Final Progress Report for Research Projects Funded by Health Research Grants

Instructions: Please complete all of the items as instructed. Do not delete instructions. Do not leave any items blank; responses must be provided for all items. If your response to an item is "None", please specify "None" as your response. "Not applicable" is not an acceptable response for any of the items. There is no limit to the length of your response to any question. Responses should be single-spaced, no smaller than 12-point type. The report **must be completed using MS Word**. Submitted reports must be Word documents; they should not be converted to pdf format. Questions? Contact Health Research Program staff at 717-783-2548.

1. **Grantee Institution:** Pittsburgh Tissue Engineering Initiative

2. **Reporting Period (start and end date of grant award period):** 1/1/2009-12/31/2009

3. **Grant Contact Person (First Name, M.I., Last Name, Degrees):** Autumn Gindlesperger, CPA

4. **Grant Contact Person's Telephone Number:** 412-624-5596

5. **Grant ME Number or SAP Number:** 4100047648

6. **Project Number and Title of Research Project: #1-** *Novel Biofuel Cell Based on High Surface Area Enzymatic Microelectrodes*

7. **Start and End Date of Research Project:** 1/1/2009 – 12/31/2009

8. **Name of Principal Investigator for the Research Project:** Sharon Marx

9. **Research Project Expenses**. 15,715

   9(A) Please provide the amount of health research grant funds spent on this project for the entire duration of the grant, including any interest earned that was spent:

   $ 15,715

   9(B) Provide the last names (include first initial if multiple individuals with the same last name are listed) of **all** persons who worked on this research project and were supported with health research funds. Include position titles (Principal Investigator, Graduate Assistant, Post-doctoral Fellow, etc.), percent of effort on project and total health research funds expended for the position. For multiple year projects, if percent of effort varied from year to year, report in the % of Effort column the effort by year 1, 2, 3, etc. of the project (x% Yr 1; z% Yr 2-3).

Last Name	Position Title	% of Effort on Project	Cost
Marx	Principal Investigator	11%	9,258

9(C) Provide the names of **all** persons who worked on this research project, but who *were not* supported with health research funds. Include position titles (Research Assistant, Administrative Assistant, etc.) and percent of effort on project. For multiple year projects, if percent of effort varied from year to year, report in the % of Effort column the effort by year 1, 2, 3, etc. of the project (x% Yr 1; z% Yr 2-3).

Last Name	Position Title	% of Effort on Project

9(D) Provide a list of **all** scientific equipment purchased as part of this research grant, a short description of the value (benefit) derived by the institution from this equipment, and the cost of the equipment.

Type of Scientific Equipment	Value Derived	Cost

10. Co-funding of Research Project during Health Research Grant Award Period. Did this research project receive funding from any other source during the project period when it was supported by the health research grant?

Yes_____ No_____X_____

If yes, please indicate the source and amount of other funds:

11. Leveraging of Additional Funds

11(A) As a result of the health research funds provided for this research project, were you able to apply for and/or obtain funding from other sources to continue or expand the research?

Yes_____X____ No_____

If yes, please list the applications submitted (column A), the funding agency (National Institutes of Health—NIH, or other source in column B), the month and year when the application was submitted (column C), and the amount of funds requested (column D). If you have received a notice that the grant will be funded, please indicate the amount of funds to be awarded (column E). If the grant was not funded, insert "not funded" in column E.

Do not include funding from your own institution or from CURE (tobacco settlement funds). Do not include grants submitted prior to the start date of the grant as shown in Question 2. If you list grants submitted within 1-6 months of the start date of this grant, add a statement below the table indicating how the data/results from this project were used to secure that grant.

A. Title of research project on grant application	B. Funding agency (check those that apply)	C. Month and Year Submitted	D. Amount of funds requested:	E. Amount of funds to be awarded:
Miniature biofuel cells	☐NIH ☒ Other federal (specify: _NSF__) ☐ Nonfederal source (specify: _____)	2/2009	$550,000	Not funded
	☐NIH ☐ Other federal (specify:_____ _____) ☐ Nonfederal source (specify: _____)		$	$
	☐NIH ☐ Other federal (specify:_____ _____) ☐ Nonfederal source (specify: _____)		$	$

11(B) Are you planning to apply for additional funding in the future to continue or expand the research?

Yes___X_____ No_____

If yes, please describe your plans:
NSF Biosensors division. Miniature biofuel cell from gold microfiber electrode. $430,000 (for 3 years). To be submitted end of Feb 2010.

12. **Future of Research Project.** What are the future plans for this research project?

We plan to further develop the project, and study the possibility of linking the glucose biosensor with an insulin pump in feedback mechanism, so that the level of glucose will be transmitted into the pump without the necessity for human intervention. We also plan to study fuel cells that use other fuel sources than glucose, for example fructose, using the appropriate bioelectocatalysts.

13. **New Investigator Training and Development**. Did students participate in project supported internships or graduate or post-graduate training for at least one semester or one summer?

Yes_____X____ No_____

If yes, how many students? Please specify in the tables below:

	Undergraduate	Masters	Pre-doc	Post-doc
Male				1
Female				
Unknown				
Total				**1**

	Undergraduate	Masters	Pre-doc	Post-doc
Hispanic				
Non-Hispanic				1
Unknown				
Total				**1**

	Undergraduate	Masters	Pre-doc	Post-doc
White				
Black				
Asian				
Other				1
Unknown				
Total				**1**

14. **Recruitment of Out-of–State Researchers**. Did you bring researchers into Pennsylvania to carry out this research project?

Yes_____X____ No_____

If yes, please list the name and degree of each researcher and his/her previous affiliation:
Dr. Sharon Marx is a visiting assistant professor from the Israel Institute for Biological Research (IIBR), Ness Ziona, Israel. Her position in Israel was biosensor group leader in the Department of Physical Chemistry in IIBR.

15. **Impact on Research Capacity and Quality**. Did the health research project enhance the quality and/or capacity of research at your institution?

Yes__X_____ No_____

If yes, describe how improvements in infrastructure, the addition of new investigators, and other resources have led to more and better research.
This project introduced electrochemical techniques to the institute and ability to characterize bioelectrocatalysts using electrochemical techniques like cyclic voltammetry. In addition, the ability to generate metal-polymer composite in microdimensions was also developed.

16. **Collaboration, business and community involvement.**

16(A) Did the health research funds lead to collaboration with research partners outside of your institution (e.g., entire university, entire hospital system)?

Yes_____ No___X_____

If yes, please describe the collaborations:

16(B) Did the research project result in commercial development of any research products?

Yes_____ No_____X____

If yes, please describe commercial development activities that resulted from the research project:

16(C) Did the research lead to new involvement with the community?

Yes_____ No____X_____

If yes, please describe involvement with community groups that resulted from the research project:

17. Progress in Achieving Research Goals, Objectives and Aims.

List the project goals, objectives and specific aims (as contained in the grant application's strategic plan). Summarize the progress made in achieving these goals, objectives and aims for the entire grant award period. Indicate whether or not each goal/objective/aim was achieved; if something was not achieved, note the reasons why. Describe the methods used. If changes were made to the research goals/objectives/aims, methods, design or timeline since the original grant application was submitted, please describe the changes. Provide detailed results of the project. Include evidence of the data that was generated and analyzed, and provide tables, graphs, and figures of the data. List published abstracts, poster presentations and scientific meeting presentations at the end of the summary of progress; peer-reviewed publications should be listed under item 20.

This response should be a DETAILED report of the methods and findings. It is not sufficient to state that the work was completed. Insufficient information may result in an unfavorable performance review, which may jeopardize future funding. If research findings are pending publication you must still include enough detail for the expert peer reviewers to evaluate the progress during the course of the project.

Health research grants funded under the Tobacco Settlement Act will be evaluated via a performance review by an expert panel of researchers and clinicians who will assess project work using this Final Progress Report, all project Annual Reports and the project's strategic plan. After the final performance review of each project is complete, approximately 12-16 months after the end of the grant, this Final Progress Report, as well as the Final Performance Review Report containing the comments of the expert review panel, and the grantee's written response to the Final Performance Review Report, will be posted on the CURE Web site.

There is no limit to the length of your response. Responses must be single-spaced below, no smaller than 12-point type. If you cut and paste text from a publication, be sure symbols print properly, e.g., the Greek symbol for alpha (α) and beta (ß) should not print as boxes (□) and include the appropriate citation(s). DO NOT DELETE THESE INSTRUCTIONS.

Novel Biofuel Cell Based on High Surface Area Enzymatic Microelectrodes
~ Final Report ~

The goal of this project was to fabricate a biofuel cell that will be mediator-less and will have high power output (i.e., more than 100 $\mu W/cm^2$).

We proposed to reach this goal by combining two approaches:

1) Use Catalase as the working oxidizing enzyme – increase theoretical power density and increase oxygen concentration in the cell
2) Increase the enzyme concentration on both anode & cathode using high surface area micro-gold electrodes.

The use of Glucose Oxidase (Gox) together with catalase (Cat) will enable the biofuel cell to operate on glucose as the sole fuel source. The direct immobilization of catalase and laccase directly on micro wires will eliminate the use of electron mediators in their various forms and will render the system more simple and applicable for implantable applications. The proof of concept of Gox and Cat immobilized on the same solid matrix and working in concert to produce oxygen from glucose was shown in the past for other applications[1, 2].

The project was divided into four distinct segments:

1. The fabrication and characterization of gold microfiber electrodes
2. Immobilization of redox enzyme on the electrode
3. Electrochemical characterization of anode and cathode half cells
4. Construction of a biofuel cell.

The progress made in the project will be reported using the above segments as guidance.

1. <u>Fabrication and characterization of gold microfibers</u>

We have become interested in the synthesis of gold microfibers because the high aspect ratio of these fibers ensures high surface area of the resulting material. The fabrication of microfibers of gold could be achieved through various techniques [3, 4] as well as through electrospinning. Electrospinning has been used recently to produce fibrous materials for a variety of applications from filtering[5, 6] to tissue scaffolding[7, 8]. Typically, fibers are spun by applying a high voltage (typically over 5 KV) to a drop of a polymer solution. The polymer drop is charged, and due to electrostatic repulsion from the source, the drop is elongated towards a grounded target. If the viscosity of the polymer solution is high enough; the drop does not break into a spray but rather stretches via a whipping process until it hits the grounded collecting plate. The solvent evaporates in the air during the stretching process, thus resulting in a nano to micrometer scale fiber diameter (based on solution viscosity, applied voltage and other parameters).

The majority of fibers are polymer based or polymer-inorganic composites such as silica [9, 10] or titania fibers [11]. Several reports have demonstrated the use of electrospinning for the fabrication of thin metal fibers [12-14]. These fibers are made by the electrospinning of a mixture of a water insoluble polymer solution and a metal precursor, and subsequent electroless deposition of metal on the fiber. The metal precursor then nucleates seeds for the growth of a metal coating. Continuous gold fibers have been produced in this way, and have been characterized to be polycrystalline and conductive [13, 15]. In this project, we use a gold fibrous material as an electrode on which an enzyme is immobilized and used as a biosensor. We demonstrate that this gold microfiber electrode shows reversible electrochemical properties and also is an excellent scaffold for enzyme immobilization.

In this project, we have succeeded to fabricate gold fiber by a three step process that included the electrospinning of a poly(acrylonitrile) polymer blended with gold chloride salt, then a step of reducing gold chloride to metallic gold by a mild reducing agent (NaBH4) and them deposition of gold nanocrystals by electroless deposition. The resulting material was characterized using electron microscopy using scanning and tunneling electron microscopes, and also by X-ray diffraction and nitrogen adsorption.

The process of the fabrication of the fibers was as follows: a solution containing PAN 8% (w/w) and $HAuCl_4$ 10% w/w was prepared by dissolving the solids in DMF and heating at 80°C until the solution was clear. Electrospinning was performed using an in-house built instrument consisting of a power sources (Gamma High voltage, Fl), and syringe pump (World Precision Instruments, Fl). Electrospinning voltage was 20 KV, the polymer flow rate was 0.5 ml/hr, and the tip-to collector distance was 8 cm. the fibers were collected on Al foil or on a gold rod rotated by a motor (in-house built set up) at 3000 rpm. Fabrication of gold microfibers: The resulting fibers were first reduced by 1 mM $NaBH_4$ solution until no H_2 evolution was apparent, washed with dilute HCl and then H_2O. Then, gold was electroless deposited on the fiber by dipping the fiber in an aqueous solution containing 0.02 mg/ml hydroxylamine hydrochloride and 0.1 mg/ml $HAuCl_4$. The fiber was incubated in the solution for 10-30 minutes and then moved to a fresh solution. This was repeated 10 times.

Figure 1 shows the scanning electron microscope (SEM) image of the fibers after the electrospinning and after the electroless deposition process. The as-spun fibers contained defined domains of $HAuCl_4$ (Figure 1A). These domains were created during the evaporation of the solvent which resulted in partial crystallization of the gold chloride salt and phase separation. The gold chloride was reduced initially by $NaBH_4$, a process leaving small metallic gold regions on the fibers. These small metallic clusters served as the nucleation seeds for the electroless gold deposition process that followed. During the electroless deposition process, Au^{+3} was reduced by the mild reducer hydroxylamine to form metallic gold [12, 16, 17]. In the presence of a nucleation seed (the gold particle in the fiber) the gold was deposited on the seed and growth of the gold microcrystal was observed (figure 1B, C). From the SEM images we observed that the gold microcrystal were ordered, and formed linear crystalline arrays. The proximity of the gold microcrystals indicated that the material is metallic.

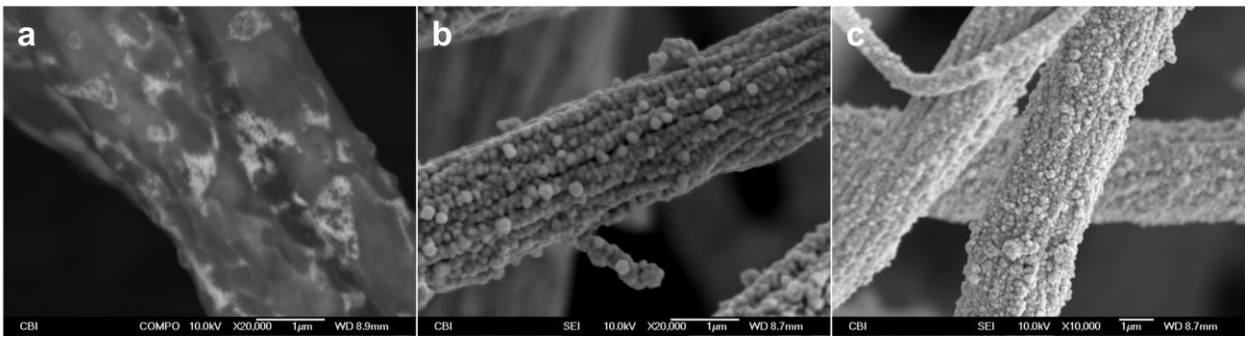

Figure 1: a- SEM of as-spun PAN-$HAuCl_4$ polymer; B- SEM of the same polymer after electroless deposition of gold using secondary emission detector; C: SEM of fiber after electroless deposition of gold using backscatter detector.

Transmission electron microscopy (TEM) reveals more information on the microcrystalline nature of the gold crystals and their continuity. For TEM imaging the fibers were imbedded in epoxy resin and section to micro meter thin slices. Since the fibers have no particular order, we were able to image both vertical and horizontal sections of the fibers, figure 2. These images reveal that the gold crystals are deposited on the surface of the polymer core in a densely packed fashion and that the nanoparticles of the gold are crystalline and were deposited with no preference in orientation. This was expected since the fiber core was an amorphous polymer. The average size of the gold particles encrusting the polymer fiber was 93.1 ±27.7 nm. The remaining gold particles in the core of the fiber that were not exposed to the electroless deposition process remained small and had an average size of 22.3 ±6.6 nm. The d spacing that was measured for the gold crystals was d = 2.38 Å, and the lattice constant from the {111} planes was 4.12 Å, in good agreement with the parameters measured for gold crystals in the literature [18].

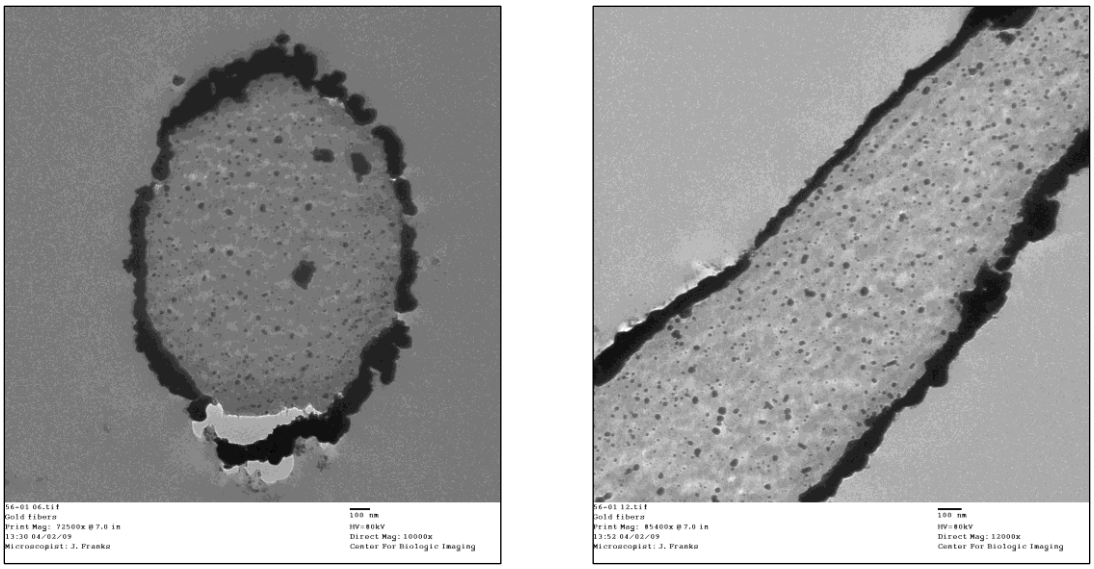

Figure 2: TEM images of horizontal (left panel) and vertical (right panel) cross section of a gold fiber.

The bulk crystallinity of the sample was evaluated using x-ray diffraction (XRD) on a bulk sample (~ 100 mg). Figure 3 shows the XRD pattern of the fiber sample compared to a gold standard. Four characteristic diffraction peaks corresponding to the (111), (200), (220) and (311) planes of a face-centered cubic gold crystal were identified, and were correlated to standard metallic gold diffraction.

The bulk surface area of the fibers was measured from the nitrogen adsorption isotherm, and was found to be 3.15m^2/gr. In comparison, the BET surface area of the PAN fibers 8% w/v without gold chloride was found to be 12.3 m^2/gr, and the surface area of the as-spun material was found to be 0.7 m^2/gr.

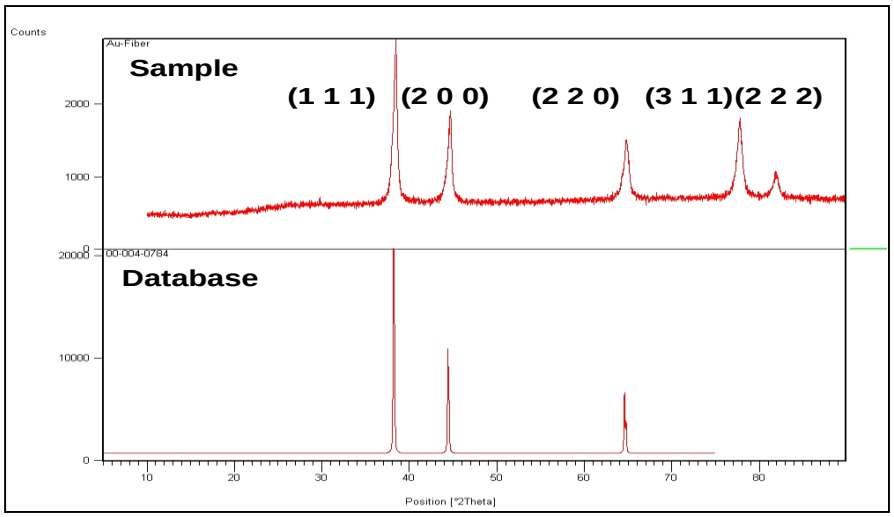

Figure 3: X-ray diffraction pattern of the fiber (top) compared to gold metal standard (bottom). Numbers in brackets indicate the appropriate crystal lattice plane.

In order to assess the suitability of the fiber electrode as a platform for electrochemical biosensor we determined the electrochemical behavior of the gold microfiber. The reversible electron transfer of ferrocene methanol, a common reversible redox probe, was investigated on the gold microfiber. The data conformed to the Randles-Sevcik equation[19]

Eq. 1 $$I = kn^{\frac{3}{2}} FAD^{\frac{1}{2}} v^{\frac{1}{2}}$$

Where k=2.6710^5 (c/mol v$^{-1/2}$) , n is number of transferred electrons, F = 96485 c/mole, A is the electrode surface area, cm^2, D= 6.7 10^{-7}[20] cm^2/sec and v= scan rate.

As expected, the peak current increased with scan rate. Figure 4 shows the plot of the peak current as a function of the scan rate as compared to the results obtained with a polished planar gold disc electrode. The linearity of both anodic and cathodic peak current with the square root of the scan rate indicated that the redox process was governed by diffusion, and that the reversibility of the probes was similar to a bulk gold electrode.

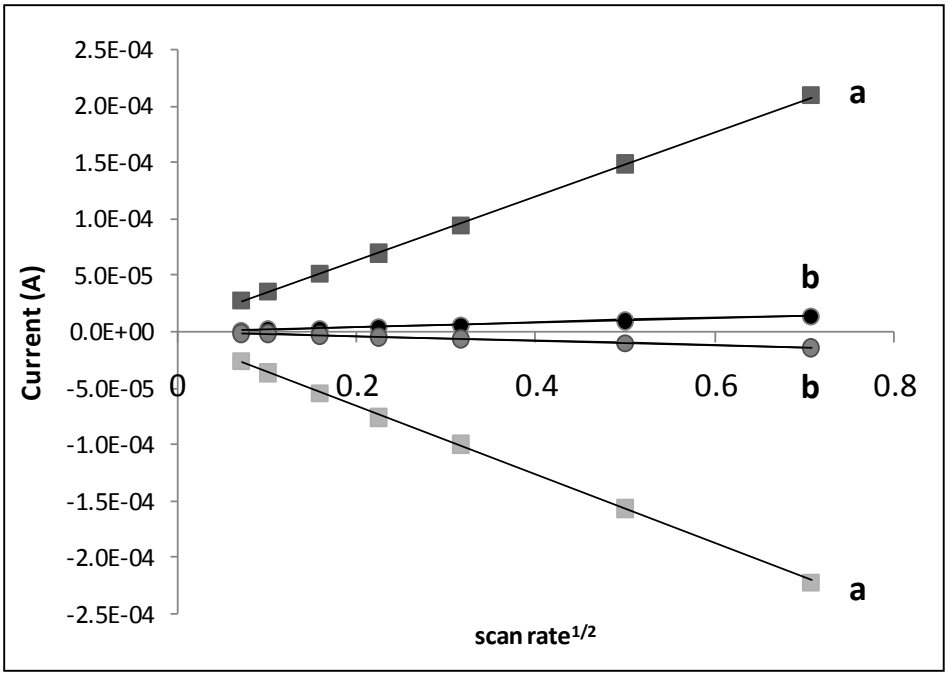

Figure 4: Anodic and cathodic currents as a function of the scan rate, measured on a gold fiber electrode (a) and on polished gold disc electrode (b) in 1 mM ferricyanide solution in 0.1 M KCl.

In summary: this milestone was completed successfully. The process was optimized to produce reproducible micron sized fibers, with metallic and electrochemical characteristics of bulk gold.

2. Immobilization of redox enzymes on the electrode

Several redox enzymes were immobilized on the gold fibers using conventional crosslinking chemistry using a self-assembled monolayer of cystamine as the chemical anchor to gold. The scheme of the immobilization is depicted in figure 5. Glucose oxidase (Gox) was crosslinked to the amine monolayer using glutaraldehyde. The enzymatic activity of the immobilized Gox was assayed using o-dianisidine assay directly on the enzyme-fiber assembly[21]. The coverage of the enzyme electrode was found to be ca. 0.005 units/cm^2, which corresponds to ca. 8.6% of surface coverage (based on the Gox footprint of 80 nm^2 [22]). Other methods of covalent immobilization were investigated: coupling of the enzyme by EDC to a carboxylic acid monolayer, cross linking with DSS or BS^3. The enzyme density achieved by the cystamine-glutaraldehyde method was the highest.

A typical procedure for the enzyme immobilization was as follows: the fibers were incubated in 0.1 M cystamine for 3 hr, washed with H_2O and the incubated for 1 hr in 5% (w/w) glutaraldehyde. The fibers were rinsed with water and then incubated in Glucose oxidase (from *aspergillus niger*), (10 mg/ml in phosphate buffer 0.1 M pH 7.0) overnight at 4°C. The electrodes were rinsed thoroughly with phosphate buffer until no Gox activity could be assayed in the washing buffer (typically, 5 washes).

In a similar fashion, other redox enzymes like fructose dehydrogenase, laccase and bilirubin oxidase were immobilized to the surface of the gold fiber. The activity of each enzyme was assayed after the immobilization to ascertain that the immobilization did not impair the biochemical activity of the enzyme.

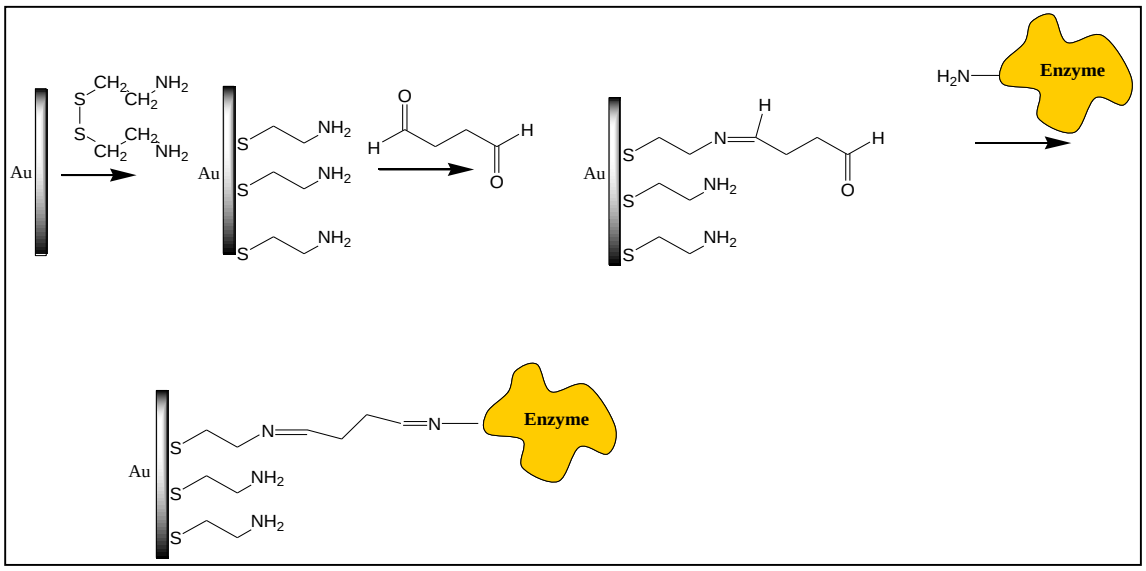

Figure 5: the scheme of the immobilization of enzyme to the gold fiber surface

In summary: this milestone was accomplished successfully. We have established an immobilization scheme that yields a stable and active monolayer of enzyme on the gold electrode surface. It is, however, desired to increase the enzyme concentration, and this will be accomplished in the future by either using multi layers of enzymes or by further increasing the surface area of the gold fibers.

3. Electrochemical characterization of anode and cathode half cells

Each half cell that constructs the biofuel cells is essential an amperometric biosensor. For example, Gox immobilized on the gold fiber surface can function as a glucose sensor. As such, the response of the immobilized enzyme to glucose should be measure by recording the catalytic current resulting from glucose oxidation. Since the electron transfer between the FAD in the active site of Gox to and from the electrode surface is inefficient, an electron transfer mediator is needed to assist in shuttling the electrons between the active site and the gold fiber. In this case, we used ferrocene methanol as the mediator. The catalytic cycle of glucose oxidation and active site regeneration by the mediator is depicted in figure 6.

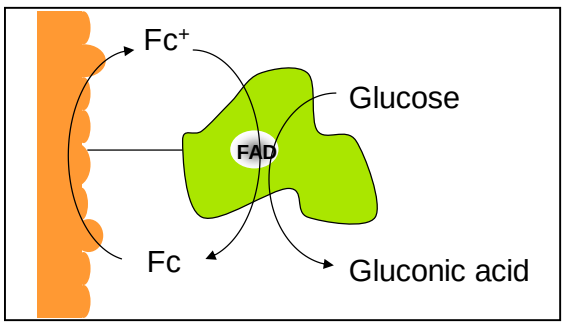

Figure 6: The electron transfer mechanism of Glucose oxidase assisted by an electron mediator, during the electro-oxidation of glucose.

When a Gox-immobilized electrode was challenged with increasing glucose concentrations, the resulting cathodic peaks were recorded, figure 7. The response of the enzyme electrode to glucose concentration was linear in the range of 0.2-30 mM glucose (figure 7, inset), which is also the clinically relevant glucose concentration range. The current- glucose relationship was used to calculate the Michaelis constant of the immobilized enzyme. Using least squares analysis and fitting of the Michaelis equation to the data above, we found that the K_M = 10 mM. This value is in good agreement with K_M of immobilized Gox in similar systems[23], and is approximately 10 times larger than the K_m of Gox measured in solution (typically, 1 mM). The reason for the increased K_M is the slower diffusion of the substrate, glucose, to the immobilized enzyme relative to the uninhibited diffusion in a homogeneous system. The limit of detection of glucose using this sensor was 0.1 mM.

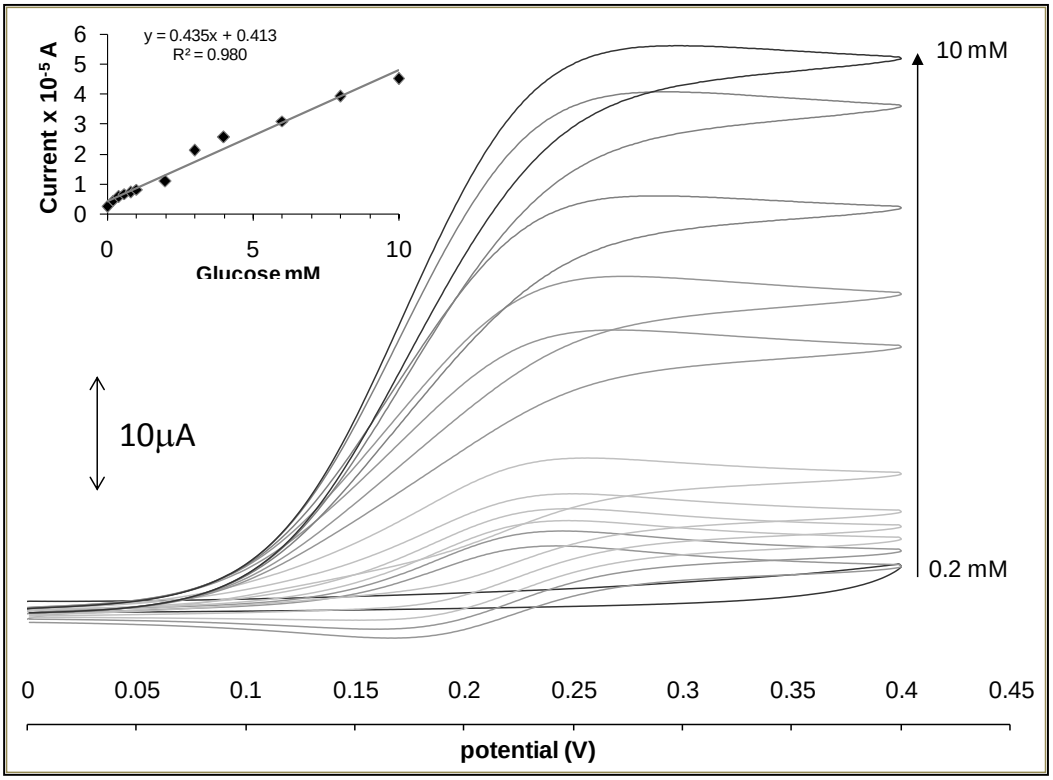

Figure 7: cyclic voltammetry of Gox immobilized on gold fiber electrode, in 0.05M phosphate buffer pH=7.0, containing 2.5×10^{-4} M ferrocene methanol. Scan rate = 5 mV/sec. Inset: the calibration graph of the response of the electrode to increasing glucose concentrations, measured for the peak of the cathodic peak.

The amperometric response of the glucose sensor to glucose can be used to optimize the amount of fiber that is used in each sensor. The hypothesis is that the more gold fiber will be used on the electrode, the more enzyme will be immobilized and thus the higher the current that can be generated for a defined glucose concentration. Figure 8 shows the relationship between the fiber weight (directly proportional to the surface area) and the current measured for glucose concentrations up to 20 mM. The results show that, as expected, the current is proportional to the amount of fiber that is used.

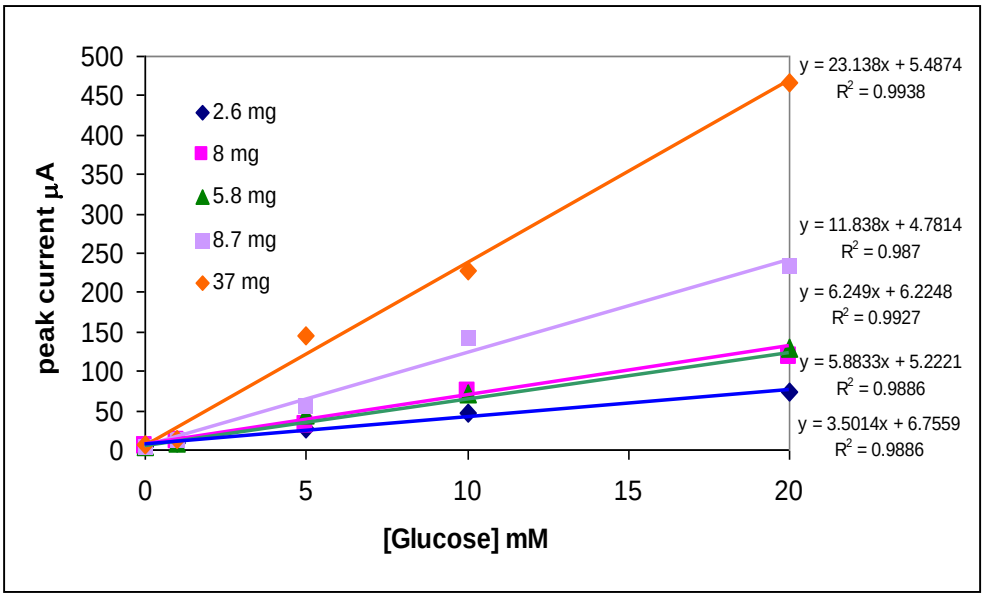

Figure 8: The amperometric response of different gold fiber weight with varying glucose concentrations. Measured in PB pH= 7.0 scan rate = 5 mV/sec, ferrocene methanol 2.5×10^{-4} M as mediator.

The immobilization procedure was performed on other redox enzymes like fructose dehydrogenase, laccase and bilirubin oxidase. Fructose dehydrogenase showed a similar linear relationship between fructose concentration and the cathodic currents measured, figure 9. The electron mediator used in this case was also ferrocene methanol. The concentration of ferrocene methanol used in the study was 5×10^{-4} M. The FDH-gold fiber electrode exhibits a proportional response to fructose concentrations in the range of 0.1 mM-1.5 mM, Figure 9. The calculated limit of detection was 11.7 µM, which was in agreement with reported values of ferrocene mediated fructose sensors (7 µM [24, 25]) The Michaelis constant (K_M) of the immobilized FDH on the fiber electrode was calculated from the electrode responses and was found to be 5 mM, with I_{max} = 142 µA (I_{max} is equivalent to V_{max}). This value is in good agreement with K_M for immobilized FDH in similar systems [23, 26], and is 50% than the K_M of FDH measured in solution (typically, 10 mM).

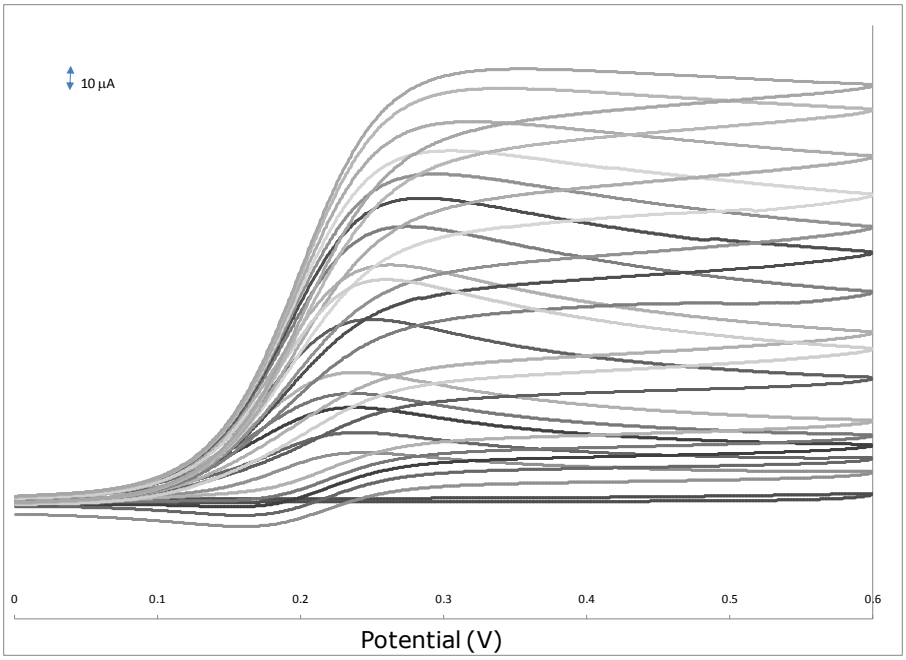

Figure 9: Cyclic voltammetry of FDH immobilized on gold fiber electrode, in McIlvaine buffer pH=4.5, containing $5x10^{-4}$ M ferrocene methanol.
Scan rate = 20 mV/sec. concentrations are from 0.1mM to 1.5 mM.

The reducing enzymes laccase and bilirubin oxidase were also immobilized using the same immobilization chemistry. The electrochemical analysis of these enzymes is performed using buffer saturated with the substrate- oxygen, and using a different mediator- in this case 2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS). Figure 10 portrays a typical electrochemical analysis of laccase immobilized on the fiber electrode. In this case the observed peak is anodic due to the reduction process that occurs in the cell. Figure 10A also shows the capability of laccase to receive the electrons directly from the electrode without the mediator, as observed from the small catalytic peak after the addition of oxygen. However, the direct electron transfer is not as efficient as with a mediator, which greatly enhances the observed current, figure 10 B.

The response of laccase to O_2 concentration was measured using a similar laccase immobilized on gold fiber electrode and different O_2 concentrations, as measures with an oxygen sensor (Orion 4 star oxygen probe). It is fairly difficult to maintain constant O_2 concentrations that are not saturation, so the calibration is not perfectly linear. However, the trend of increase anodic current with increased O_2 concentration is clearly evident from the results, figure 11.

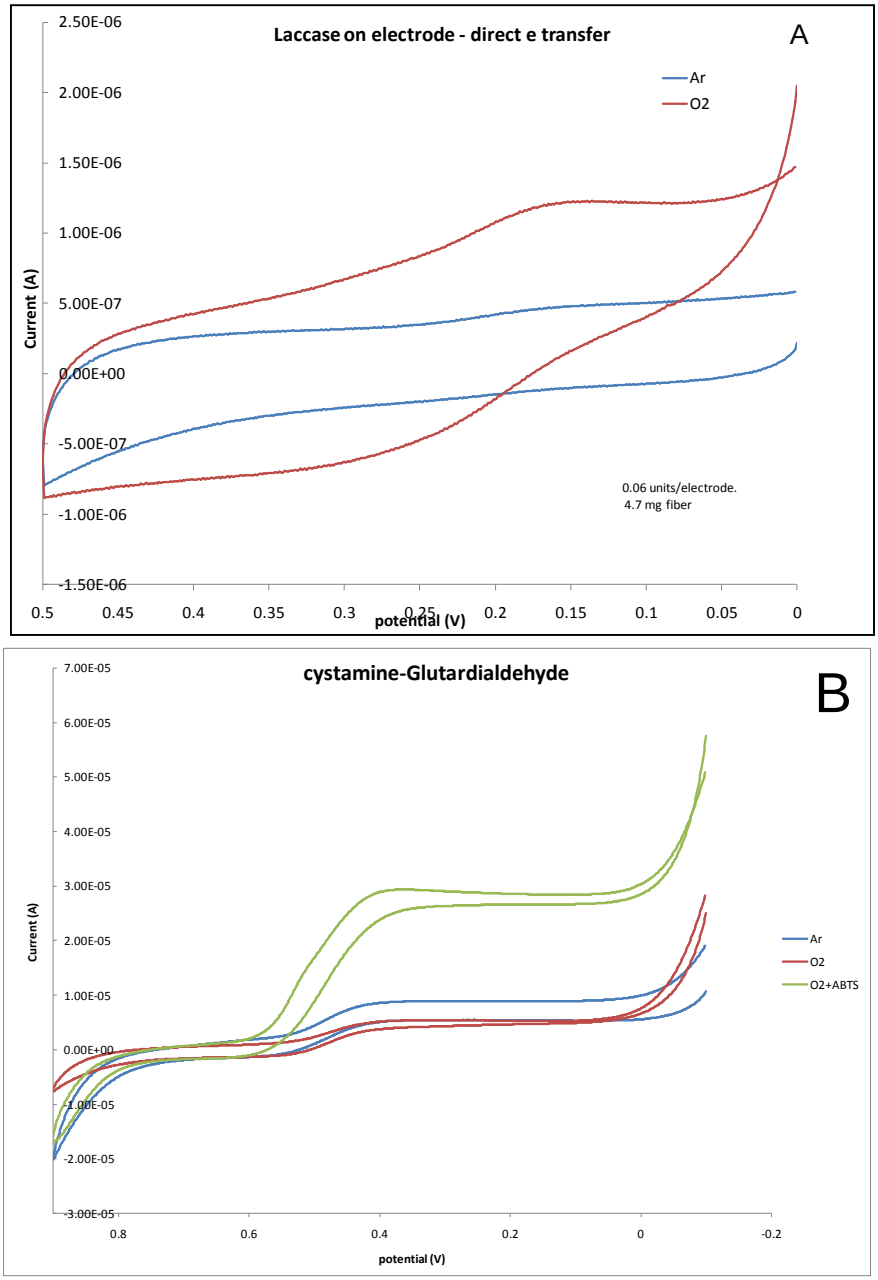

Figure 10: A- laccase from *t. versicolor* immobilized on gold fibers. Blue curve- in Ar saturated citrate buffer pH = 4.5, Red curve- after saturation with O_2. B: laccase electrode after addition of ABTS 10 μM to O_2 saturated citrate buffer. Scan rate 5 mv/sec.

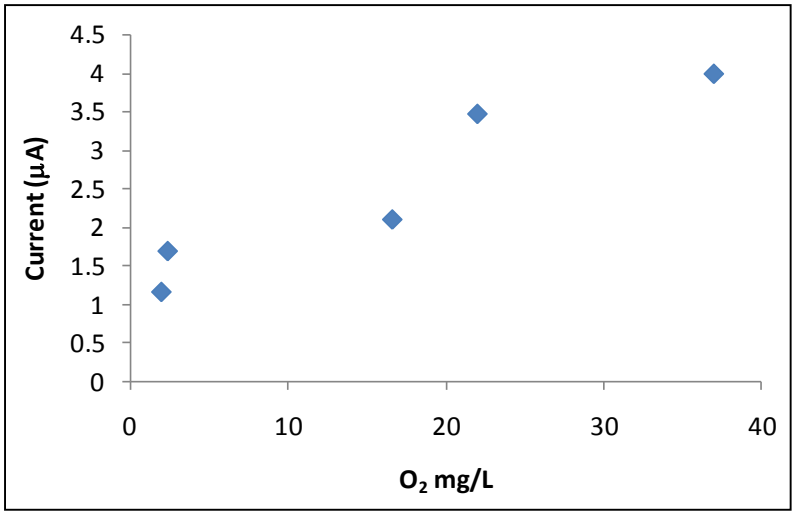

Figure 11: anodic peak currents of laccase immobilized on gold fibers challenged with increased O_2 concentrations. O_2 measured by independent oxygen probe. Performed in citrate buffer 0.1M pH = 4.5. Scan rate = 5 mV/sec.

The last enzyme that will be reported is catalase. This enzyme is a *heme* protein, and is responsible in nature for the scavenging of hydrogen peroxide that is formed as a byproduct in several biochemical processes. H_2O_2 can be damaging to proteins, thus catalase is responsible for the rapid disintegration of H_2O_2 to O_2 and water. This enzyme is known by its efficient electron transfer to carbon electrodes[4, 27], but the electron transfer to gold metals is less reported[1, 28]. We found that catalase adsorbs strongly to the gold fiber surface and no chemical immobilization is needed. When catalase is adsorbed on the gold fiber electrode and H_2O_2 is added to the buffer- it is possible to immediately detect O_2 bubbles formed on the surface. The anode design that is unique in this project is a combination of catalase- Gox immobilized on the surface of the gold fiber, where Gox will oxidize glucose to gluconic acid subsequently followed by the regeneration of the FAD active site by the reduction of O_2 to H_2O_2. H_2O_2 will then be consumed by catalase and transformed into O_2 and H_2O and two electrons that will be transferred to the electrode. Figure 12 shows the amperometric response of catalase adsorbed on the gold fiber electrode. When only catalase is present- no special peaks are observed. Addition of Gox and glucose- does not change the observed cyclic voltammogram. Only the saturation of the solution with oxygen, the co-substrate of Gox, starts the cascade of enzymatic reactions and leads to the appearance of the desired catalytic current with a peak at -0.4 V (figure 11, purple trace).

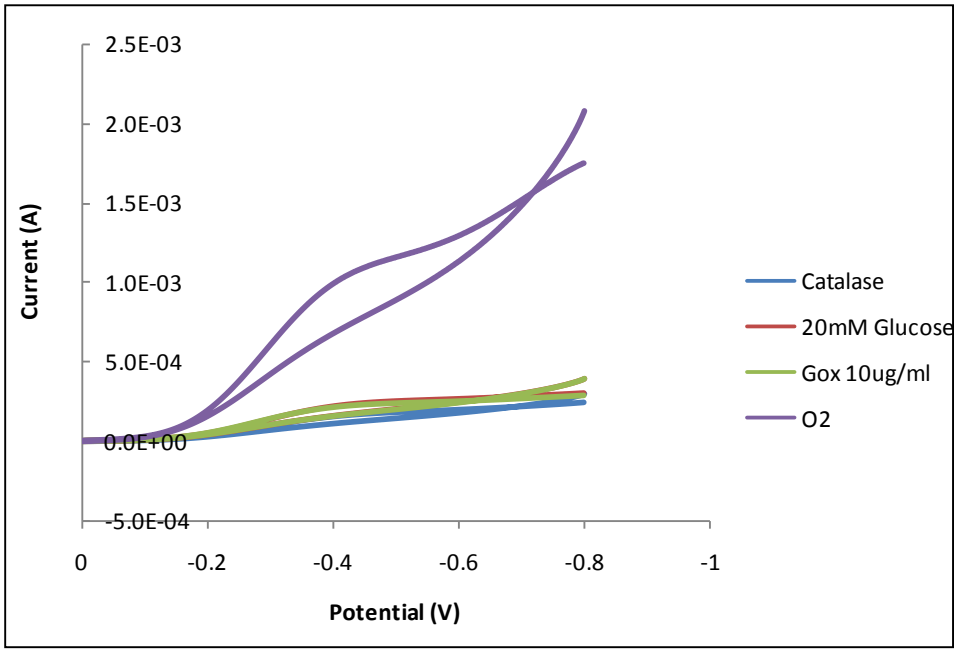

Figure 12: cyclic voltammogram of catalase adsorbed on gold fiber. Blue trace- catalase in phosphate buffer 0.1 M pH = 7.0; green- addition of 10 mg/ml Gox; red: addition of 20 mM glucose; purple- same solution, after saturation with O_2. Scan rate = 100 mV/sec.

In summary: this milestone was accomplished successfully. All redox enzymes that were of interest to the study were characterized electrochemically using a 3-electrode half cell electrochemical set up. The response of each enzyme to its substrate was measured and analyzed in terms of limit of detection, linear response range, K_M, and ultimate buffer and mediator composition. Catalase was adsorbed on the surface of the electrode successfully and the direct electron transfer to the gold electrode was observed when Gox, glucose and O_2 were added to the enzyme.

4. Construction of a biofuel cell

The successful immobilization and electrochemical characterization of both oxidizing and reducing enzymes on the fold microfiber electrode led to the next step, namely to combine two half cells into a biofuel cell. The scheme of the wiring of a full cell is depicted in figure 13. Typically, the two electrodes, anode and cathode, are connected to a multimeter through a variable resistor. The potential that is produced in the cell is measures in several external resistances loading and the resulting current is calculated using Ohm's law. The power output is calculated from the potential and current at each resistance. The peak power is the maximal power density of the specific cell. In cases where a mediator is involved, a physical separation between the anode and the cathode is needed to avoid the poisoning of the enzymes by the other enzyme's mediator. In this case- a nafion membrane, made from a polysulfonated polymer is dividing the two half cells, and thus each half cell contains the electrode. The mediator and can

be saturated with oxygen or completely Argon purged, according to the specific enzyme used. Since direct electron transfer was not established at this point, we have tested the enzyme immobilized electrodes in a membrane separated setup. an example biofuel setup included an anode with immobilized fructose dehydrogenase, working in McIlvain buffer pH = 4.5, using methylene blue as mediator (0.3 mM), and a cathode with bilirubin oxidase immobilized on the gold fiber electrode, with ABTS as mediator (0.3 mM) and phosphate buffer 0.1M pH = 7.0 saturated with oxygen. The two half cells were separated by a 0.9 mm thick nafion membrane.

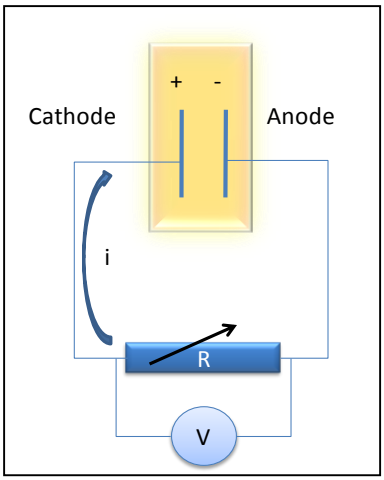

Figure 13: a scheme for the wiring of a biofuel cell

The response curve of the biofuel cell is depicted in figure 14. The peak power output of this cell was a little over 6 μW, and when taking into account the surface area of the working electrodes, the power density was 20 μW/cm^2. This peak power output was measured at 0.5 V. the maximum current output was 13 μA. the power was constant for over 2 hours, and after 24 hours, only 20% decrease in the power was observed. Similar results are observed using laccase on the anode or Gox on the cathode.

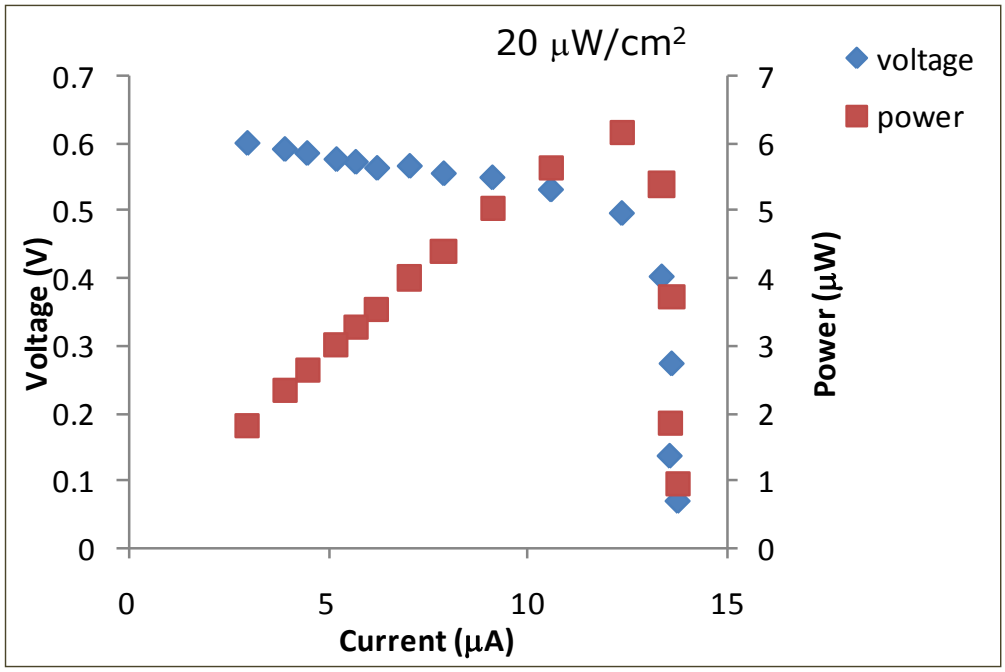

Figure 14: biofuel cell power output measurement. Anode: Fructose dehydrogenase on gold fibers, in McIlvain buffer pH = 4.5, with methylene blue 0.3 mM and fructose 0.2 M. cathode: bilirubin oxidase immobilized on gold fiber electrode, in phosphate buffer 0.1 M pH = 7.0, saturated with O_2, and 0.3 mM ABTS. ♦- voltage; ■- power.

In summary: preliminary results showing a biofuel cell from the enzyme electrodes that were developed is shown, the cell produced power for a prolonged time without the regeneration of the substrate and without a significant of loss of activity. The power generation is lower than the desired goal (100μW/cm^2).

During the first year of this project, we have succeeded to fulfill almost all the project goals as specified in the proposal. A platform for the immobilization of enzymes was developed in the form of electrospun fibers that were transformed into gold microfibers. We have established the immobilization technique for all redox enzymes of interest in this study and show that ability to assay the enzymes both biochemically and electrochemically. The enzyme electrodes were analyzed as independent biosensors. A biofuel cell prototype was built and tested using pairs of anode and cathode enzyme electrodes that were developed. Each pair was characterized by it maximum power out pot and maximal voltage output. We have succeeded to achieve maximum power output of 20 μW/cm^2, which is lower than what we anticipated. We plan to reach and even exceed our initial power output goal by increasing the amount of enzyme on the electrode, use a gold-carbon composite material and increase the available surface area of the fibers by using hollow fibers.

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18. Extent of Clinical Activities Initiated and Completed. Items 18(A) and 18(B) should be completed for all research projects. If the project was restricted to secondary analysis of clinical data or data analysis of clinical research, then responses to 18(A) and 18(B) should be "No."

18(A) Did you initiate a study that involved the testing of treatment, prevention or diagnostic procedures on human subjects?

_____Yes
___X__No

18(B) Did you complete a study that involved the testing of treatment, prevention or diagnostic procedures on human subjects?

_____Yes
_X____No

If "Yes" to either 18(A) or 18(B), items 18(C) – (F) must also be completed. (Do NOT complete 18(C-F) if 18(A) and 18(B) are both "No.")

18(C) How many hospital and health care professionals were involved in the research project?

_____Number of hospital and health care professionals involved in the research project

18(D) How many subjects were included in the study compared to targeted goals?

_____Number of subjects originally targeted to be included in the study
_____Number of subjects enrolled in the study

18(E) How many subjects were enrolled in the study by gender, ethnicity and race?

<u>Gender:</u>
_____Males
_____Females
_____Unknown

<u>Ethnicity:</u>
_____Latinos or Hispanics
_____Not Latinos or Hispanics
_____Unknown

<u>Race:</u>
_____American Indian or Alaska Native
_____Asian
_____Blacks or African American
_____Native Hawaiian or Other Pacific Islander

_____White
_____Other, specify:_____
_____Unknown

18(F) Where was the research study conducted? (List the county where the research study was conducted. If the treatment, prevention and diagnostic tests were offered in more than one county, list all of the counties where the research study was conducted.)

19. Human Embryonic Stem Cell Research. Item 19(A) should be completed for all research projects. If the research project involved human embryonic stem cells, items 19(B) and 19(C) must also be completed.

19(A) Did this project involve, in any capacity, human embryonic stem cells?
_____Yes
_X____ No

19(B) Were these stem cell lines NIH-approved lines that were derived outside of Pennsylvania?
_____Yes
_____ No

19(C) Please describe how this project involved human embryonic stem cells:

20. Articles Submitted to Peer-Reviewed Publications.

20(A) Identify all publications that resulted from the research performed during the funding period and that have been submitted to peer-reviewed publications. Do not list journal abstracts or presentations at professional meetings; abstract and meeting presentations should be listed at the end of item 17. **Include only those publications that acknowledge the Pennsylvania Department of Health as a funding source** (as required in the grant agreement). List the title of the journal article, the authors, the name of the peer-reviewed publication, the month and year when it was submitted, and the status of publication (submitted for publication, accepted for publication or published.). Submit an electronic copy of each publication, listed in the table, in a PDF version 5.0.5 format, 1,200 dpi. Filenames for each publication should include the number of the research project, the last name of the PI, the number of the publication and an abbreviated research project title. For example, if you submit two publications for PI Smith for the "Cognition and MRI in Older Adults" research project (Project 1), and two publications for PI Zhang for the "Lung Cancer" research project (Project 3), the filenames should be:

Project 1 – Smith – Publication 1 – Cognition and MRI
Project 1 – Smith – Publication 2 – Cognition and MRI
Project 3 – Zhang – Publication 1 – Lung Cancer
Project 3 – Zhang – Publication 2 – Lung Cancer

If the publication is not available electronically, provide 5 paper copies of the publication.

Note: The grant agreement requires that recipients acknowledge the Pennsylvania Department of Health funding in all publications. Please ensure that all publications listed acknowledge the Department of Health funding. If a publication does not acknowledge the funding from the Commonwealth, do not list the publication.

Title of Journal Article:	Authors:	Name of Peer-reviewed Publication:	Month and Year Submitted:	Publication Status (check appropriate box below):
1.				☐Submitted ☐Accepted ☐Published
2.				☐Submitted ☐Accepted ☐Published
3.				☐Submitted ☐Accepted ☐Published

20(B) Based on this project, are you planning to submit articles to peer-reviewed publications in the future?

Yes_____x____ No_____

If yes, please describe your plans:
Indeed, we plan to submit articles to peer-reviewed publications in the future.

21. Changes in Outcome, Impact and Effectiveness Attributable to the Research Project.
Describe the outcome, impact, and effectiveness of the research project by summarizing its impact on the incidence of disease, death from disease, stage of disease at time of diagnosis, or other relevant measures of outcome, impact or effectiveness of the research project. If there were no changes, insert "None"; do not use "Not applicable." Responses must be single-spaced below, and no smaller than 12-point type. DO NOT DELETE THESE INSTRUCTIONS. There is no limit to the length of your response.

The outcome of this project will be the development of implantable biofuel cells. These can in principle replace batteries in devices like pacemakers, implantable insulin pumps or other electronic based implantable devices that are being developed. The advantages of these biofuel cells over conventional batteries are that they run on substances that are abundant in the body and they are not toxic. These devices can come to the aid of the huge population of diabetics in the U.S. who rely on insulin pumps.

22. Major Discoveries, New Drugs, and New Approaches for Prevention Diagnosis and Treatment. Describe major discoveries, new drugs, and new approaches for prevention, diagnosis and treatment that are attributable to the completed research project. If there were no major discoveries, drugs or approaches, insert "None"; do not use "Not applicable." Responses must be single-spaced below, and no smaller than 12-point type. DO NOT DELETE THESE INSTRUCTIONS. There is no limit to the length of your response.

None

23. Inventions, Patents and Commercial Development Opportunities.

23(A) Were any inventions, which may be patentable or otherwise protectable under Title 35 of the United States Code, conceived or first actually reduced to practice in the performance of work under this health research grant? Yes_____ No____X

If "Yes" to 23(A), complete items a – g below for each invention. (Do NOT complete items a - g if 23(A) is "No.")

a. Title of Invention:

b. Name of Inventor(s):

c. Technical Description of Invention (describe nature, purpose, operation and physical, chemical, biological or electrical characteristics of the invention):

d. Was a patent filed for the invention conceived or first actually reduced to practice in the performance of work under this health research grant?
Yes_____ No____

If yes, indicate date patent was filed:

e. Was a patent issued for the invention conceived or first actually reduced to practice in the performance of work under this health research grant?
Yes_____ No____
If yes, indicate number of patent, title and date issued:
Patent number:
Title of patent:
Date issued:

f. Were any licenses granted for the patent obtained as a result of work performed under this health research grant? Yes_____ No____

If yes, how many licenses were granted?_____

g. Were any commercial development activities taken to develop the invention into a commercial product or service for manufacture or sale? Yes___ No____

If yes, describe the commercial development activities:

23(B) Based on the results of this project, are you planning to file for any licenses or patents, or undertake any commercial development opportunities in the future?

Yes_____ No____X_____

If yes, please describe your plans:

24. Key Investigator Qualifications. Briefly describe the education, research interests and experience and professional commitments of the Principal Investigator and all other key investigators. In place of narrative you may insert the NIH biosketch form here; however, please limit each biosketch to 1-2 pages. *For Nonformula grants only – include information for only those key investigators whose biosketches were not included in the original grant application.*

Sharon Marx- biosketch

Professional Preparation

Hebrew University, Jerusalem Israel	Chemistry	B.A.	1990
Hebrew University, Jerusalem Israel	Chemistry	Ph.D	1996

Appointments

8/2008-presnet	visiting assistant professor, University of Pittsburgh, McGowan institute for regenerative medicine.
1996-Present	Research scientist, Department of physical chemistry, Israel Institute for Biological Research Israel. Head of the thin film sensor group.

Relevant Publications

1. Shapiro L, **Marx S**, Mandler D. Preparation and characterization of ultrathin sol gel films. Thin solid films 2007; 515; 4624-8.
2. Fireman-Shoresh S, Turyan I, Mandler D, Avnir D, **Marx S**. Chiral Electrochemical Recognition in Ultrathin Molecularly Imprinted Sol-Gel Films. *Langmuir*. 2005; 21; 7842-7.
3. Shustak G, **Marx S**, Turyan I, Mandler D. Application of sol gel technology for electroanalytical sensing. *Electroanalysis*, 2003; 15(5-6); 398-408.
4. **Marx-Tibbon S**. Katz E. Willner, I. Chiral recognition in mediated electron transfer in redox proteins. *J Am Chem Soc*. 1995; 117; 9925-6.
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transduction of recorded optical signals using monolayer modified Au-electrodes. *J Am Chem Soc*. 1995; 117; 6581-92.

Other Significant Publications

1. **Marx, S**. Chiral Molecular Imprinting as a tool for chiral drug sensing. In Cellular and Biomolecular recognition. R. Jelinek, Ed. Wiley intersicence; 2009; p. 97-109.
2. Bromberg A, Marx **S**, Frishman G. Kinetic study of the thermal inactivation of ChE enzymes trapped in solid matrices. Biochim Biophys Acta. 2008; 1784; 961-966.
3. Raichlin Y, **Marx S**, Katzir, A. The state of water in polymeric films probed by MIR evanescent wave sensors. J Phys Chem B. 2007; 111; 6131-4.
4. Avnir D, **Marx S**. The induction of Chirality in sol gel materials (special Sol Gel issue).
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Synergistic Activities

1. Service on Ph.D. committees; School of Chemistry, Hebrew University, Jerusalem Israel.
2. Organizer/Program Committee for 43rd oholo conference, 1997; Organized all aspects of these symposia, which included inviting local, national, and international scholars to give research talks; also planned budgets and organized event locations.
3. Recipient of the "Katzir" Fellowship for young researchers (1996-1999).
4. Scientific advisor toe “Popular Science”, Hebrew edition (2000-2009)

Collaborators and Other Affiliations

Collaborators:

Katz, Evgeny (Clarkson University, NY, USA)
Katzir, Abraham (Tel Aviv University, Israel)
Avnir, D,. (Hebrew University, Jerusalem, Israel)

Graduate and Postdoctoral Advisors:

Fireman-Shoresh, S. (Hebrew University, Jeruslaem Israel)