Response Form for the Final Performance Review Report – Pittsburgh Tissue Engineering Initiative, Inc. 2009F*

1. Name of Grantee: Pittsburgh Tissue Engineering Initiative, Inc.

2. Year of Grant: 2009 Formula Grant

A. For the <u>overall grant</u>, briefly describe your grant oversight process. How will you ensure that future health research grants and projects are completed and required reports (Annual Reports, Final Progress Reports, Audit Reports, etc.) are submitted to the Department in accordance with Grant Agreements? If any of the research projects contained in the grant received an "unfavorable" rating, please describe how you will ensure the Principal Investigator is more closely monitored (or not funded) when conducting future formula funded health research.

PTEI provides grant oversight to all Formula Grants in the following manner. Weekly update meetings are conducted between the PI (Dr. Rick Koepsel) and the Grant Coordinator (Dr. Alan Russell) to ensure progress of milestones. The Annual and Final Progress Reports are submitted to PTEI two weeks prior to the State's submission deadline to allow for review, comments, and questions, by the Program Manger and the Grant Coordinator. Once reports are finalized and approved they are submitted in accordance with the Grant agreement. If the project receives an unfavorable rating, the Grant Coordinator meets with the PI to address the comments and craft the appropriate response. PTEI will then continue to closely monitor the progress of the project.

In addition, PTEI monitors the grant through the review of invoices from the subcontractor, the University of Pittsburgh, along with a review of its own internal administrative expenses. PTEI is invoiced monthly for expenses of the previous month by the subcontractor. Once received, the invoice is coded by the Grants Coordinator and checked against the original budget for variances. If no variances have occurred that need follow up, the invoice is submitted for additional review to the Senior Research Administrator and the Controller. Once these approvals have been obtained, the invoice is prepared for payment. Using this information the audit report is prepared and submitted accordingly.

* Please note that for grants ending on or after July 1, 2007, grantees' Final Performance Review Reports, Response Forms, and Final Progress Reports ***will be made publicly available on the CURE Program's Web site.***

Project Number: 0990601
Project Title: Novel Biofuel Cell Based on High Surface Area Enzymatic Microelectrodes
Investigator: Koepsel, Richard

B. Briefly describe your plans to address <u>each</u> specific weakness and recommendation in Section B using the following format. As you prepare your response please be aware that the Final Performance Review Report, this Response Form, and the Final Progress Report will be made publicly available on the CURE Program's Web site.

Reviewer Comment on Specific Weakness and Recommendation (*Copy and paste from the report the reviewers' comments listed under Section B - Specific Weaknesses and Recommendations)*:

Response (*Describe your plan to address each specific weakness and recommendation to ensure the feedback provided is utilized to improve ongoing or future research efforts*):

SPECIFIC WEAKNESSES AND RECOMMENDATIONS

<u>Reviewer 1:</u>

1) *No publications, patents or significant collaborations resulted from the work. Goals were only partially met.*

Response: The comment that the goals of the project were not completely met is true. It often happens in science that things don't work exactly as planned. The PI's role in those instances is to direct the effort of the project to problem solving. Our efforts to develop an electrode using fructose dehydrogenase were an effort to understand enzyme loading on the electrode surface and the potential to increase the performance of a fuel cell by altering the enzymatic activity. In this, the project did succeed with the construction of a fructose sensing electrode which was being developed as the anode of the fuel cell.

The work did eventually result in a peer-reviewed publication:

Marx S, Jose MV, Andersen JD, Russell AJ. Electrospun gold nanofiber electrodes for biosensors. Biosens Bioelectron. 2011;26(6):2981-6.

2) This grant was too small to expect much. It should have been reviewed in the beginning not just at the end of the work. The results were small, but so was the funding.

Response: We agree. The funding supported the PI at approximately 10% effort. This effort was spent in planning experiments, reviewing data with the researchers, writing reports, writing grant applications, and trouble-shooting protocols. All of these are vital functions but most have a smallish impact on the outcomes.

3) *A functioning and improved fuel cell was not made.*

Response: It was not clear from the report but we had made and tested functioning fuel cells prior to the start of the project reported here. It is true that they were not improvements over those previously reported in the literature but we had developed a novel electrode with the microfibers gold. The, perhaps understated, goal became the optimization of the anode. Functioning fuel cells were also reported in the article listed above.

4) *There were no publications or patents. This would be the most significant indicator of progress.*

Response: See comment 1) above. Additionally, another manuscript resulting from the further funding of the project by the NSF is currently under review. The funding was a direct consequence of the Formula Grant Program.

5) *The impact of Ph.D. recruits was not indicated. This should have been noted.*

Response: As noted the scope of this project was much grander than the funding provided. The PhD recruits were vital to the realization of the project which continues outside of, but as an outcome of, the Formula Grant program.

6) *There were no collaborations.*

Response: We believe that the final product will result in and from collaborations.

Reviewer 2:

1) *The project proposed to take their preliminary/previous work on fructose bioanodes and develop a laccase biocathode in the first six months; and in the second six months, they proposed to marry the bioanode with biocathode and test a full functioning biofuel cell. They did continue their bioanode work, but they did not develop a biocathode or test a full functioning biofuel cell. Also, their bioanode was tested as a sensor but was not evaluated as a fuel cell electrode. In addition, this project is not particularly novel; the enzymes used are commercially available and common in the field. The team utilized a gold electrode that was interesting, but the data did not show any particular advancement in the field.*

Response: We agree with much of this critique. We have been working on a biocathode in the half cell mode rather than as a full fuel cell. We have abandoned the laccase electrode because of issues with enzyme loading half-cell potential and the lack of a clear pathway to development of a mediator free electrode. We have been working on a biocathode using bilirubin oxidase but its development lags behind the bioanode. We believe, however, that current development of the bioanode will be equivalently useful to the biocathode. (This response is expanded in the response to comment 3) below.)

2) *The team should develop a biocathode and test both electrodes via polarization in half cell mode before testing a complete biofuel cell. Biofuel cell testing should be done galvanostatically instead of with external resistors and a multimeter*.

We agree and thank the reviewer for the suggestion of the galvanometer.

3) *Mediators are a clear issue for implantable power sources. The team should address how they will eliminate mediators or protect from leaching mediators from the biofuel cell.*

Response: We could not agree more. The ultimate goal of this work has been an implantable device. Our development efforts since the writing of the report for this Formula Grant have been directed at loading glucose oxidase onto the electrode in a manner compatible with mediator-less activity. The manuscript currently under review describes that work. A relevant finding is that at relatively low enzyme loading rates we are able to get upwards of 25% of the bound enzyme directly transferring electrons to the electrode. The new electrode fabrication protocol should result in similar activities at the biocathode. The results further indicate that enzyme loading is very far from saturated pointing to direct avenues for improvement.

4) *The team should build both intellectual property and peer-reviewed publications in the field.*

Response: New publications are in process.

5) *Bioanode and biocathode stability should be addressed if they are considering implantable applications.*

Response: Bioanode stability in *in vitro* and *in vivo* conditions are underway with encouraging preliminary results.

Reviewer 3:

The general design of the research is flawed. A basic understanding of the principles of fuel cells is needed. More convincing preliminary results are needed. The potential applications and impacts of the work need to be studied carefully.

Response: While I understand the instructions for this Response Form state that the response is to the reviewer's comments in section B of the report, in order to respond to Reviewer 3's comment here I need to refer to specific comments this reviewer made in previous sections of the report. These comments are:

1) From **criterion 1** "*The problem might be related to the design of the work. They assume that the formal potential of the enzyme fructose dehydrogenase is about -0.35 V, but their results showed a 0.2 V potential for the oxidation of fructose. This is too high for an anode. It is difficult to find a cathode providing a potential higher than this value so that the two electrodes can work together as a fuel cell*."

Response: The formal potential was not "assumed;" it was obtained from the literature and was based on the reaction in solution. We looked at fructose dehydrogenase because of this potential with the full understanding that immobilized enzymes may not act the same as soluble enzymes. In fact the reason one does research is to avoid assumption and reveal reality. The response of the enzyme immobilized on the electrode was measured at 0.2V as the change in amperage from the catalytic curve in a ferrocene methanol mediated reaction. It should be mentioned that this electrochemical determination was very dissimilar to the native reaction and environment of the enzyme and is thus not surprisingly different from the derived value. We have in fact made fuel cells using the fructose dehydrogenase electrode and a bilirubin oxidase cathode. The cells produced power with mediator but not by direct electron transfer.

2) From **criterion 2:** "*The principal investigator talked about implantable power sources using fructose as the fuel source. Would they be implantable in plants? And, which plants need a fuel cell inserted in them? The principal investigator should explain."*

Elementary biochemistry should be enough explanation but was apparently not considered. Glucose is converted to fructose by the direct activity of the enzyme glucose isomerase. A major industrial enzyme used to convert glucose to fructose in high fructose corn syrup. It works with the fructose dehydrogenase electrode to allow the electrode to do glucose measurements and when added to a fuel cell allows the electrode to use glucose as a fuel source.

C. If the research project received an "unfavorable" rating, please indicate the steps that you intend to take to address the criteria that the project failed to meet and to modify research project oversight so that future projects will not receive "unfavorable" ratings.

Response: The project as funded by the Formula Grant was for one year; other funding has continued. Most of the critical comments here have been resolved by the continuation of the project. Some of the failings noted were inevitable outcomes of research process but resulted in a change of focus that will be successful.

D. Additional comments in response to the Final Performance Review Report (OPTIONAL):