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14. ABSTRACT

Glucose sensor arrays were fabricated on gold electrodes on flexible polyimide sheets by cross-linking glucose oxidase and redox polymer using UV-initiated free radical reaction. Using conventional silicon fabrication methods, five-element array Au microdisks were initially fabricated using mid-UV photolithography. Active glucose oxidase was entrapped in hydrogel by UV-initiated photo polymerization with poly(ethylene glycol) diacrylate or PEG-DA on the array electrodes. The fabricated microarray sensors were individually addressable and with no cross-talk between adjacent array elements as assessed using cyclic voltammetry. We have fabricated an array of glucose sensors on flexible polyimide sheets that exhibit the desired linear response in the biological range. We have also tested the sensors using other electrochemical methods including amperometry and square wave voltammetry (which is known for its background reduction).

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Introduction

Intensive treatment with the goal of maintaining blood glucose concentrations close to the normal range can prevent or delay the occurrence of diabetic related complications.[1] Diabetic patients, therefore, have to frequently monitor their blood glucose levels by drawing blood necessary for conventional glucose monitoring. The blood data obtained gives no indication of direction or trend of blood sugar levels. Even the most motivated diabetic patient performing frequent tests may miss reoccurring highs or lows, particularly at night. Development of simple and painless techniques of monitoring glucose with increased frequency would be beneficial to diabetic patients.

A number of strategies for measuring the blood glucose are continuously under development to allow pain free, more frequent glucose monitoring. Transdermal spectroscopic[2, 3] and interstitial fluid sampling[4, 5] are some of the techniques under investigation. A major advantage of transdermal spectroscopic techniques is painless testing; however, their main drawback is measurement of glucose in a highly complex matrix of water, proteins polysaccharides and lipids. The result is a complex signal with glucose measurement having to be extracted through other mathematical programs hence introducing errors at every stage. Reverse iontophoresis [6] and sonophoresis[7] techniques for interstitial blood sampling have been studied. One advantage of these techniques is that a physiologically relevant fluid sample with glucose concentration closely related to glucose in blood is collected. There still exists significant difference between the blood plasma glucose and interstitial fluid glucose.[8]

Implantable sensors for glucose have been under investigation for nearly three decades with mixed and promising results. Implantation of sensors can be done either in subcutaneous tissue to measure glucose in interstitial fluid[9-12] or intravascular.[13] These sensors have enormous potential for long term monitoring of glucose in humans as demonstrated from limited clinical trials. As with any foreign object introduced into the body, biocompatibility is one of the most important requirements of these sensors. For blood-contacting devices, hemocompatibility of *in vivo* sensors is also an important consideration, as well. Adsorption of proteins on surfaces of implanted sensors and devices constitutes the first step of several biological responses, including the activation of the coagulation cascade. Following protein adsorption, cell adhesion occurs. This is not a desirable event, since it could lead not only to the alteration of the sensor output but can also cause harmful side effects on the subject, e.g., thrombi formation after adsorption and activation of platelets.[14] In this work we have used PEG-DA hydrogel networks that provide an ideal three-dimensional, aqueous in vivo-like surrounding[15-18] to entrap both the redox polymer and glucose oxidase on polyimide sheets. We have used biocompatible PEG-DA hydrogel networks that have shown to be provide an ideal three-dimensional environment[15-18] to entrap both the redox polymer and glucose oxidase on polyimide sheets.

We have used silicon microsensor fabrication technologies to fabricate sensor arrays and using mid-UV photolithography to polymerize and pattern on a micrometer scale three-dimensional redox polymer hydrogels containing glucose oxidase for glucose monitoring. An osmium-based redox polymer replaced oxygen as a non-physiological electron acceptor. We have used square wave voltammetry, amperometry, and cyclic voltammetry to monitor catalytic oxidation of glucose by glucose oxidase enzyme in a reaction mediated by a redox polymer.

Body

In the proposal for this project there were many different aspects that were to be examined for the implantable continuous monitoring sensors. The first of which was to do the bench-top work on the glucose sensors and test and optimize the sensor, which is described in the glucose sensor section of the report. Other aspects have been and are still currently being examined, such as the design of the sensor, lactate sensors, and animal testing, and are discussed in subsequent sections.

Glucose Sensors

In this report we will discuss glucose sensors developed with photolithographic methods and using PEG-DA as the hydrogel encapsulating sensing elements. In the proposal we wanted to encapsulate the redox polymer (POs-EA) and the enzyme (*e.g.* GOX) in the PEG-DA hydrogel simultaneously, but this method proved to have difficulties. We altered the sensor by electrostatically attaching POs-EA to the surface of the self-assembled monolayer on the gold and then used photolithography methods to encapsulate GOX in the PEG-DA hydrogel. This method proved to work and the results for glucose sensors below is using this method.

Materials and Methods

Glucose oxidase (GOX, EC 1.1.3.4, Type X-S, 128 units/mg solid from *Aspergillus niger*), Ammonium hexachloroosmate(IV), 11-mercaptoundecanoic acid (MUA), poly(4-vinylpyridine), β-D-glucose, acetone, ammonium hexafluorophosphate, sodium dithionite, ether, 2-bromoethylamine hydrobromide, N,N-Dimethylformamide, anion exchange beads, hydrochloric acid, poly(ethylene glycol) diacrylate (MW 575), and 2,2′-dipyridyl (bpy) were obtained from the Aldrich Chemical Co. (Milwaukee, WI). Ethyl alcohol, ethylene glycol and acetonitrile were obtained from Fisher Scientific Co. (Pittsburgh, PA). DAROCUR, the photo initiator, was obtained from Ciba. All reagents, unless otherwise stated, were used as received. Polyimide sheets (1/16” thick) were purchased from McMaster Carr (New Brunswick, NJ). Phosphate buffered saline (PBS) consisted of 1.1 mM potassium phosphate monobasic, 3 mM sodium phosphate dibasic heptahydrate. Conductive silver epoxy was obtained from Ladd Research (Williston, VT). Ma-P 100, positive photoresist, and ma-D 330, Developer, were purchased from MicroChem Corp. (Newton, MA). The polycationic redox polymer, poly[vinyl pyridine $Os(bipyridine)_2Cl$]-co-ethylamine (POs-EA as seen in Figure 1) was synthesized according to a procedure described previously.[10]

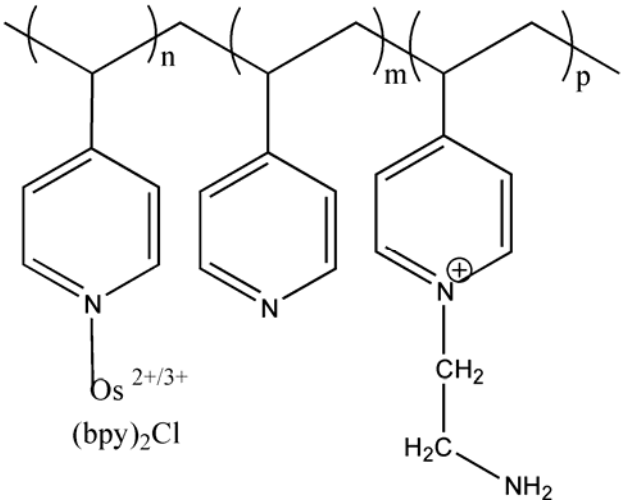


Figure 1: Structure of poly[4-vinylpyridine Os(bipyridine)$_2$Cl]-co-ethylamine (POs-EA) molecule containing osmium redox polymer backbone (where n=1, m=4, p=1.2)[19]

Synthesis of poly[vinylpyridine Os(bipyridine)$_2$Cl]-co-ethylamine (POs-EA)

Synthesis of poly[vinylpyridine Os(bipyridine)$_2$Cl]-co-ethylamine (POs-EA), an osmium based polycationic redox polymer was done following modifications of established protocols.[19] Os(bpy)$_2$Cl$_2$ was synthesized according to a standard procedure with minor modifications.[20] In brief two equivalents of bipyridine (1440 mg) were mixed with one equivalents ammonium hexachloroosmate (IV) (2000 mg) in 100mL ethylene glycol. The mixture was heated to reflux for 45 minutes and then precipitated with supersaturated sodium diothionate. The precipitate was repeatedly washed with water and finally with ether.

Os(bpy)$_2$Cl$_2$ (0.988 g, 1.728 mmol) and poly(4-vinyl-pyridine) (0.860 g, 8.18 mequiv) were heated under nitrogen at reflux in 36 mL of ethylene glycol for 2 hours. The solution was then cooled down to room temperature and 60 mL of DMF and 3.0 g of 2-bromoethylamine hydrobromide (14.6 mmol) were added and then stirred overnight at 45 ^{o}C. A crude polymer precipitate was formed by pouring the solution into rapidly stirred acetone. The hygroscopic precipitate was collected and dissolved in water. The solution was filtered and precipitated as the PF_6^- salt by addition of a solution of ammonium hexafluorophsophate. The precipitate was dried in a vacuum at 40^{o}C. Dry PF_6^- salt (0.98 g) was dissolved in 40 mL of acetonitrile and then diluted with 100 mL of water and stirred over 10.4 g of anion exchange beads for 2 hours. The solution was filtered and evaporated under vacuum to ~20 mL. Concentrated HCl was added to the solution to adjust to pH 2. The solution was then dripped into rapidly stirred acetonitrile. A precipitate that formed was filtered and dried in a vacuum desiccator. The pure product was analyzed.

Fabrication of electrode arrays on polyimide sheets

Polyimide sheets are flexible insulating materials and ideal platforms for electrode manufacturing. Square pieces of polyimide were cut and washed with ethanol before coating with positive photoresist (ma-P 100). Modified literature procedure was used to fabricate the electrode arrays.[21] In brief positive photoresist (ma-P 100) was deposited on square polymer sheets and spin coated at 4000 rpm for 30 seconds and then soft baked at 100 ^{o}C for 5 minutes. These polymer sheets with dry photoresist were brought in close contact with the photo-mask

and exposed to 365 nm UV light. The polymer sheets were then placed in developer solution (ma-D 330) for seventy seconds to remove the portions of photoresist that were exposed to UV light. The sheets were then rinsed with distilled water.

The photoresist patterns were then sputter coated with 10 nm adhesion layer of chrome followed by 150 nm layer of gold (done at Pennsylvania State University using Lesker CMS-18 sputtering tool and by Lance Goddard Associates at Foster City, CA). The photoresist was removed using acetone to lift-off chrome and gold from all non-patterned areas. The result was distinct patterns of gold with 500 µm diameter electrodes with leads 10 µm and contact pads at 2.5 mm x 2.5 mm; a completed sensor array can be seen in Figure 2. Wires were attached to the contact pads by using conductive silver epoxy resin (Ladds Research, Williston, VT).

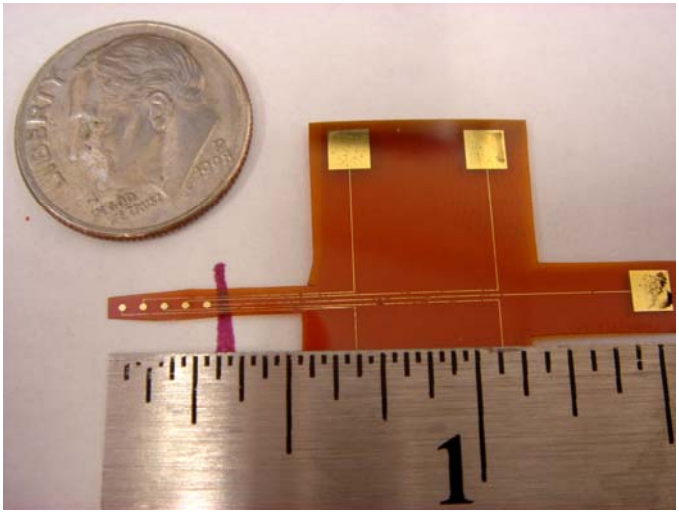


Figure 2: Fabricated Electrode Array where only the part to the left of the line will be inserted into the skin (where the scale is in inches).

Immobilization and pattering of biosensor films on gold surfaces

The electrode arrays were functionalized with a carboxylic end group by immersing in 2 mM MUA in ethanol for 20 minutes and then washed with ethanol. The electrodes were then dried under nitrogen. The thiol end group was chemisorbed to gold to provide an anchor. Next, the redox polymer was immobilized on the electrodes by depositing 2µL of 10 mg/mL POs-EA, solution on electrode surface and left to cure overnight in the dark. Strong electrostatic interactions occur between the negative MUA and the positive POs-EA. The excess POs-EA was removed by washing with water and dried with nitrogen. The PEG-DA precursor was made by mixing 5 µL of a GOX solution (50 mg/mL in HEPES buffer), 5 µL PEG-DA, and 0.5 µL DAROCUR and then placed onto the electrode surface. UV light was then applied and PEG-DA hydrogels were formed on the five different electrode array elements.

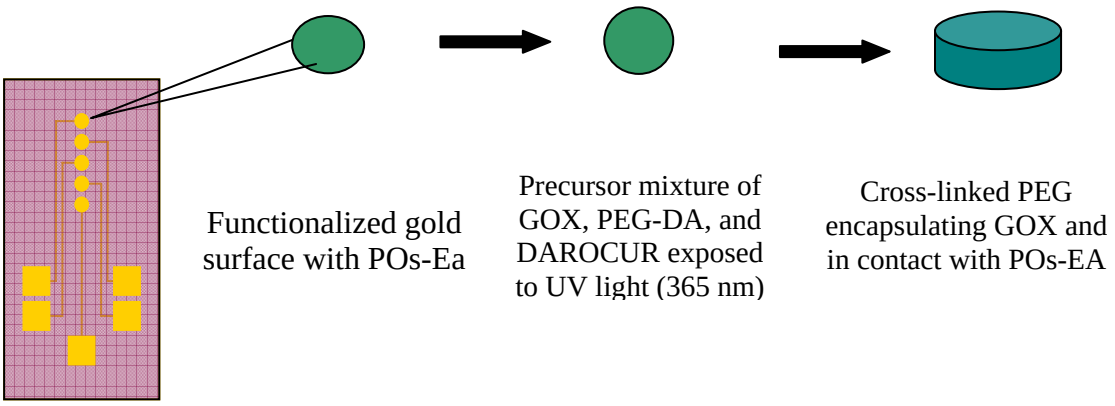


Figure 3: Schematic of the fabrication of sensor

Electrochemical Techniques

All electrochemical experiments were performed using Princeton Applied Research (PAR) 273 potentiostat on a three electrode system with platinum wire used as counter electrode and Ag/AgCl as reference electrode. The array electrodes with redox polymer and enzyme encapsulated with a PEG-DA hydrogel were used as working electrodes. All experiments were conducted in 0.1 M PBS buffer pH 7.0 with varying amounts of a glucose solution (200 mM glucose in 0.1 M PBS) added for the desired concentration.

Results and Discussion

Amperometric biosensors based on redox polymer/enzyme complexes were shown to be miniaturizable and implantable.[22-24] Previously, enzymes entrapped in redox hydrogels using photo polymerization were shown to retain their activity.[25-29] Enzyme-containing redox polymer films were formed through the UV-initiated free radical cross linking of a redox polymer and PEG-DA in each electrode array element. Each sensor array element after microfabrication was smooth and without any discontinuities.

Cyclic Voltammetry

To determine whether the array elements had any defects or whether they functioned independently, cyclic voltammetry was performed initially without the analyte (glucose) to evaluate the reproducibility of micro-fabrication process and the stability of the film. The micro-fabrication process was quite reproducible as observed from almost overlaid cyclic voltammograms for each element of the array (not shown). The entrapment of the redox polymer, glucose oxidase and PEG-DA was also uniform on all the electrodes, which is especially important when summing sensor signals of the arrays. The signal from all the array sensor elements mimics the behavior of one large sensor with electrode area equal to the sum of all the electrode array elements. Figure 4 shows cyclic voltammograms of increasing number of array elements. Measurements done after combination of different array members show increasing peak currents with increasing number of array elements combined. With such a sensor array, multiple analytes can be measured simultaneously and through the introduction of

redundancy, measurements can be derived from the average of the signal resulting from each array element. There was no cross-talk between the array members.

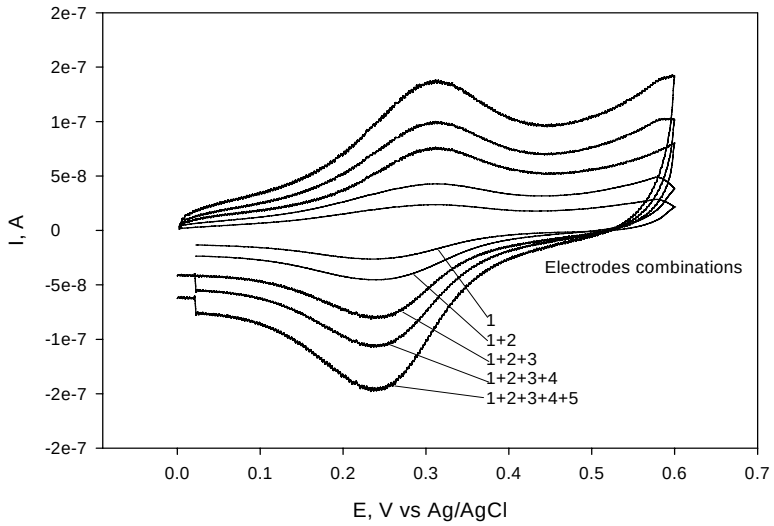


Figure 4: Cyclic voltammograms of different combinations of micro-array electrodes at 20 mV/s scan rate

The formal potential of this redox polymer was around 0.3 V versus Ag/AgCl electrode about the same as what has been reported for this molecule.[30] Anodic and cathodic peaks were observed at 0.32 and 0.25 V versus Ag/AgCl reference electrode at 20 mV/s scan rate. The peak separation was approximately 60 mV. Figure 5 shows cyclic voltammograms of one element of microarray at different scan rates. Plots of peak current versus scan rate were linear at scan rates 0.01-0.50 V s^{-1}. At high scan rates (above 50 mV/s) a deviation from linearity was observed (not shown). A plot of peak current (i_p) versus square root of scan rate ($v^{1/2}$) yielded a straight line (Figure 6). The dependence of the current function i_p on the scan rate, $v^{1/2}$, is an important diagnostic criterion for establishing the type of reaction mechanism by cyclic voltammetry.[31] The ratio of anodic to cathodic peak currents was unity indicating chemical reversibility. The CV is consistent with non-ideal, reversible, thin layer electrochemistry.[32] Cyclic voltammetry of the electroactive polymer film showed a reversible response for the Os^{II}/Os^{III} redox couple, with forward and reverse peaks at similar potentials (Figure 4-6).

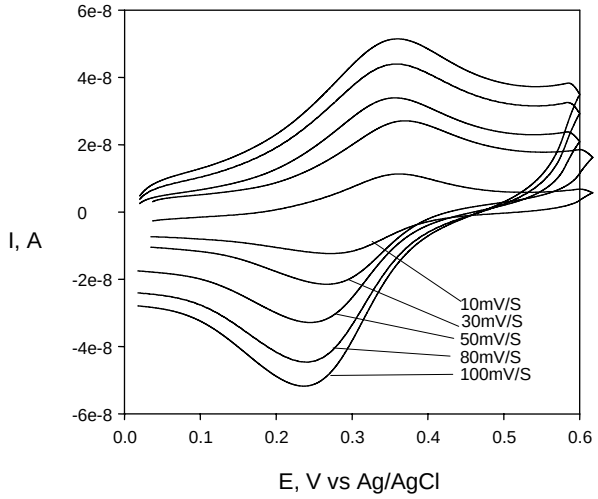


Figure 5: Cyclic voltammograms of one element of the micro-array at different scan rates.

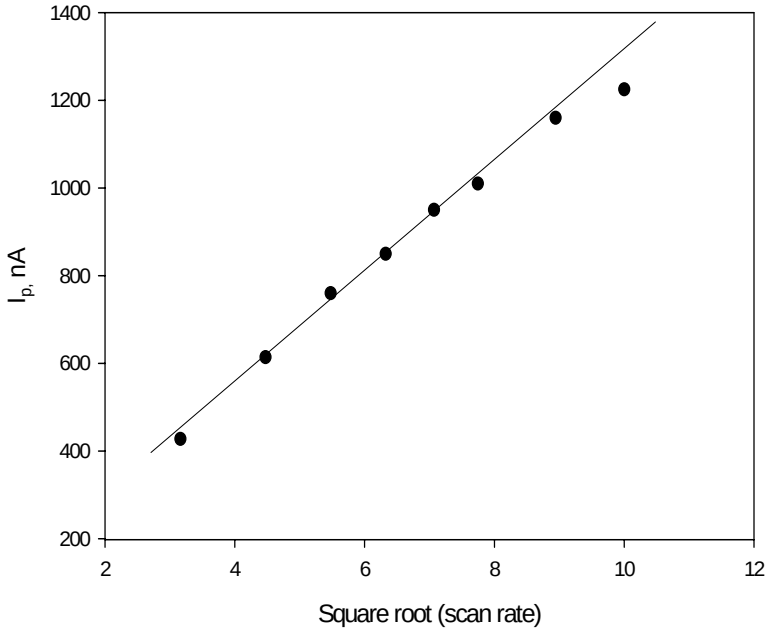


Figure 6: Scan rate dependence of film containing GOX, redox polymer and PEG-DA in phosphate buffer

Properly cross-linked GOX and POs-EA molecules are expected to retain the activity of glucose oxidase and to present signal stability. Signal stability would degrade if enzyme leaching from the hydrogel occurred. Figure 7 shows cyclic voltammograms of five combined elements of

the microarrays in buffer and in buffer containing 5, 10, and 20 mM glucose solutions. In the presence of increasing glucose concentration, the current is found to increase. The rate of this catalytic reaction is proportional to the concentration of glucose present. Flavin adenine dinucleotide of glucose oxidase GOX(FAD) reacts with β-D-glucose to form a reduced form GOX($FADH_2$) and gluconic acid inside the hydrogel. The reduced form of GOX($FADH_2$) is in turn oxidized by the electrochemically generated Os^{3+} form of the redox polymer, setting up a catalytic pathway which produces an enhanced oxidation peak.

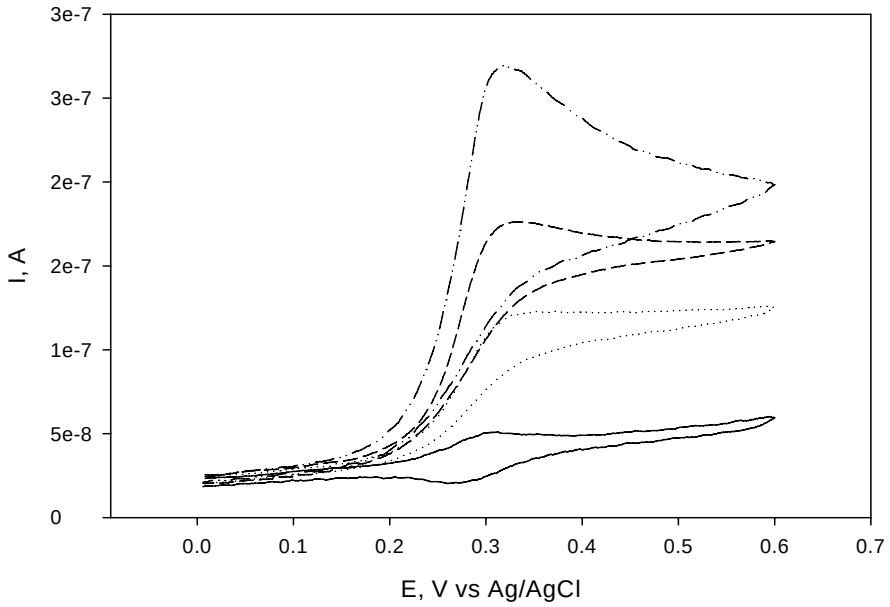


Figure 7: Cyclic Voltammograms of one sensor element at different glucose concentrations (— in buffer, … in 5mM glucose, - - in 10 mM glucose, and --.. in 20 mM glucose).

The electrons are transferred from the enzyme to the redox polymer, shuttled between the redox sites in self exchange reaction until being transferred to an electrode surface. The catalytic current produced is proportional to the glucose concentration. Presence of oxygen does not influence the rate of the reaction and hence the catalytic current obtained. Glucose oxidase is securely trapped in the hydrogel network and glucose diffuses through to access the glucose oxidase sites. Photopolymerization of the acrylate end groups of the PEG-DA by the photo initiator DAROCUR occurs and entraps the glucose oxidase enzyme on the redox polymer film. We are yet to find out how different thicknesses of the hydrogel affect the glucose movement in and out of the hydrogel and how the catalytic signal is affected. We believe that the thickness of the hydrogel network ultimately affects the communication of enzyme and the redox polymer. Hence optimum thickness will be very important in this sensor.

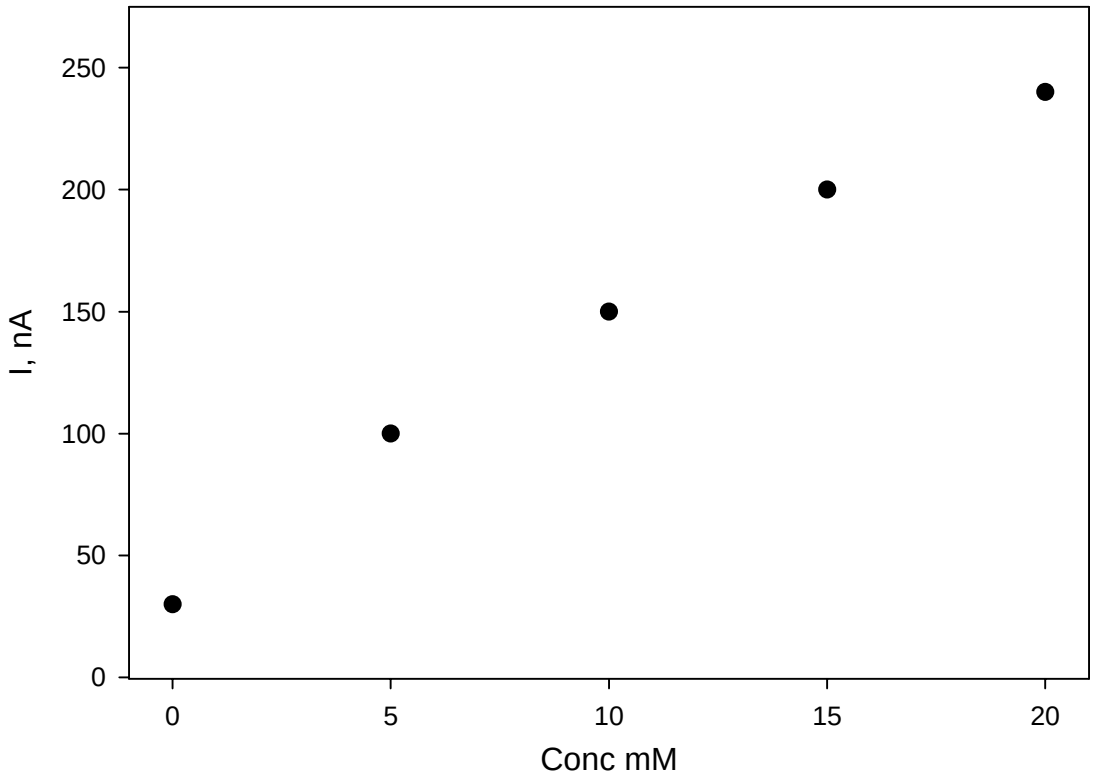


Figure 8: Collective response of five sensor elements to increasing glucose concentration

Figure 8 indicates that the sensor signal is proportional to the concentration of glucose (0-20 mM).

Amperometry

Another electrochemical method for testing is amperometry where a constant potential is applied over a given time and then the current is monitored. With amperometry the current is proportional to the reaction rate, which in this case is proportional to the glucose concentration. In Figure 9 the potential is maintained at 0.35 V and the current is monitored when the amount of glucose in solution is increased. In Figure 10 the steady state value of the current density versus the glucose concentration is graphed and displays the expected linear relationship. At higher concentrations the data is not as linear due to the nature of the enzyme.

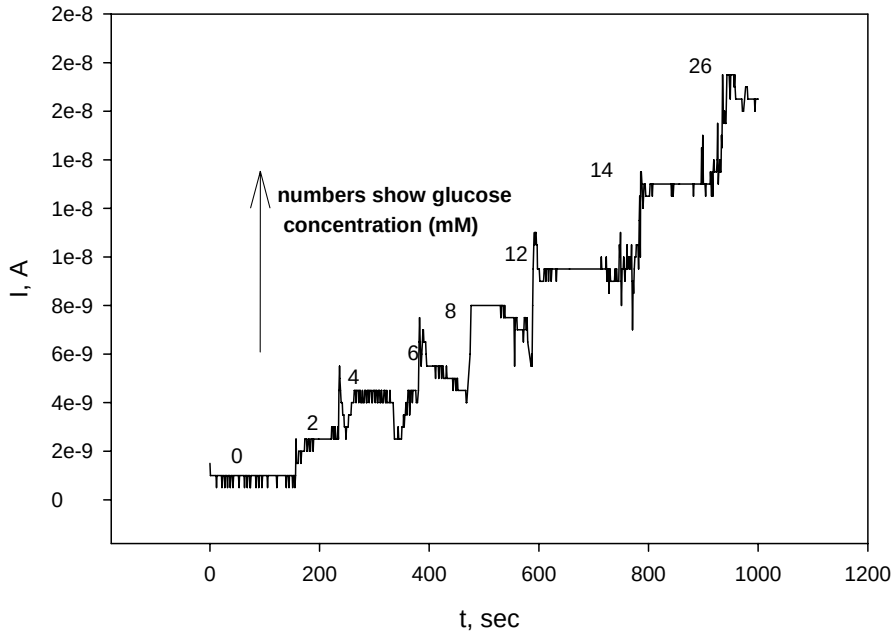


Figure 9: Amperometric response of one sensor array element to varying concentration of glucose

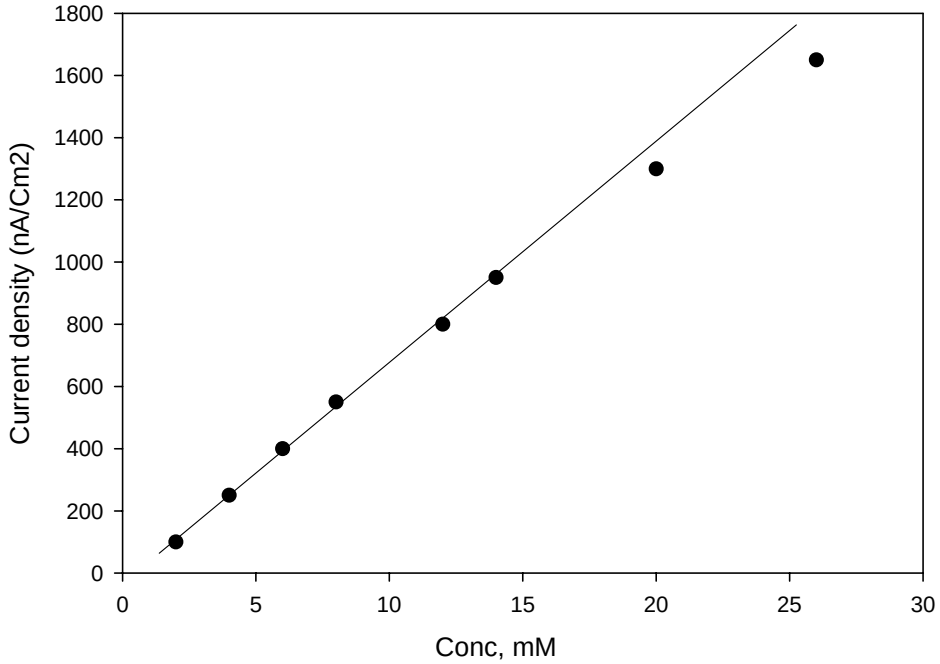


Figure 10: Current density versus concentration for the amperometric response for one sensor array element

Square Wave Voltammetry (SWV)

Square wave voltammetry (SWV) is an electrochemical technique suitable for both qualitative and quantitative analysis. In SWV the current signal is repeatedly sampled at two points relative to the time of application of a square wave voltage signal to the electrode. The difference between the two current values can be plotted as a function of the applied potential. Forward, reverse and difference scans are obtained. The resultant peaks correspond to the electroactivity of the species in the electrochemical cell. The major component of this difference current is the faradaic current, which flows due to an oxidation or reduction at the electrode surface. One major advantage of this technique is that the charging current component, due to electrical charging of electrode double layer, is largely eliminated and the signal to noise ratio is enhanced.

In this work redox polymers exchanged electrons with glucose oxidase entrapped in biocompatible poly (ethylene glycol) diacrylate (PEG-DA) hydrogels. SWV of the electroactive polymer film showed a reversible response for the Os^{II}/Os^{III} redox couple, with forward and reverse peaks at similar potentials (figure 11). The formal potential of this redox polymer entrapped in PEG-DA hydrogel was around 0.3 V versus Ag/AgCl.

Figure 12 shows square wave voltammograms of one element of the micro-array at different frequencies. The SWV difference peak current was proportional to frequency from 1 to 5 Hz consistent with thin-layer electrochemical behavior.[33] At frequencies higher than 15 Hz, the plots plateau (Figure 13).

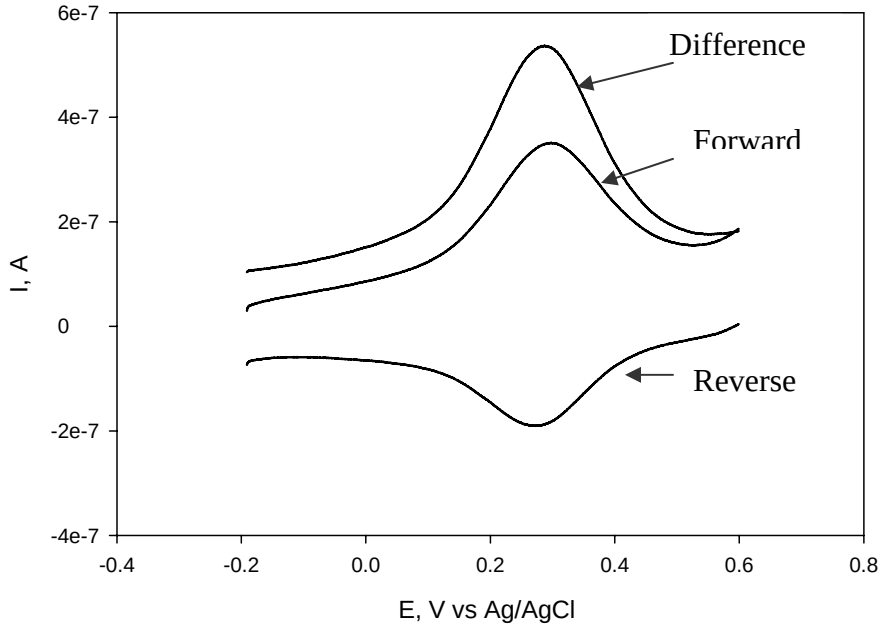


Figure 11: Forward, reverse and difference curves of SWV of redox polymer of one element of the array with GOX entrapped in PEG-DA hydrogel

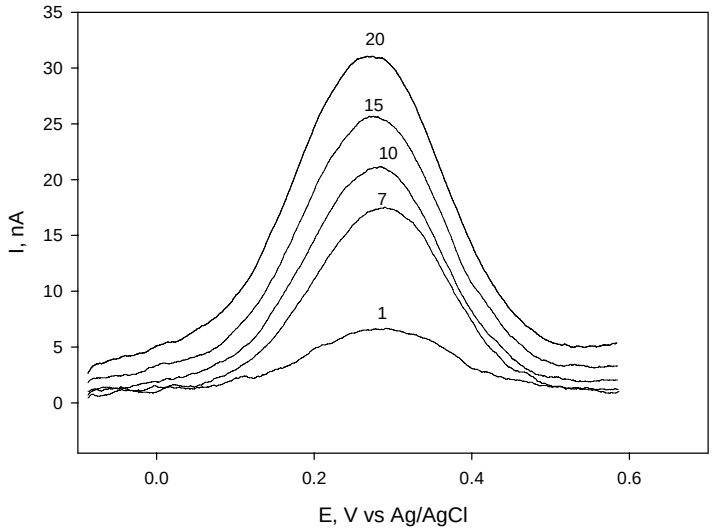


Figure 12: Square wave voltammograms of sensor electrodes at different frequencies

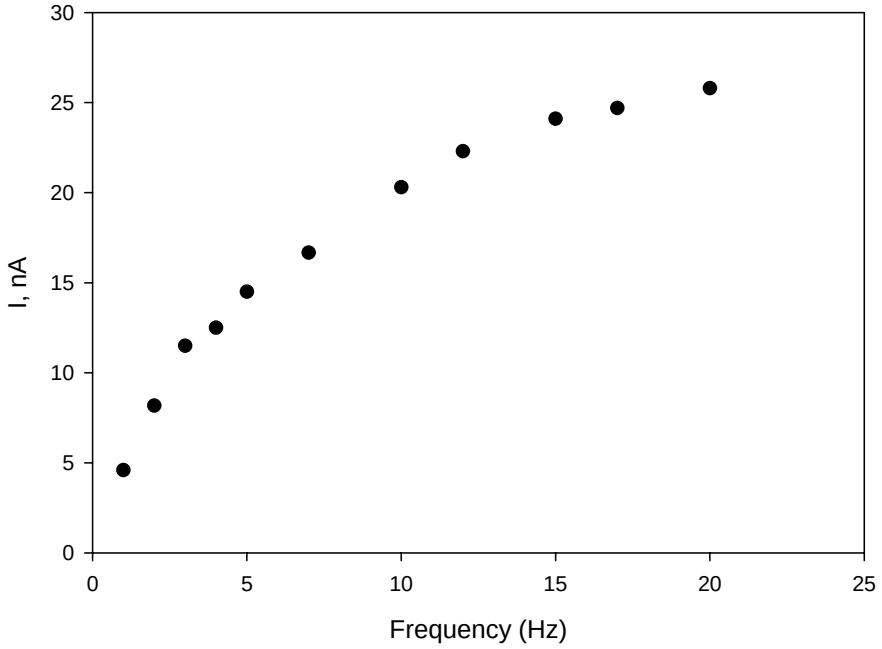


Figure 13: Frequency dependence of film containing redox polymer and glucose oxidase enzyme entrapped in PEG-DA hydrogel

Each sensor array element was smooth and without any discontinuities. To determine whether the array elements had any connectivity problems, or whether they functioned independently, SWV was done on each array member. Each electrode 500 μm in diameter was individually addressable. The micro-fabrication process was quite reproducible as observed from almost overlaid square wave voltammograms for each member (data not shown). The

entrapment of the redox polymer, glucose oxidase and PEG-DA was uniform on all the electrodes, which is especially important when the arrays sensors signals are to be summed. The signal from all the array sensor elements mimics the behavior of one large sensor with electrode area equal to the sum of all the electrode array elements. Figure 14 shows square wave voltammograms of different combinations of increasing number of array elements. Measurements done after combination of different array members show increasing peak currents with increasing number of array elements combined (Figure 15). The sensor arrays portray a situation where the area of the array elements is almost the same and the film assembly on each element being reproducible. With such a sensor array, multiple analytes can be measured simultaneously and through the introduction of redundancy, measurements can be derived from the average of the signal resulting from each array element. There is no evidence of cross-talk between the array members in these sensors.

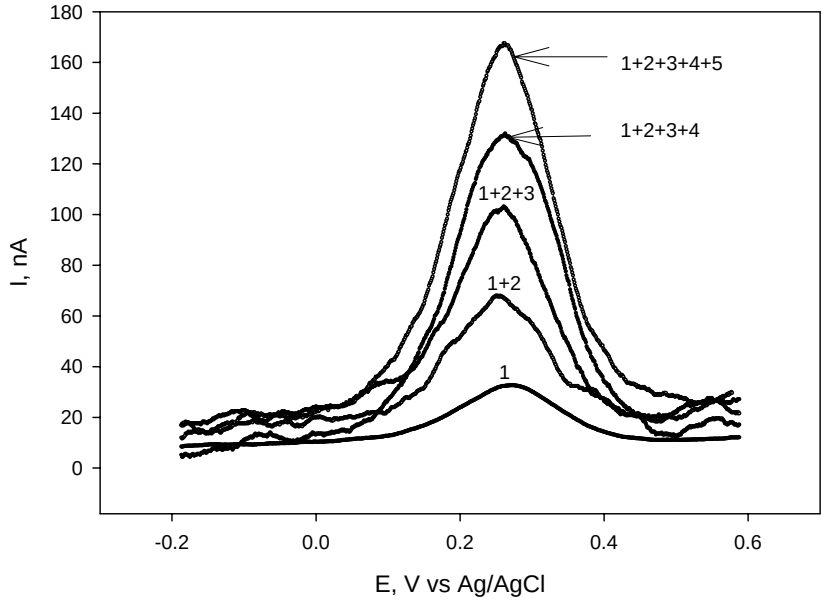


Figure 14: SWV of different combinations of array sensor elements in PBS at pH 7

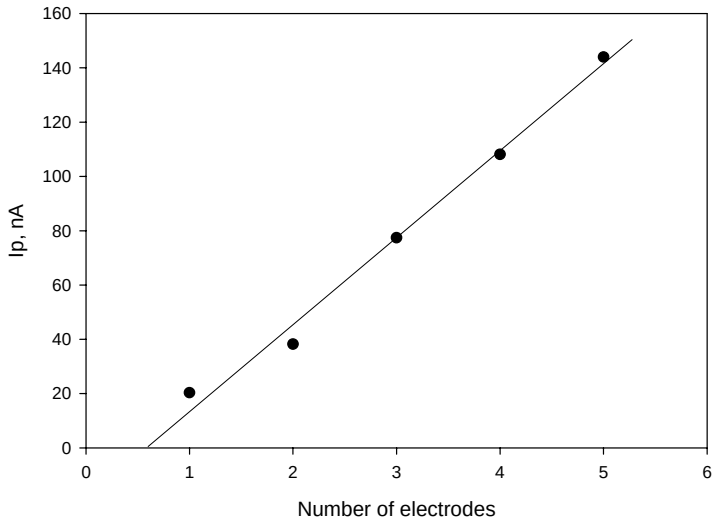


Figure 15: Change of peak currents with different combinations of electrodes in the array sensor

Biocompatible PEG-DA was used to cross-link glucose oxidase enzyme with electroactive redox polymer, POs-EA. Properly cross-linked molecules are expected to retain the activity of glucose oxidase enzyme and to present signal stability. If enzyme is lost through leaching from the electrode surface the signal stability would suffer. Figure 16 shows square wave voltammograms of one element of the micro-array in buffer without glucose and in buffer containing 10 mM, 20 mM and 50 mM glucose solutions. In the presence of increasing glucose concentrations the current is found to increase. Flavin adenine dinucleotide of glucose oxidase GOX(FAD) reacts with β-D-glucose to form a reduced form GOX($FADH_2$) and gluconic acid. The reduced form of GOX ($FADH_2$) is in turn oxidized by the electrochemically generated Os^{3+} form of the redox polymer, setting up a catalytic pathway which produces an enhanced oxidation peak. The electrons are transferred from the enzyme to the redox polymer, shuttled between the redox sites in a self exchange reaction until being transferred to an electrode surface. The catalytic current produced is proportional to the glucose concentration (Figure 17). As expected high concentrations of glucose (above 30 mM) there is deviation of linearity due to the nature of enzymes.

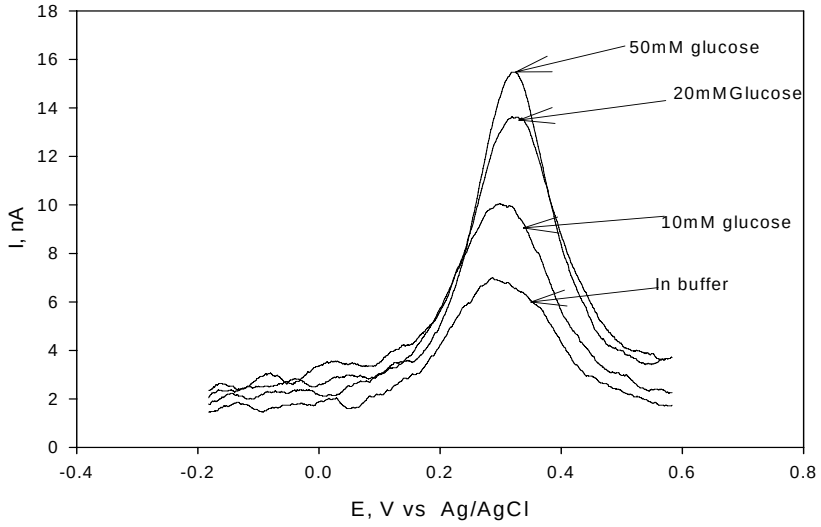


Figure 16: Square wave voltammograms of one sensor element at different glucose concentrations.

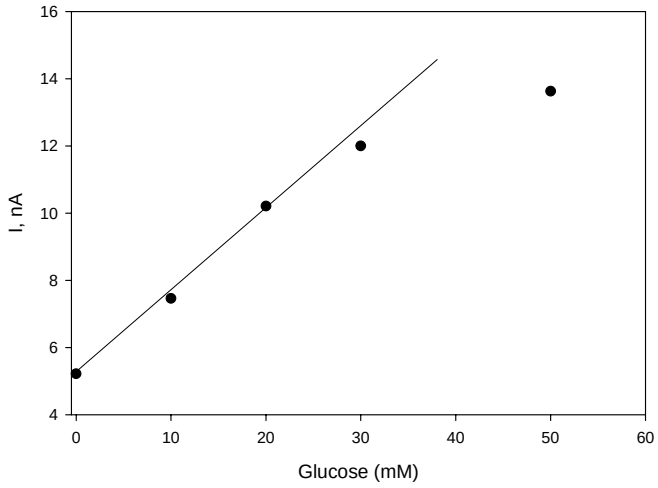


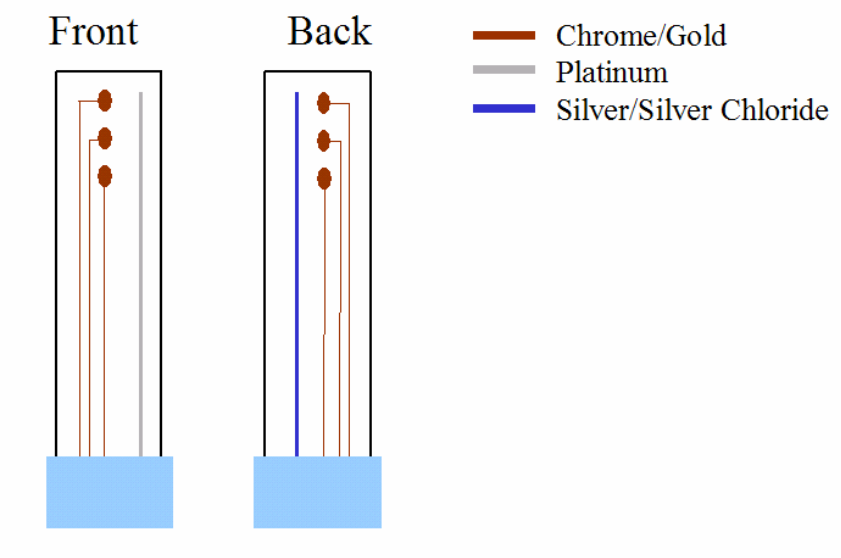
Figure 17: Sensor response to increasing glucose concentration.

Glucose oxidase is securely trapped in the hydrogel network and glucose diffuses inside the hydrogel to access the glucose oxidase sites. Photopolymerization of the acrylate end groups of the PEG-DA by the photoinitiator DAROCUR occurs and entraps the glucose oxidase enzyme on the redox polymer film. This reaction does not diminish the activity of the glucose oxidase enzyme.

Sensor Design

We have worked with a company, State of the Art, to design a new sensor design that will include all electrodes necessary for the sensors to work and can be implanted. The newly designed sensor will include the working (Chrome/Gold, as an array for redundancy to increase

reliability), reference (Silver/Silver Chloride), and counter (Platinum) electrodes and a schematic of the sensor design can be seen below. The current projected diameter for the working electrodes is 500 μm, but can be miniaturized for more electrodes per sensor.



Lactate Sensors

To develop the lactate sensors we are using the enzyme lactate oxidase. This enzyme loses activity quickly when in solution[34] and therefore has been difficult to test with. Currently we are lyophilizing the enzyme into small aliquots for use and testing stabilizers with the enzyme inside the PEG-DA hydrogel to determine optimum storage and testing conditions.

Animal Testing

We have recently received IACUC approval (#23204) for our testing protocol (the previous one expired) and we are currently under review by the DEA for authorization to purchase and distribute the anesthetics to anesthetize the rats for animal studies. The graduate student on the project has also taken a Rat Handling and Biomethodology seminar for training to use rats for experiments.

Key Research Accomplishments

- We have determined the sensor fabrication techniques for optimum sensor performance and developed a glucose sensor and tested it with cyclic voltammetry, amperometry, and square wave voltammetry.
- We have developed a new design for the sensor that includes all electrodes that need to be included to test inside the body in on one platform and are currently working to fabricate it.
- We have begun work on the lactate sensors and are currently working on making the enzyme stable for the sensors.
- We have recently received approval from IACUC for the animal studies and are currently under review for a DEA license to purchase and administer the anesthetics.

- Becky, the graduate student on this grant, recently received Rat Handling and Biomethodology training, which will be used when the animal studies are conducted.
- Currently there is one undergraduate student on the project that has been trained in many aspects and is learning the basics of conducting research.

Reportable Outcomes

Submission of 2 manuscripts:

Amos Mugweru, Becky L. Clark and Michael V. Pishko. Electrochemical sensor array for glucose monitoring fabricated by rapid immobilization of active glucose oxidase within photochemically polymerized hydrogels. *Manuscript in Review by Diabetes Technology and Therapeutics.*

Amos Mugweru, Becky L. Clark and Michael V. Pishko. Implantable Electrochemical Redundant Micro-sensor Arrays for Glucose Monitoring Patterned Polymer Films. *Manuscript in Review by Electroanalysis.*

Oral Presentations:

Becky L Clark, Amos Mugweru and Michael V Pishko. Electrochemical Glucose Sensor for Diabetes Management: Fabrication of Redundant Electrochemical Array. American Institute of Chemical Engineers Conference. November 2005.

Becky L Clark, Amos Mugweru and Michael V Pishko. Electrochemical Glucose Sensor for Diabetes Management: Use of Voltammetric Techniques to Improve Sensitivity. American Institute of Chemical Engineers Conference. November 2005.

Amos Mugweru, Becky L Clark and Michael V Pishko. Electrochemical redundant micro-sensor arrays for glucose monitoring micro-fabricated on photo-polymerized and micro-lithographically patterned polymer films. American Chemical Society. August 2005.

Amos Mugweru, Becky Clark and Michael V Pishko. Implantable Electrochemical Redundant Micro-sensor Arrays for Glucose Monitoring Patterned Polymer Films. Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy. March 2006.

Poster Presentations:

Becky L Clark, Amos Mugweru and Michael V Pishko. Electrochemical Glucose Sensor for Diabetes Management. Crossover 2005.

Amos Mugweru, Becky L Clark and Michael V Pishko. Electrochemical redundant micro-sensor arrays for glucose monitoring micro-fabricated on photo-polymerized and

micro-lithographically patterned polymer films. American Chemical Society. August 2005.

Progression of people on the grant:

Amos Mugweru, a postdoc supported by this grant, applied for and obtained a position as an Assistant Professor in the Department of Chemistry at Rowan University in New Jersey.

Becky Clark, the graduate student supported by this grant, completed her comprehensive exam.

Conclusions

We have fabricated a glucose sensor array on flexible polyimide sheets. Using conventional silicon micro-fabrication methods, we have initially formed a five-element microelectrode array consisting of gold microdisks. Enzyme-containing redox polymer films on five micro-arrays were formed through the UV-initiated free radical cross linking of a redox polymer and PEG-DA. These microarray sensors were individually addressable and were without discontinuities. There was no cross-talk between adjacent members. When sampled together the micro-array electrodes behaved as one large electrode with peak current equivalent to the sum of the individual elements of array, especially important when diagnosing any array element failure. We have shown catalysis of glucose oxidation resulting from glucose oxidase enzyme exchanging electrons with redox polymer in PEG hydrogel. We are exploring other parameters such as the extent of free radical cross-linking to ensure optimum catalytic performance of the enzyme and also electron exchange between enzyme and the redox polymer.

Currently, we are working to progress the glucose sensors we have already developed and to develop lactate and pyruvate sensors, and then combine all three sensors into one platform. We are working on fabricating a new sensor platform that will include all electrodes necessary to test within the body. We are also waiting for DEA approval to purchase and dispense anesthetics to begin the animal studies portion of this research.

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