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, Inc.

2. **Reporting Period (start and end date of grant award period):** 1/01/2010 – 12/31/2010

3. **Grant Contact Person (First Name, M.I., Last Name, Degrees):** Richard R. Koepsel, PhD

4. **Grant Contact Person's Telephone Number:** 412-624-5298

5. **Grant SAP Number:** 4100050906

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:** 1/01/2010 – 12/31/2010

8. **Name of Principal Investigator for the Research Project:** Richard Koepsel, PhD

9. **Research Project Expenses**.

   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:

   $ 12,265.10

   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
Koepsel	Principle Investigator	10% (7-1-10 to 12-31-10)	7,039.24

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
Jose	Research Associate	60
Andersen	Technician	20

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
None		

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 X No_____

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

Armed Forces Institute for Regenerative Medicine match funds. $56,000

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 Cell from Gold Microfiber Electrodes	☐NIH x Other federal (specify:_NSF__) ☐ Nonfederal source (specify: _____)	09-2010	$396,454	$200,000 NSF award Number: 0019645.

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

Yes_____ No____X_____

If yes, please describe your plans:

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

This project will develop a fully implantable fuel cell. We plan to use that fuel cell to power a fully implantable insulin pump that will be developed with future funding.

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			1
Female				
Unknown				
Total	**1**			**1**

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

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

    Sharon Marx, PhD (Visiting Professor), Israeli institute for Biological Research
    Moncy Jose, PhD University of Alabama Birmingham

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.

    We gained the expertise to build and test biofuel cells. This expertise will allow the development of implantable self-powered medical devices.

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.

Project Title and Purpose

Novel Biofuel Cell Based on High Surface Area Enzymatic Microelectrodes - The purpose of this project is to continue our development of a miniaturized biofuel cell, a device that can turn available substances like sugars and oxygen to energy, by using an assembly of biocatalysts and a special gold fiber electrode. The device will be designed to utilize fructose as the fuel, and turn the sugar into energy by a series of enzymatic reactions, using commercial enzymes as the catalytic elements. The process will be carried out on the surface of a new type of gold microfiber electrode, which will be developed. In this project continuation we would like to expand the fuel sources and study a biofuel cell based on fructose conversion into electrical energy by fructose dehydrogenase.

Project Overview

The research objectives are to develop a biofuel cell, with high current density, using fructose as the source of fuel. In addition, the anode and the cathode of the cell will be new high surface area gold fiber electrodes which will be fabricated using an electrospun gold-polymer scaffold.

This research program began in the previous grant, and this project is a continuance. The research will be divided into 3 parts: (1) fabrication of gold electrodes; (2) immobilization of the enzymes on the electrodes; and (3) optimization of experimental conditions for maximal current density. Gold electrodes will be prepared by a multi step process using a composite gold - polymer microfiber scaffold to construct the gold microelectrodes. First, electrospun gold salt-polymer fibers will be fabricated. Gold electroless deposition or direct electrochemical reduction will form a gold coating on the fiber. Increased surface area will also be attempted by dissolving the polymer core and creating gold hollow fibers. Characterization will be by scanning electron microscopy (SEM) and transmission electron microscopy (TEM), X-ray diffraction (XRD), BET surface area and cyclic voltammetry (CV).

The enzymes to be used will be fructose dehydrogenase at the anode, and bilirubin oxidase or laccase at the cathode. The enzymes were chosen after examining their redox potentials to have the highest difference between the oxidizing and reducing enzyme in order to achieve the highest open circuit potential, and perform at acidic pH. There are two important features in the planned design: direct electrical communication of the enzymes with the electrode, without highly toxic electron mediators, and glucose as the only fuel source. Fructose will be oxidized by the PQQ cofactor in the enzyme, resulting in two electrons that will be transferred to the electrode. Fructose dehydrogenase, in contrast to the more popular glucose oxidase, does not use oxygen in the oxidation process, thus not depleting the oxygen from the solution; on the cathode, oxygen will be reduced by bilirubin oxidase or laccase to water, using two electrons. These processes will be probed using CV and by standard biochemical enzymatic assays to evaluate enzyme loading. Enzymes will be immobilized on the gold electrode using conventional cross linking techniques (thiol SAM with subsequent covalent cross linking, or direct physical adsorption).

The biofuel cell assembly will be constructed of a two- electrode cell connected through an external resistor load and a multi-meter. The potential that will be developed between the anode and cathode will be measured.

Expected Research Outcomes and Benefits

The ultimate outcome of this research will be a miniaturized biofuel cell, a device that will essentially act as a power source made from a composite of biomaterials and metal. This power source will use fructose as the single source of energy, and will transform the fructose to energy by using enzymes- nature's catalysts. The end product will be able to generate energy from fructose containing substances - such as fruit juices and high fructose corn syrup - a cheap commercial fructose source. It is highly desired to fabricate such devices that utilize commonly available fuel sources to produce energy. It is also possible to add another enzyme, invertase, to the anode and then use this anode to convert the endogenous glucose to fructose, thus applying the fuel cell in an implanted fashion. These power sources are not toxic like those commonly used in batteries, they do not contain any foreign materials that the body does not recognize and can, in principle, operate for extended periods of time (weeks up to several months). Preliminary studies by other groups have shown the feasibility of such devices to be able to generate power that can operate a small electrical device (such as a Sony Walkman). The design proposed is better, since it does not contain any toxic material – while most of the other designs rely on toxic electron mediators or toxic polymeric gels. In addition, the proposed design will enable the production of a smaller device with higher power output, due to the novel high-surface area electrodes that we will use. Preliminary results show that the gold microfiber electrodes indeed have a high surface area that is available for enzyme immobilization. In addition, we have shown that redox enzymes can be immobilized on the surface and portray electron transfer capabilities with the gold fiber electrode.

Summary of Research Completed

A new form of high surface area bio-electrode, based on nanofibers of electrospun gold with immobilized fructose dehydrogenase was developed. The gold fibers were prepared by electroless deposition of gold nanoparticles on an electrospun poly(acrylonitrile)-$HAuCl_4$ fiber. The material was characterized using electron microscopy, XRD and BET, as well as cyclic voltammetry and biochemical assay of the immobilized enzyme. The surface area of the gold microfibers was 0.32 ± 0.04 m^2/g. Fructose dehydrogenase was covalently coupled to the gold surface through glutaraldehyde cross links to a cystamine monolayer. The enzyme exhibited mediated electron transfer directly to the gold electrode and catalytic currents characteristic of fructose oxidation in the presence of a ferrocene methanol mediator were observed. The limit of detection of fructose was 11.7 μM and the K_m of the immobilized enzyme was 5 mM. The microfiber electrode was stable over 20 cycles with a 3.05% standard deviation. The response time of the sensor was less than 2.2 seconds and reached half maximum value within 3.6 seconds. The sensor was proven to be accurate and precise in both serum and popular beverages containing high fructose corn syrup.

Results

Material characterization.
Electrospun fibers were imaged using SEM with both secondary emission and backscatter detectors to image both the topography and the difference in material density of the fiber. 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 subsequent phase separation. The gold chloride was reduced by $NaBH_4$, to deposit small metallic gold regions on the fibers. These metallic clusters served as nucleation seeds for the electroless gold deposition process that followed. Au^{+3} was reduced by hydroxylamine to form metallic gold. The deposited gold and growth of the micro crystals was observed microscopically (Figure 1B). From the SEM images we observed that the gold microcrystals were ordered and formed linear crystalline arrays. The proximity of the gold microcrystals indicated that the material was metallic. The XRD analysis (Figure 1C) showed four characteristic diffraction peaks corresponding to the (111), (200), (220), (311) and (222) planes of a face-centered cubic gold crystal. After the electroless deposition procedure, the mean diameter of the fibers in figure 1 was 990±490 nm. Fiber size was dependant of flow rate, voltage and solvent. Cross sectioned fiber TEM showed that the particles formed a crust around the polymer core, and were adherent to one another, imply that the metallic surface would be conductive (Figure 1D). HR-TEM confirmed that the nanoparticles of the gold were 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 2.38 Å, and the lattice constant from the (111) planes was 4.12 Å, in good agreement with known dimensions.

A nitrogen adsorption isotherm was used to determine the bulk surface area of the fibers and was found to be 3.15 m^2/g. In comparison, the BET-measured surface area of the PAN fibers (8% w/v without gold chloride) was found to be 12.3 m^2/g, and the surface area of the as-spun material was found to be 0.7 m^2/g. The electrochemical surface area, which can be distinct from the physical surface area, measured by chronocoulometry using potassium ferricyanide as the probe was 0.32±0.04 m^2/g. A possible explanation for the difference between the BET and electrochemically measured surface areas could be linked to the difference in the medium that the measurement was performed in. BET was performed in the gas phase, while choroncoulometry was performed in solution. Both methods relied on the adsorption of a molecular probe to the surface (N_2 or ferricyanide). In solution, ferricyanide was solvated, and the molecular footprint of the probe on the gold surface was larger than that of the nitrogen, so fewer molecules were actually used to cover the surface than when in the gas phase. The surface area calculation by chronocoulometry takes into account only the diffusion coefficient of the probe and not the size of the probe, and hence the difference in the observed values.

Electrochemical characterization of the gold microfiber.

In order to assess the suitability of the fiber electrode as a platform for an 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:

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

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= $6.7x10^{-7}$ cm^2/sec and v = scan rate.

As expected, the peak current increased with scan rate (Figure 2). 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. The data also showed that the probes behaved in a similar manner with bulk gold electrodes and the nanofibers.

Enzyme immobilization on the fiber.

Fructose dehydrogenase (FDH) was immobilized on the gold fibers via glutaraldehyde crosslinking to a self-assembled monolayer of cystamine which served as the chemical anchor to the gold. The enzymatic activity of the immobilized FDH was assayed using a Prussian Blue assay directly on the enzyme-fiber assembly. The coverage of the enzyme electrode was found to be 0.36± 0.03 enzyme molecules/nm^2 averaged on three electrodes. In comparison, when the same immobilization procedure was performed on a polished gold disc electrode of 2 mm diameter, 1.8±0.14 molecules of enzymes/nm^2 were detected (n=3). The density of the enzyme on the fiber electrode is likely reduced because only the outer layers of the fibers were immobilized. The total protein content of the electrode was assayed using a highly sensitive fluorescent assay. The gold fiber electrode contained on average 0.50±0.02 molecules of protein/nm^2, when the gold disc electrode contained 9.46±0.43 molecules of protein/nm^2**.** Compared to other published results, immobilization of FDH on the gold nanofibers resulted in doubling the amount of immobilized protein, and quadruples the amount of enzyme units immobilized, per cm^2. Using the fluorescent assay, we can estimate the amount of active enzyme that was retained on the surface and the ability of the gold fibers to stabilize the immobilized enzyme. When taking into account the specific activity of the soluble enzyme (426 u/mg), it is possible to estimate the level of expected activity based on the protein content. We calculated that the protein weight that was found for the gold microfiber electrode should produce approximately 2.5 molecules of FDH per nm^2 of electrode. In comparison, the protein weight found for a gold disk electrode should translate to 46.8 molecules of FDH per nm^2. This means that after immobilization of FDH on gold microfibers, 14.4% of the enzyme remains active, while immobilization on the flat gold disc surface resulted in less than 4% activity of the immobilized enzyme. A possible explanation of this improved activity retention may lie in the micro-structure of the gold fibers.

Electrochemical evaluation of FDH on gold fibers.

The electrochemical activity of FDH was measured using the catalytic cathodic current observed when the electron mediator was electrochemically regenerated by the PQQ active site after fructose oxidation. At high mediator concentrations the heme *c* prosthetic group of the enzyme is reoxidized in an internal redox process. The electron mediator used in our study was ferrocene methanol, a commonly used mediator for redox enzymes. At a ferrocene methanol concentration study $5x10^{-4}$ M the anodic current measured was found to be optimal. The FDH-gold fiber electrode exhibited a proportional response to fructose concentrations in the range of 0.1 mM - 3 mM (Figure 3). The calculated limit of detection was 11.7 μM, which was in the same range of reported values of ferrocene mediated fructose sensors (7 mM). The Michaelis constant (K_M) of the immobilized FDH on the fiber electrode was calculated from the electrode responses (figure 3, inset), and was found to be 5 mM, with I_{max} = 142 μA. This value was in good agreement with K_M for immobilized FDH in similar systems and was 50% less than the K_m of FDH measured in solution (typically, 10 mM).

The sensor response to fructose was also measured by the oxidative current resulting from the injection of 20 mM of fructose to a solution of buffer containing ferrocene methanol, at a constant potential of 0.2 V (vs. Ag/AgCl). The detection of fructose was observed within 2.2 sec of injection and reached its half maximal value after 3.6 seconds, and steady state plateau within 20 sec. (Figure 4). The response time observed was at least 2-4 times shorter than other reported response times.

The stability of the enzyme electrode was measured by repeatedly determining the current at 20 mM fructose concentration. The relative standard deviation for 20 successive measurements of 20 mM fructose was 3.05 %, a significant improvement on previously described FDH immobilized on gold electrodes, which were reported to reduce response after as much as a single measurement cycle. The nanofibrous fructose sensor was tested in bovine calf serum and popular beverages. The test of the sensor in full serum was intended to show the application of the sensor in complex environments containing interferents like ascorbate and high concentration of proteins. The measurement in beverages was to show the practical application of the sensor in fructose containing media. The FDH sensor electrode was tested in undiluted bovine calf serum that was spiked with 20 mM fructose. The measured current decreased by 21% by changing the medium from MIB to the serum. The decrease in the current can be attributed both to the different pH (the serum is more basic than the MIB used, thus the sensor is performing far from its optimal pH) and to the presence of interfering materials.

Cyclic voltammograms of the FDH electrode challenged with several popular beverages containing high-fructose corn syrup were collected. The concentration of fructose was measured using our sensor and with a commercial fructose kit (F-20, sigma) (Table 1). The correlation between the fructose sensor and the commercial fructose kit demonstrated the precision and accuracy of the sensor.

Gold/CNT electrodes

Preliminary results have shown that the gold microfiber electrodes have a high surface area that is available for enzyme immobilization. The results have also shown that, in the presence of chemical mediators, Glucose oxidase (GOX) can produce electrons that can feed into the gold microfibers and generate potential and current. The necessity for the presence of the chemical mediators of electron transfer is problematic if the electrodes were to be implanted in the body as the mediators are not bound to the electrode and would therefore diffuse away and further most if not all of the efficient mediators are toxic to cells. We have been working to bypass the necessity of chemical mediators by creating a direct connection between the enzyme active site and the gold microfibers of the electrode. We had initially tried to attach GOX directly to the gold via thiol intermediates. While this resulted in GOX attached to the surface (as seen by enzyme activity assays (data not shown), there was little or no electrical connection. Connecting active surface groups of GOX to the surface of the gold did not place the enzyme active site in a proper orientation to transfer electrons to the electrode. Carbon nanotubes (CNT) are known to conduct electricity and have been used as supports for enzymes in electrodes. The research in this project period thus centered on attaching CNT to the gold microfibers followed by attaching GOX to the CNT. The basic construction of the electrodes is shown in Figure 5.

Deposition of CNT on gold microfibers was accomplished through electrophoretic deposition. CNTs are exceedingly hydrophobic and adhere tightly to one another especially in the presence of water. Even deposition of CNT on a surface from a suspension requires a homogeneous suspension of individual CNT. Several methods were investigated to achieve a suspension of CNT. The protocol that yielded the best suspension involved the addition of the detergent triton X100 to the CNT suspension followed by several hours treatment with a sonic probe. The sonic energy dispersed the CNT and the Triton X100 acted to keep them apart and aided in the deposition onto the gold fibers. Figure 6 shows a picture of a gold microfiber electrode before and after CNT deposition. The fibers are evenly coated with few obvious clumps of CNTs.

To confirm that the morphology of the electrodes was not significantly altered by the deposition of the CNT, samples of the electrode/CNT combinations were subjected to electron microscopy. SEM images showed that the gold microfibers were intact and that there were no significant areas of accumulation of CNT. The even coating of the fibers was confirmed by transmission electron microscopy (Figure 7). The images clearly show a layer of nanotubes about 100 nm thick covering the gold microfiber.

The final part of the electrode was the enzyme GOX. The experiments conjugating GOX to the CNT are preliminary but encouraging. The enzyme was incubated with the activated CNT and the electrodes were washed and tested for electrochemical and enzymatic activity.

The response of the electrode to various concentrations of glucose was evaluated in an oxygen saturated PB solution (0.1 M, pH 7.0). When the solution is saturated with oxygen a large reduction peak of oxygen is observed (curve b in Fig 8A) along with the reduction of the FAD cofactor in GOX. When glucose is added GOX consumes the dissolved oxygen (glucose oxidation) which results in the decrease of peak current (curve c to I in Fig 8A). The data (as replotted in Fig. 8B) show that as the glucose concentration increases reduction peak decreases and the decrease is linearly proportional to the glucose concentration up to 6 mM which is the physiological glucose range. The system showed a sensitivity of 390 $\mu A\ mM^{-1}$ (143 $\mu A\ mM^{-1} mg^{-1}$) which is the highest observed for a GOX electrode system with direct electron transfer capability. This suggests the enzyme specificity is maintained on the electrode system while maintaining direct electron transfer.

Conclusions

We have fabricated a hybrid gold/carbon nanotube electrode that has a high surface area capable of binding to active enzymes. When glucose oxidase is conjugated to the electrode current is produced using glucose as the electron source. These results demonstrate that a glucose powered biofuel cell can be developed that will not only provide power but that will be directly responsive to glucose concentration.

Methods

Electrospinning of PAN/$HAuCl_4$ composite: 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 ^oC until the solution was clear. Electrospinning was performed using a power source (Gamma High voltage, Fl), and

syringe pump (World Precision Instruments, Fl). The 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 aluminum foil (for material characterization) or on a gold rod (1 mm diameter, for the sensor preparation) rotated by a motor 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, and then washed with dilute HCl and then H_2O. Next, gold was electrolessly deposited on the fibers by dipping the fibers in an aqueous solution containing 0.02 mg/ml hydroxylamine hydrochloride and 0.1 mg/ml $HAuCl_4$. The fibers were incubated in the solution for 10-30 minutes and then moved to a fresh solution. This was repeated 10 times. The resulting electrodes were electropolished by cycling 0-1.5 V (vs. Ag/AgCl) in 0.1 M H_2SO_4 until a constant cyclic voltammogram (CV) was observed, were rinsed thoroughly in water and stored dry until used.

Enzyme modification of gold microfibers: Fibers were incubated in 0.1 M cystamine for 3 hr, washed with H_2O and then incubated for 1 hr in 6.25 % glutaraldehyde. The fibers were rinsed with water and then incubated in FDH, (5 mg/ml in phosphate buffer 0.1 M pH 7.0) overnight at 4^oC. The electrodes were rinsed thoroughly with phosphate buffer until no FDH activity could be assayed in the washing buffer (typically, 5 washes). FDH activity on the fibers was measured using a Prussian Blue assay. Protein assays were performed using the fluorescent measurement of the adduct of o-phthaldialdehyde to amino acids, using BSA for calibration.

Electrophoretic deposition (EPD) of CNTs onto gold fibers: Our design exploited the conductivity of the gold-coated electrospun fiber as a base upon which to perform electrophoretic deposition of CNTs. Commercial CNTs with carboxyl functionalities at the end of the CNTs and at defect sites along the length of the tubes (0.5 mg/ml) were dispersed in water along with TX-100 as surfactant (1.0 mg/ml) by sonication. The combination of surfactant and sonication gave a highly dispersed CNT solution with ink-like consistency. The negative charge (carboxyl functionalization) on the CNT was then utilized to disperse the CNTs under DC voltage (electrophoresis) and deposit them on the oppositely charged gold fiber. Controlling the voltage, time and distance between electrodes during EPD (allowed us to uniformly coat of CNTs on the fiber surface. We found that 20V applied to the sensor material (positive) held in the middle of parallel copper plates (negative), gave an electrode gap of 0.5 cm and EPD was performed for 30s. The CNT-coated gold electrospun fiber was then dried under vacuum at room temperature before use.

Enzyme Immobilization to gold/CNT microfibers: Enzyme was covalently coupled to the CNTs via the carboxyl groups present on the CNTs using the coupling reagents N-(3-dimethylaminopropyl) -N´-ethyl carbodiimide hydrochloride (EDC)/ N-hydroxysuccinimide (NHS). We soaked the fiber in a solution of EDC/NHS (molar ratio of EDC (50 mM) to NHS = 1:1) in acetonitrile for 30 minutes. This was followed by drying the electrode in an inert atmosphere before immersing it in a 500 µl enzyme (10 mg/ml) in phosphate buffer (0.1 M, pH 7.0) for 18 hours at 4 °C. The resultant GOX electrode was washed with buffer until no enzyme was found in the washings, and then characterized for electrochemical and enzyme activity.

Electrochemical characterization: electrochemical experiments were run on a CHI 660 electrochemical station, equipped with a standard 3-electrode cell. The auxiliary electrode was a

Pt wire, and an Ag/AgCl electrode was used as a reference. Cyclic voltammetry to measure the response to fructose was performed by scanning between 0-0.6 V versus Ag/AgCl at a scan rate of 20 mV/sec, after adding $5x10^{-4}$ M ferrocene methanol as a diffusional electron mediator. The electrochemical surface area of the gold microfibers was determined by chronocoulometry using 1 mM ferricyanide in KCl.

Physical characterization of the fibers: Scanning Electron Microscopy (SEM) was performed on carbon coated fibers, at 10 KV acceleration voltage using a JEOL 6330F SEM. TEM images were obtained on epoxy embedded samples that were sectioned, and imaged on JEOL JEM1210. A Micromeritics ASAP 2010 instrument, N_2 adsorption device was used to determine surface area. Fiber diameter and gold particle size distribution were measured using ImageJ software from SEM/TEM images, using at least 50 objects for reliable statistics.

Table 1: Fructose measurement using fructose electrode and commercial kit

	fructose sensor fructose mg/ml	F-20 kit fructose mg/ml
Coca - cola	22.7±*3.1*	23.*4*±*0.7*
Pepsi	21.8±*2.6*	24.4±*1.1*

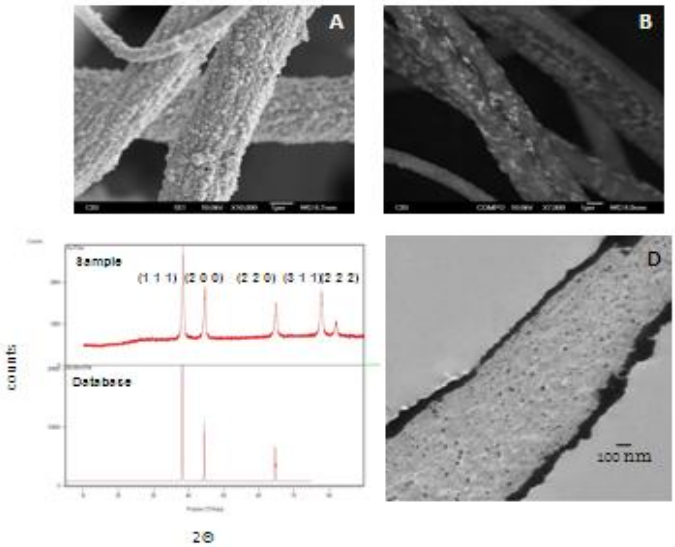

Figure 1. (A) SEM of as-spun PAN-$HAuCl_4$ polymer; (B) SEM of the same polymer after electroless deposition of gold; (C) XRD of the fiber after electroless deposition; (D) TEM of a cross section of the fiber through its length.

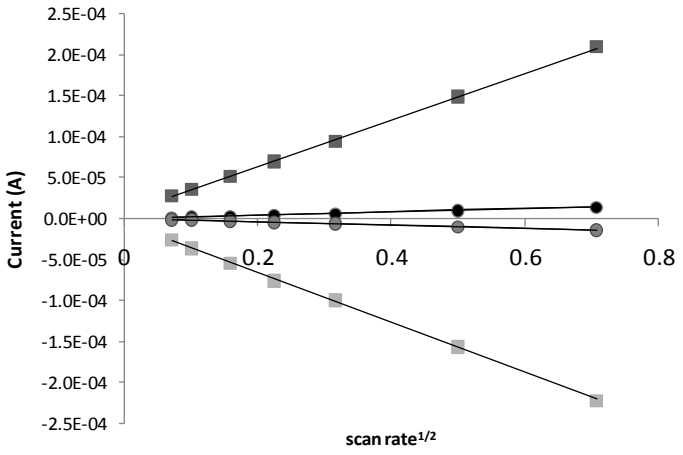

Figure 2. Anodic and cathodic currents as a function of the scan rate, measured on a gold fiber electrode (a) and on polished gold disc electrode (b) in 1 mM ferricyanide solution in 0.1 M KCl.

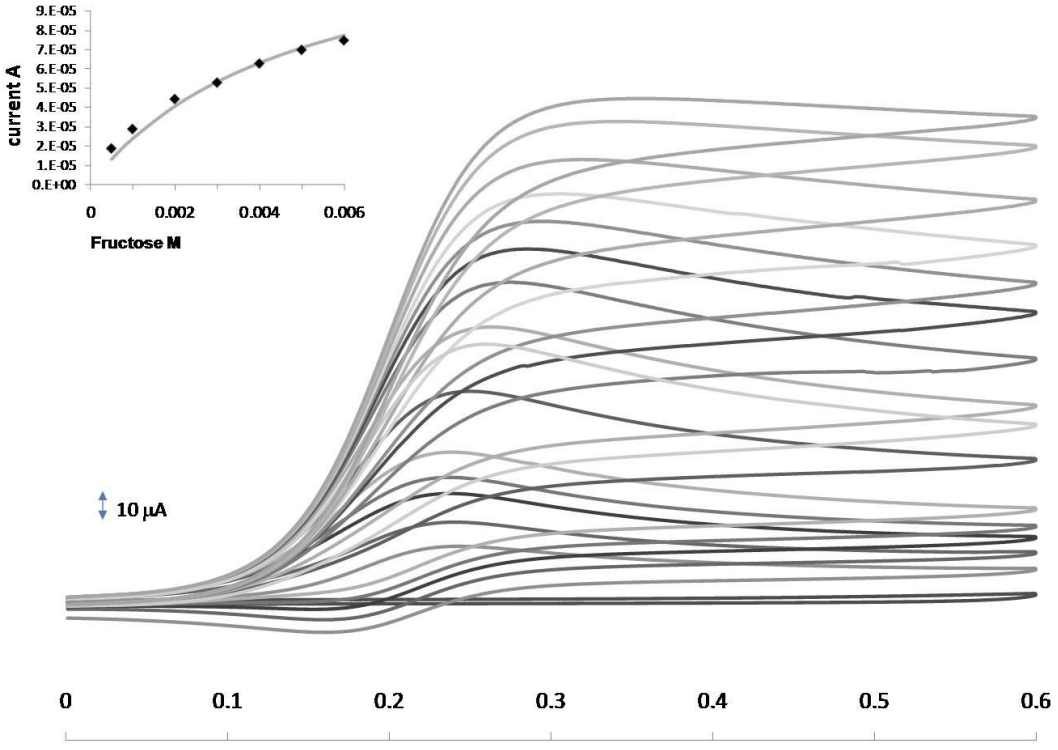

Figure 3. Cyclic voltammetry of FDH immobilized on gold fiber electrode, in McIlvaine buffer pH = 4.5, containing $5x10^{-4}$ M ferrocene methanol. Scan rate = 20 mV/sec. Concentrations are from 0.1 mM to 1.5 mM. Inset: Michalies-Menten kinetics of electrochemical activity of FDH as a function of fructose concentration.

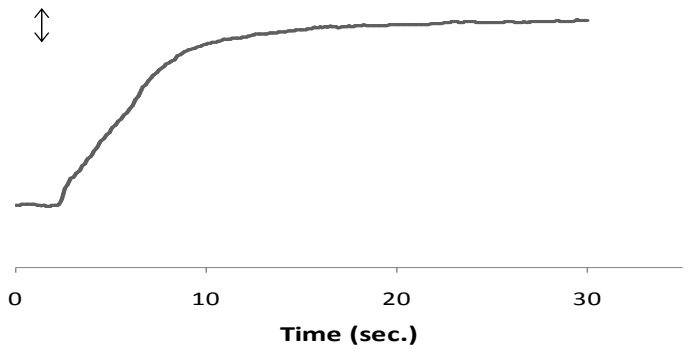

Figure 4. I-t curve of FDH response to 20 mM fructose injection into McIlvaine buffer pH = 4.5 containing $5x10^{-4}$ M Ferrocene methanol. Potential was held at 0.2 V vs. Ag/AgCl.

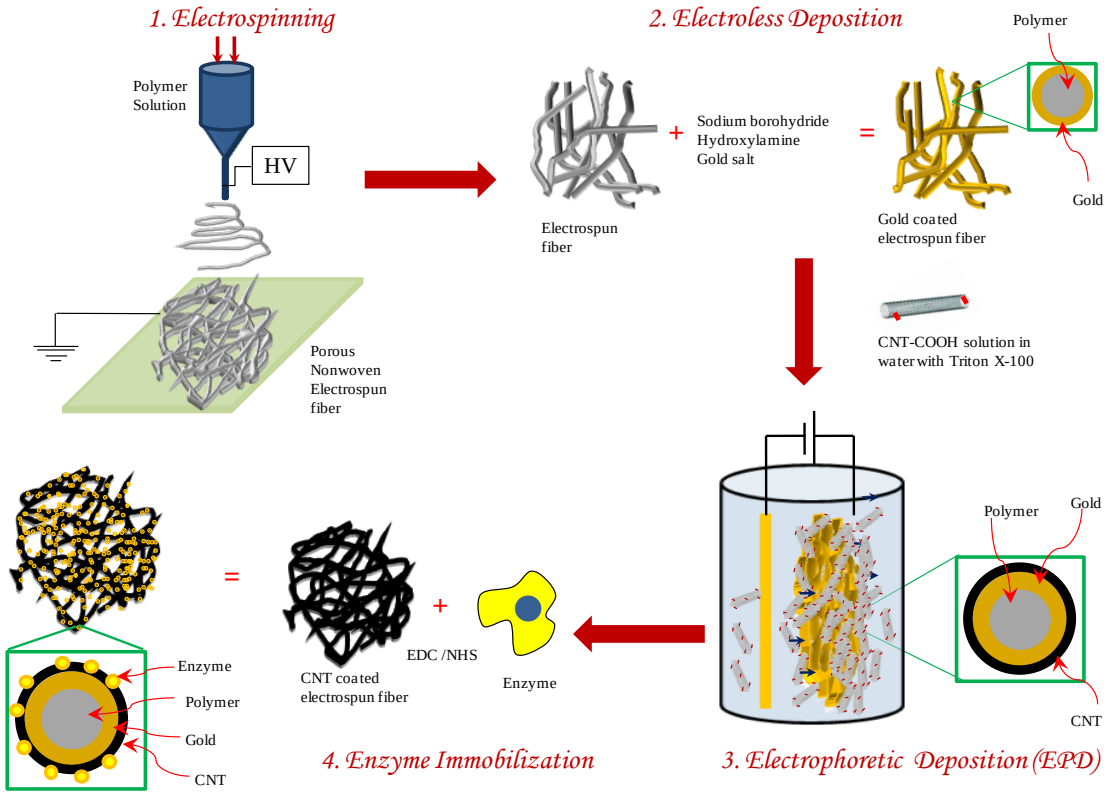

Figure 5. Schematic of electrode fabrication

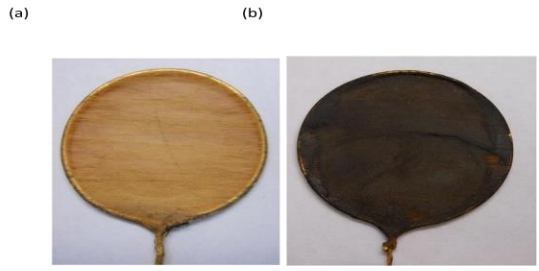

Figure 6. Photographs of gold fiber paddle before (a) and after (b) CNT coating by electrophoretic deposition

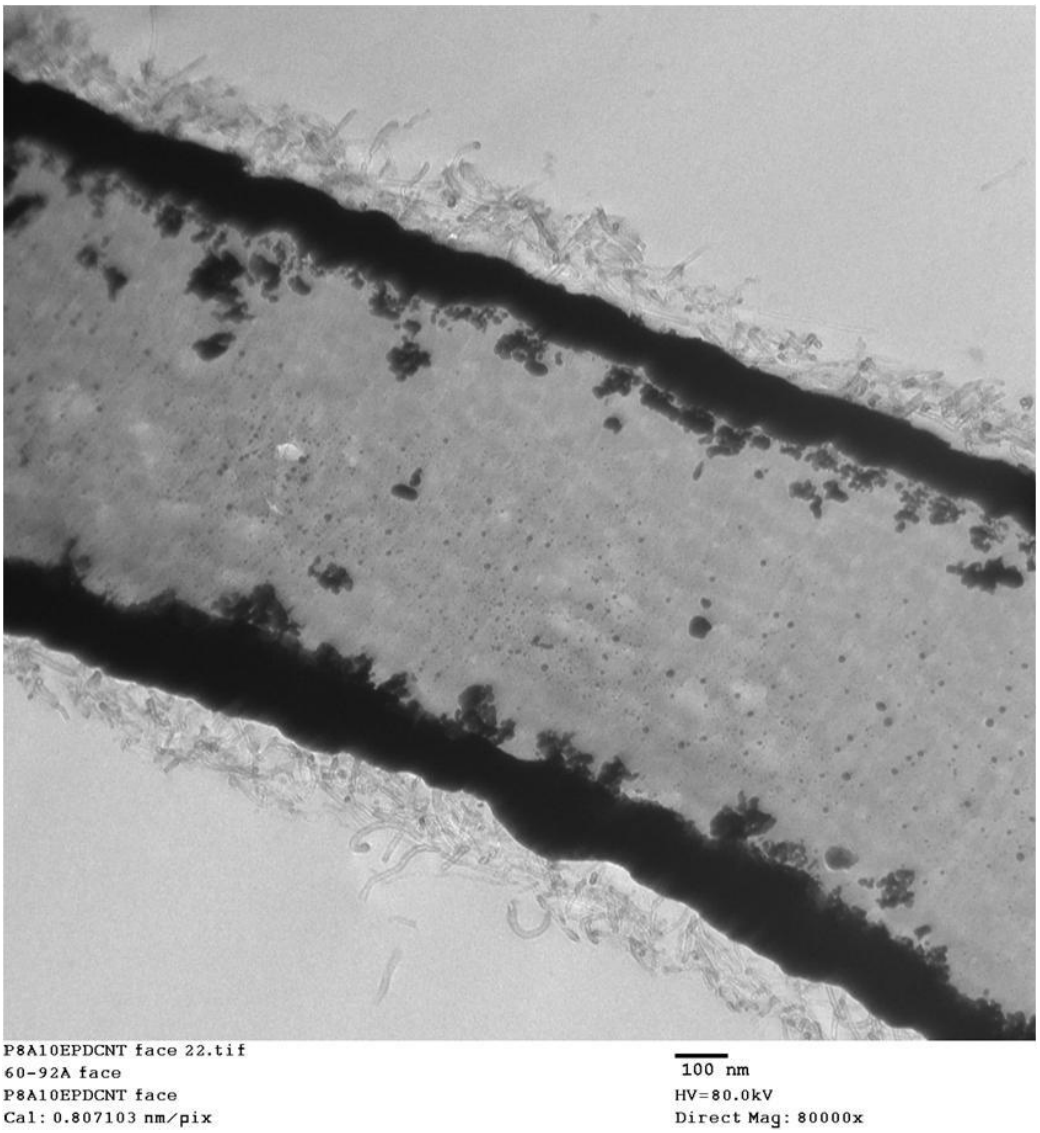

Figure 7. TEM image of a gold microfiber. The structure of the fiber consists of a layer of CNT on a layer of gold each about 100nm thick, covering the approximately 1 micron diameter polymer fiber.

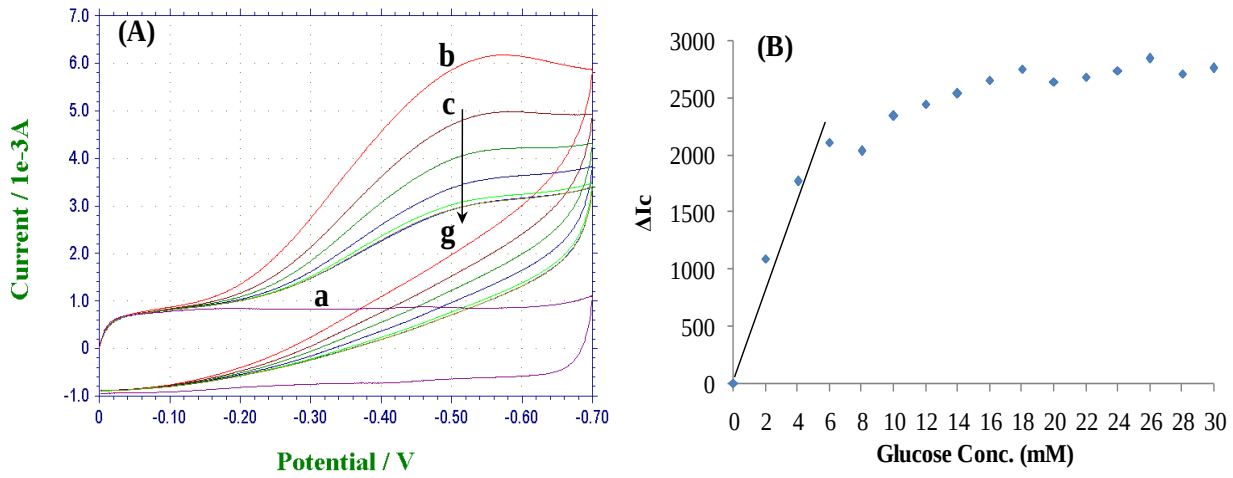

Figure 8. (A) Cyclic voltammograms of electode in 0.1M phosphate buffer, pH 7.0 at a scan rate of 100 mV/s in the presence of different concentration of glucose: (a) Ar-saturated without glucose, (b) O_2-saturated without glucose, and (c-g) with of 2,4, 10,16,24 and 30 mM glucose**;** (B) calibration curve corresponding to the peak currents measured at -436 mV at different glucose concentration**.**

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?

Gender:
_____Males
_____Females
_____Unknown

Ethnicity:
_____Latinos or Hispanics
_____Not Latinos or Hispanics
_____Unknown

Race:
_____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. None				☐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:

An article describing the gold/carbon nanotube electrodes is in preparation.

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.

None.

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.*

NAME	POSITION TITLE
Richard R. Koepsel, Ph.D.	Research Professor
eRA COMMONS USER NAME koepsel	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
University of Wisconsin, Madison WI	BS	1974	Bacteriology
University of Wisconsin, Madison WI	MS	1977	Pharmacognosy
University of Wisconsin, Madison WI	Ph.D.	1981	Bacteriology
University of Pittsburgh, Pittsburgh PA	Post-doc	1982-1987	Molecular Biology

A. Personal Statement

I have over 30 years of experience in research projects involving bacteria and antimicrobials. Over the past 10 years I have worked with Dr Alan Russell studying antimicrobial surfaces and polymeric antimicrobial materials. We have been able to synthesize antimicrobial polyquaternary amines and to characterize their mechanism of action. Additionally we have recently synthesized a new class of antimicrobial materials which generate active halogens using immobilized enzymes acting on glucose and halide salts.

B. Professional Experience

February 1987 – June 1999
Assistant Professor
Department of Microbiology-Biochemistry
School of Dental Medicine, University of Pittsburgh

July 1999 – July 2009
Research Associate Professor
University of Pittsburgh
Department of Chemical and Petroleum Engineering
Center for Biotechnology and Bioengineering
300 Technology Drive
Pittsburgh PA 15219

July 2009 – present
Research Professor
University of Pittsburgh
Department of Surgery
McGowan Institute for Regenerative Medicine
450 Technology Drive
Pittsburgh PA 15219

C. Selected Publications

Amitai G, Murata H, Andersen J, **Koepsel R**, Russell AJ. Decontamination of chemical and biological warfare agents with a single multifunctional material.
Biomaterials. 31:4417–4425. 2010.

Amitai G, Andersen J, Wargo S, Asche G, Chir J, **Koepsel R,** Russell AJ. Polyurethane-based leukocyte-inspired biocidal materials. *Biomaterials*. 30:6522-6529. 2009.

Liu Z., Bartlow P., Dilmore R.M., Soong Y., Pan Z., **Koepsel R**., Ataai M. Production, purification, and characterization of a fusion protein of carbonic anhydrase from Neisseria gonorrhoeae and cellulose binding domain from Clostridium thermocellum. *Biotech Progress.* 2009. 25:68-74.

Huang J, **Koepsel RR**, Murata H, Wu W, Lee SB, Kowalewski T, Russell AJ, and Matyjaszewski K. Nonleaching Antibacterial Glass Surfaces via "Grafting Onto": The Effect of the Number of Quaternary Ammonium Groups on Biocidal Activity. *Langmuir* 2008 24:6785-6795.

Cunningham D, Liu Z, Domagalski N**, Koepsel RR,** Ataai MM, and Domach MM. Pyruvate Kinase-Deficient *Escherichia coli* Exhibits Increased Plasmid Copy Number and Cyclic AMP Levels *J. Bacteriol.* 2009. 191: 3041–3049.

Murata H, **Koepsel RR**, Matyjaszewski K and Russell AJ,. Permanent, non-leaching antibacterial surfaces How high density cationic surfaces kill bacterial cells *Biomaterials* 2007, 28: 4870-4879*.*

Huang J, Murata H, **Koepsel RR**, Russell AJ, and Matyjaszewski K. Antibacterial Polypropylene via Surface-Initiated Atom Transfer Radical Polymerization. *Biomacromolecules* 8:1396-1399, 2007**.**

Ravikumar T, Murata H, **Koepsel RR**, and AJ Russell. Surface-Active Antifungal Polyquaternary Amine. *Biomacromolecules* 7: 2762-2769, 2006

Lee SB, **Koepsel RR**, Russell AJ Surface dispersion and hardening of self-assembled diacetylene nanotubes *NANO LETTERS* 5: 2202-2206, 2005

Zhu T, Pan Z, Domagalski N, **Koepsel R**, Ataai MM, Domach MM. Engineering of Bacillus subtilis for enhanced total synthesis of folic acid. *APPLIED AND ENVIRONMENTAL MICROBIOLOGY* 71: 7122-7129, 2005

Lee SB, **Koepsel RR**, Morley SW, Matyjaszewski K, Sun YJ, Russell AJ. Permanent, nonleaching antibacterial surfaces. 1. Synthesis by atom transfer radical polymerization BIOMACROMOLECULES *5:877-882* 2004

Lee SB, **Koepsel R,** Stolz DB, Warriner HE, Russell AJ. Self-assembly of biocidal nanotubes from a single-chain diacetylene amine salt JOURNAL OF THE AMERICAN CHEMICAL SOCIETY *126: 13400-13405* 2004

Russell, AJ, Drevon GF, Berberich, JA, and **Koepsel RR**. Biomaterials for mediation of chemical and biological warfare agents. *Annual Review of Biomedical Engineering*. 5:1-27, 2003

Koepsel RR, Russell AJ. Directed capture of enzymes and bacteria on bioplastic films. *Biomacromolecules* 4: 850-82755 2003

Fry B., Zhu T., Domach M.M., **Koepsel R.R.,** Phalakornkule C., and Ataai M.M. Characterization of growth and acid formation in a pyruvate kinase mutant of *Bacillus subtilis*. *Appl. Environ. Microbiol.* 66:4045-4049. 2000