FINAL REPORT
SCHOLARLY ACTIVITY AND IMPROVEMENT FUND (SAIF)
# 102-271106-2

Title:  PROLIFERATION & ACCUMULATION OF EMBRYONIC AND ADULT STEM CELL LINES FOR DIFFERENTIATION ANALYSIS

Project Director: Dr. Esther N. Ofulue, Professor of Biology, UW-Platteville

Date of Report: September 30, 2011

Project Objectives:
The three objectives were: 1) to optimize and calibrate equipment and learn new software for the research data analysis, 2) to optimize protocols and culture, freeze and accumulate human embryonic and mouse bone marrow stem cells, and 3) to evaluate the frozen stem cells for viability and differentiation capacity using growth hormones as activators and the new equipment in data analysis.

Summary of Project Outcomes:
The overall goal to standardize and optimize protocols for culture, passaging, freezing and thawing of human and mouse embryonic stem (ES) cells and required feeder cells in order to generate large pools of viable frozen stem cells was accomplished. Well over twenty frozen vials of mouse ES cells and five vials of human ES cells were frozen (Please see attached pdf image file). The optimized protocols were documented for further research on stem cell differentiation and also for training in UW-Platteville student independent research. The protocols were also used by the Principal Investigator in training faculty, undergraduate and graduate students at the University of Ibadan, Nigeria where she spent one year of Fulbright sabbatical Teaching and Research leave.

In a broader goal of the Principal Investigator’s sabbatical leave research project, the activation of mouse adult bone marrow stem cell differentiation using pharmaceutical plant extracts indigenous to Ibadan, Nigeria was investigated as a possible replacement for the problematic standard activation using animal growth factors and hormones. The results of this study when completed will have relevance in human medicine.

The SAIF project done in the summer of 2010 afforded us time to systematically accumulate cells of similar lineage that will be used for at least the next two years at UW-Platteville without reoptimizing the protocols. This ultimately saved cost and time while engaging us in intensive professional activity. Two UW-Platteville student helpers were trained in the process with the knowledge and skill to pursue their career goal and interests.

Outcome of Objective Parts 1 & 2:
We were able to calibrate the carbon dioxide regulating system using Fyrite analyzer to maintain a steady flow at 5% and incubation temperature of 37°C in humid atmosphere. I also learned to use the ElementD 3.1 DSU2 imaging software connected to an inverted microscope and camera. I trained the student helpers to perfect their skills in mode switching, camera time lapse, resolution, stitching and image grabbing functions. The attached cell image document was obtained throughout the study period using this software. We prepared a simplified step-by-step protocol for training new users in future.

Once protocols and image documentation systems were standardized, we began work of systematic culture, freezing, thawing and accumulation of cells. The images shown from 6/28/10 to 6/30/10 were samples of mouse fibroblast feeder and embryonic stem cells that were successfully frozen and then thawed. Viability of cells was evaluated based on number of cells that attached to the flask (dead cells usually float in the media) after 12 hours and increase in number of attached cells due to cell division after 24 hours. Cell count was done using the software images instead of hemocytometer.
Cells in 1 of 9 squares x \(10^4 = \text{cells/milliliter.}\) High level of cell viability was indicative of perfected freezing and thawing techniques/protocols. The culture, freezing and thawing protocols of WiCell and Dr. Gary Lyons of UW-Madison (Lyons et al, 2000) were adjusted and optimized for these the cell manipulations. Adjustments usually involve glucose, trypsin and amino acid levels and timings.

Accumulated feeder cells and human and mouse embryonic stem cells were stored frozen in cryovials in liquid nitrogen at about -200°C (-320°F).

**Outcome of Objective Part 3:**
We were unable to carry out the study of stem cell differentiation by growth hormones at UW-Platteville because of depleted funds for payment of PI and the student helpers. However, this study and evaluation of differentiation by medicinal plant extract were initiated during my sabbatical leave and is on-going as part of two graduate student research projects in Nigeria. The patent for this idea will be prepared and submitted for processing in the near future.

**Benefit of the Project:**
**To Project Director:**
1) As stated earlier, this research was professional development to help me to stay abreast and current with technology in my field.
2) Also, results from this and the graduate research projects will provide a strong position to write and submit manuscripts.
3) Information and skills gained will certainly enrich my curriculum and enhance my teaching by providing concrete examples to relate to real-life.
4) The project provided funds, as well as skills to the student helpers. My students in class will also learn the skills to better prepare them to enter the workforce or professional schools.
5) Furthermore, investigation of the inexpensive medicinal plant for harnessing tissues will generate positive interest in medical research and tissue therapy.
6) Finally, a variety of UW-Platteville courses and disciplines – Animal Tissue Culture, Genetics Biotechnology, Cell & Molecular Biology, chemistry, biomedical engineering, nanotechnology, and pre-medical courses - will especially benefit from techniques and contents of this and future research. Discussions on ethics of stem cell technology will enhance understanding and enable students to make informed judgment. In 2009 I was invited to the Chancellor’s Cabinet to present on my Stem Cell research work at UW-Platteville and the impact of Federal Policies.

**Dissemination of results**
I have done presentations and seminars on this project at the Redeemer University and the University of Ibadan (my sabbatical host campus), both in Nigeria. I also trained and have engaged the collaboration of faculty and graduate students in the extension of this project.

I am scheduled to present the research project at my Departmental level next month (November 4, 2011). I also intend to submit an abstract to share the outcome of the project in the Spring of 2012 at the Research & Poster Event sponsored by the UW-Platteville Office of Sponsored Program.

Once enough data have been collected from the graduate project, I will prepare and submit a manuscript for publication in a peer-reviewed journal.

**Budget:**
- Salary: $3145.50
- Student Help Salary 60 hours @ $7.25: $435.00
- Student Help Salary 20 hours @ $7.25: $145.00
- Travels 2 X 150 miles @ $0.32: $96.00 (trip back and forth Platteville to UW-Madison)
Supplies $274.50 (other minor supplies were funded by Dept)
TOTAL SAIF Request $4000.00
TOTAL Amount Spent $4096.00 (travel was covered out of personal funds)

**Justification of Budget:**
**Salary:** The amount $3145.5 covered my salary for approximately 2½ full-time weeks at my pay scale at the time of the project. However, the project involved a total of about 6 weeks of work. Student Helpers were paid as shown above.

**Supplies & Other:** $274.50 was spent on materials such liquid nitrogen tank refill and purchase of 15 milliliter and 50 milliliter sterile test tubes and 22 micron filters. The Department of Biology provided funds for purchase of cultures media, antibiotics, New Calf serum and Fetal Bovine Serum.

**Travels** between the UW-Madison lab and UW-Platteville two times (to pick up mouse STO feeder cells and embryonic stem cells) was unreimbursed out of pocket expense by the principal investigator.

**List & Results of Previous SAIF Grants**
1. **SAIF A 2000** - “Analysis of Gene Expression in Nuclear Transfer Cloned & Transgenic Bovine Embryos”. **Results:** publications, presentations, training of UW-Platteville students.
   a. Publication/Presentation- “Analyses of Gene Expressed During Preimplantation Bovine Embryo Development”. *Biology of Reproduction* 64(Supplement 1) p. 269
   c. Minority Faculty Research Grant Proposal titled “Timing and Levels of Gene Expression in Nuclear Transfer Cloned and Transgenic Bovine Embryos” funded for ~$13,000.
   **Results:** Publication, presentations, training of a UWP undergraduate student.
   **Result:** NSF-NUE grant "Interdisciplinary, Upper-Division Lab Modules in Nano/ Biomaterials" for ~$250,000 was submitted with collaborators but not funded.
   **Results**
   a. NSF-MRI grant “MRI: Acquisition of Nanonics NSOM/SPM at UW-P” for $161,900 was submitted with collaborator but not funded.
   c. The contents of both NSF proposals & this publication formed part of the NSF proposal submitted by a group at College of EMS in Fall 2009 which was funded for ~$500,000.00.

Finally, students that helped with my 2000-2001SAIF research developed interest in the area and not only carried out independent studies under my instruction but also turned around their original career goal at UWP. One was hired in a biotech industry immediately after graduation, and two others recently completed their Ph.D in Transgenic Technology and Genetics, respectively.