Response Form for the Final Performance Review Report—
Pittsburgh Tissue Engineering Initiative 2008F*

1. Name of Grantee: Pittsburgh Tissue Engineering Initiative

2. Year of Grant: 2008 Formula Grant

A. For the overall grant, 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.

For each research project contained in the grant, please provide a response to items B-D as listed on the following page(s). When submitting your response please include the responses for all projects in one document. The report cannot be submitted as a ZIP file, because the Department's exchange server will remove it from the email. If the report exceeds 2MB, please contact the Health Research Program for transmittal procedures: 717-783-2548.

* 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: 0864801
Project Title: Novel Biofuel Cell Based on High Surface Area Enzymatic Microelectrodes
Investigator: Marx, Sharon

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.

<u>Reviewer 1:</u>

1. The team has successfully fabricated gold microelectrodes through electrospinning; however, no comparison was made with other nano gold electrodes to highlight the benefits in electrical properties (if any). It is also unclear how this microelectrode structure is more beneficial for enzyme immobilization when compared to a bundle of nano scale electrodes.

Response: The major advantage is that the electrodes have a very high surface area per volume of fiber. Additionally, the porosity of the material is high enough that the access to substrate is not diffusion limited.

2. Enzyme immobilization was successfully demonstrated using well-known cross-link chemistry such as glutaraldehyde. Although the immobilized enzymes were shown to be active, no assessment was made to investigate the level of deactivation due to the cross linking. Clearly, the Km values of the enzymes are significantly affected. In addition, no information was provided to test the effect of enzyme loading on biofuel performance. An indirect experiment on the effect of fiber weight was reported.

Response: We agree with the reviewer that the above are limitations of the system. Later versions of the electrodes address all of these issues and a report of the latest version is currently under review. [Jose, M., Marx, S., Koepsel, R.R**.,** Murata, H., Russell, A.J. "Direct Electron Transfer in a Mediator-Free Glucose Oxidase-based Carbon Nanotube-Coated Biosensor" *Carbon.* Submitted.]

3. They have successfully demonstrated the generation of electricity using the proposed biofuel format. Again, some comparison here with other setups will be very helpful. The current power density of 20 μW/cm2 is lower than the desired goal (100μW/cm2). They propose to address this by increasing the enzyme density. How much can they increase without seeing any diffusional limitation? Some quantitative assessment must be provided.

Response: We believe we are currently in a position to answer these questions. In short, our latest iteration of the electrode has increased the enzyme density to nearly monolayer coverage of the electrode fibers and we do not see any diffusional limitations. Further, by comparing the electrical output on a per enzyme molecule basis we can achieve power densities higher than any yet reported for an enzyme based electrode. A report on these data is being prepared.

Reviewer 2:

1. The power generated did not meet the desired level.
   Recommendation: This project is worthy of additional investment. Extension with a larger budget will yield better return.

Response: The investment has indeed continued, and very encouraging results have been achieved (please see response to Reviewer 1, #3 above)

2. Some of the immobilization agents or their component is considered toxic.

   Recommendation: Evaluate the toxicity of all components in the system should the system be considered for in vivo application.

Response: The latest iterations of the electrode do not use toxic cross-linkers relying of physio-adsorption to achieve high enzyme densities.

Reviewer 3:

1. The PI is encouraged to submit proposals centered on the biological response of the novel biofuel cell since a detrimental biological response could severely inhibit its function leading to redesigning.

Response: Such a proposal has recently been funded by the NSF.

2. The PI is encouraged to present and publish the results of this study.

Response:

A paper has recently been published: Marx, S.; Jose, M. V.; Andersen, J. D.; Russell, A. J., Electrospun Gold Nanofiber Electrodes for Biosensors. *Biosensors and Bioelectronics* **2011**, 26, 2981-2986.

A second paper is under review: Jose, M., Marx, S., Koepsel, R.R., Murata, H., Russell, A.J. "Direct Electron Transfer in a Mediator-Free Glucose Oxidase-based Carbon Nanotube-Coated Biosensor" *Carbon.* Submitted.

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: N/A

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

Response: We Thank the reviewers for their efforts.