASE DETECTION

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INTRODUCTION

The past decade has witnessed a wave of ingenuity sweeping towards fabrication of biosensors for disease detection. Biosensors offer numerous advantages: portability for on sight diagnostics, quick turn around time, and cheaper costs for both patient and health organizations. One hurdle in this technology has been the specificity and sensitivity of these sensors. In the field of electrochemical disease diagnosis, the selectivity of the sensor is often based on selective molecular binding between the targeted protein and the bio-recognition element. In this work, the selectivity of the binding molecule has been enhanced through the use of aptamers: molecular receptors which, due to their particular 3 dimensional shapes, bind tightly and selectively to their targets. The aptamer is situated in a disrupted double helix, breaking the electrochemical conductive path of the molecular chain. A blood sample is reacted with this molecular chain and upon analyte binding, the electrical conductivity of the helix is restored. This modulation in current is measured using cyclic voltammetry (CV). We have designed and built a portable potentiostat to perform such tests as well as the sub-millimeter electrodes on which the reaction takes place.

EXPERIMENTAL

Materials

The electrodes were fabricated on standard 3x1 inch glass slides. The glass was RCA cleaned for organics and sputtered with 50/50nm Cr/Au. A layer of positive photoresist was then spun on at 3500rpm for 30 s and soft baked at 100°C for 15 min. The electrodes were patterned using a UV exposure of 40 s, followed by 60 s development in MF319. The Au and Cr were etched using respective etchants for 60 s. The remaining photoresist was again exposed for 40 s and developed in MF319 for 60 s.

Of the three electrodes used to perform cyclic voltammetry, two of the electrodes, the counter electrode (CE) and working electrode (WE) are gold. The reference electrode (RE) is Ag|AgCl. The RE was fabricated by first silver plating a gold electrode. The gold electrode was submerged into a solution of 0.3M Ag₂SO₄ and 1.0M NH₃ for 4 min, with a voltage of -0.6 V applied between the electrode and a sacrificial silver source. To convert the Ag to Ag|AgCl, the plated electrode was submersed into a solution of FeCl₃ for 3 min. However, we found that after 48 hrs, the electrode was showing signs of oxidation. We have since tried using CuCl₂ for conversion to Ag|AgCl by placing the plated electrode into a solution of 1M CuCl₂ in water. (*No change in the electrode's surface has been noted*).

Methods and Apparatus

The WE must be isolated from the other electrodes while being prepared. Therefore, the CE and RE are situated on one slide while the WE occupies another slide. The working electrode slide was cleaned using a freshly prepared solution of "piranha" (3:1 mixture of concentrated H₂SO₄ and 30% H₂O₂. **WARNING:** use care in handling piranha solutions as it reacts violently to organic solvents.) at 90°C for 8 min and rinsed thoroughly with water. The freshly prepared gold surface of the electrode is then modified by spreading a 100µM solution of 11-mercaptoundecanoic acid onto the gold for an hour at 100% relative humidity. The slide was then thoroughly rinsed with water and dried with nitrogen gas.

The potentiostat (schematic shown in Figure 1) is operated via an Arduino Duemilanove microcontroller which connects to the potentiostat via ribbon cable. The output voltage is applied across two alligator clips which connect to the WE and RE, while a third alligator clip, which connects to the CE, picks up the resulting current. Both input and output are updated to a computer via USB.

Cyclic voltammetry was performed in a 30mL electrochemical cell using the 11-mercaptoundecanoic acid modified electrode as the working electrode, a

gold counter electrode and with either the plated Ag|AgCl electrode as the reference electrode or with a commercially available Ag|AgCl, 1M KCl reference electrode as the reference electrode. CV was performed in a deoxygenated solution of 5 μ M Ru(NH₃)₆Cl₃, 10mM Tris, pH 7.4. The CV was performed in the voltage range of -0.1 V to -0.5 V against the commercial reference electrode or in the range of 0 V to -0.7 V against the plated Ag|AgCl reference electrode at a scan rate of 0.1 V/s. These CVs were obtained from both the bench top potentiostat as well as the portable circuit. The results are discussed in the following section.

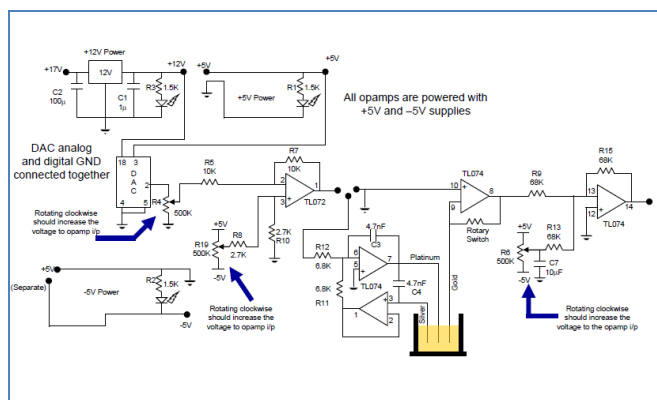


Figure 1. Potentiostat schematic

TESTING AND RESULTS

Cyclic voltammetry was chosen as the method of choice for the characterization of our electrochemical system due to its ability to provide considerable information about the thermodynamics and kinetics of the reactions at the electrode surfaces [1]. It also allows for the rapid determination of redox potentials of the reaction versus the potential of the reference electrode. In brief, CV is a diffusion controlled method where a linearly changing potential is applied to the working electrode. As the applied potential nears the redox potential of the electroactive species in solution, the current increases until the concentration at the electrode surface is depleted. In turn, this causes the current to decrease once again forming a peak. After the linear potential gradient reaches a certain point that is determined by the researcher, the potential gradient reverses and the potential scans in the opposite direction. The potential at the maximum height of the peak corresponds to the redox potential of the electrochemical reaction, which is measured

against the potential of the reference electrode.

The potential of the half-cell of the reference electrode is dependent on the chloride ion concentration of its environment. The commercially available silver-silver chloride electrode is filled with a solution of 1M KCl so that its potential remains the same ($E = 0.228$ V vs SHE – standard hydrogen electrode) [2]. The potential of our plated electrode will depend on the concentration of chloride ions in our electrolyte (~10mM). And thus, cyclic voltammetry performed using different reference electrodes will be shifted with respect to the difference in the potential of the reference electrodes.

To ensure reliable functionality of the portable potentiostat, we performed a series of tests using both the plated and the commercially available reference electrodes, and compared the results. First, to verify accuracy of the plated reference electrode, we compared CVs produced by the bench top potentiostat, for each RE. The results are shown in Figure 2. As mentioned previously, the peaks of the curves are slightly shifted but the peak's relative position is correct.

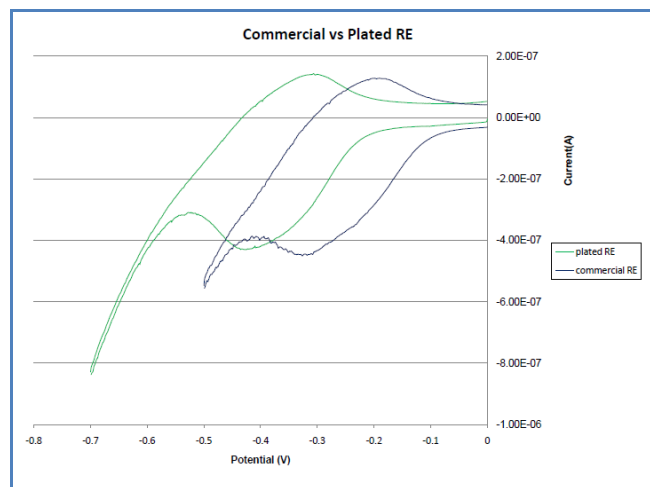


Figure 2. Commercial RE vs Plated RE

Then, to compare the potentiostats in a controlled environment, we used non miniaturized electrodes: we used the commercial RE, a pure gold electrode as the WE and a platinum wire for the CE. The CVs were performed in a solution of 1mM K₃Fe(CN)₆ and 100mM NaCl. The results of this test are shown in Figure 3. The portable potentiostat circuit is on a PCB and currently not contained, therefore, the CVs produced show greater noise than those obtained from the bench top potentiostat. Plans to enhance the signal to noise ratio will be discussed later.

CONCLUSION AND FUTURE WORK

We have demonstrated that separately, our miniature electrodes and our portable potentiostat, can produce comparable results to those obtained from the bench top system. In order to reduce the noise created when using our system as a whole, (our electrodes with our portable potentiostat), we are revising several aspects of the system. We are now beginning work on performing the same tests all on one slide. We hope to create removable PDMS chambers that may be placed onto the slide and adhere to the glass to form a seal. We plan to use a smaller chamber, diameter $\sim 0.2\text{mm}$, to isolate the WE from the other electrodes during incubation. Cyclic voltammetry tests will be performed within a larger PDMS chamber of diameter $\sim 0.6\text{mm}$ which will be water tight to the slide and encompass all three electrodes. In addition, once the tests are performed on a single slide we will implement a more secure connection to the electrodes than alligator clips in hopes to reduce the noise. We will also encase the circuit to cut down on interference from external noise sources.

Once the fabrication process of the chambers has been finalized, we will miniaturize the system further. The unit will be completely portable and battery operated. We will no longer use cyclic voltammetry but chronoamperometry instead so that analysis may be performed without an external computer.

ACKNOWLEDGEMENTS

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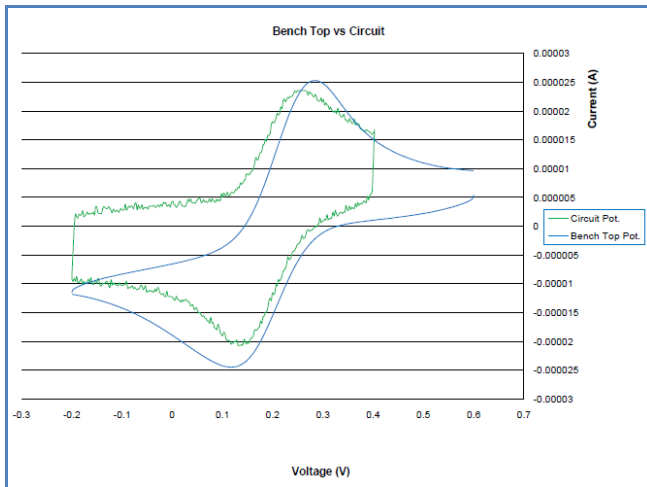


Figure 3. Benchtop vs Circuit Potentiostat

And finally, we compared our system (the plated RE and portable potentiostat) with the bench top potentiostat using the plated RE. These results are shown in Figure 4.

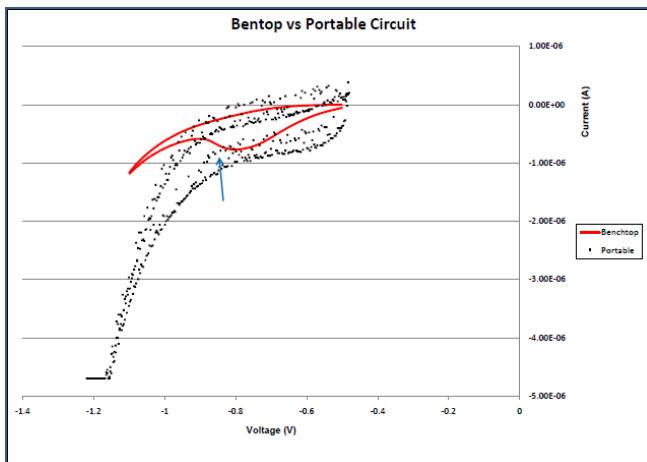


Figure 4. Bench top vs portable potentiostat

When using both the miniaturized electrodes, and the portable potentiostat, the noise interference is too great. Here in Figure 4, the negative peak is lost in the noise. The arrow indicates where the peak is actually occurring when viewed by an oscilloscope. Although there are data points visible, tracing the curve of this peak, there are too many other data points interfering. We are currently working on reducing this noise to obtain clearer results.