

REPORT DOCUMENTATION PAGE					<i>Form Approved OMB No. 0704-0188</i>	
The public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing the burden, to Department of Defense, Washington Headquarters Services, Directorate for Information Operations and Reports (0704-0188), 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number.						
PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.						
1. REPORT DATE (DD-MM-YYYY)		2. REPORT TYPE			3. DATES COVERED (From - To)	
4. TITLE AND SUBTITLE				5a. CONTRACT NUMBER		
				5b. GRANT NUMBER		
				5c. PROGRAM ELEMENT NUMBER		
6. AUTHOR(S)				5d. PROJECT NUMBER		
				5e. TASK NUMBER		
				5f. WORK UNIT NUMBER		
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)					8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)					10. SPONSOR/MONITOR'S ACRONYM(S)	
					11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
12. DISTRIBUTION/AVAILABILITY STATEMENT						
13. SUPPLEMENTARY NOTES						
14. ABSTRACT						
15. SUBJECT TERMS						
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON	
a. REPORT	b. ABSTRACT	c. THIS PAGE			19b. TELEPHONE NUMBER (Include area code)	



425 River Road
Athens, GA 30605

**Quarterly Report
Human Neural Cell-Based Biosensor**

Date: October 31, 2012

Reporting Period: July 1, 2012–September 30, 2012

Prepared for:
Office of Naval Research (ONR)
Director, Naval Research Lab
Attn: Code 5596
4555 Overlook Avenue, SW
Washington, D.C. 20375-5320

Contract Number: N00014-11-C-0011, Amendment/Modification P00002

Submitted by:
Dr. Steven L. Stice, Principle Investigator
ArunA Biomedical, Inc.
425 River Road
Athens, GA 30602
Phone: 706-583-0071
Fax: 706-262-2821
Email: ssstice@arunabiomedical.com

Distribution Statement A:
Approved for public release; distribution is unlimited.

UNCLASSIFIED

Distribution of Quarterly Report

ADDRESSEE	DODAAC CODE	REPORT ENCLOSED	NUMBER OF COPIES	
			UNCLASSIFIED/ UNLIMITED	UNCLASSIFIED/ LIMIT ED AND CLASSIFIED
Program Officer: Dr. Laura Kienker ONR Code: 342 E-Mail: laura.kienker@navy.mil	N00014	Full technical report	1	1
Administrative Contracting Officer: Office of Naval Research ONR 0254: Russelle Dunson 875 North Randolph St. Arlington, VA 22203-1995 E-mail: russelle.dunson@navy.mil	S1103A	SF 298 only	1	1
Director, Naval Research Lab Attn: Code 5596 4555 Overlook Avenue, SW Washington, D.C. 20375-5320 E-mail: reports@library.nrl.navy.mil	N00173	Full technical report	1	1
Defense Technical Information Center 8725 John J. Kingman Road STE 0944 Ft. Belvoir, VA 22060-6218 E-mail: tr@dtic.mil	HJ4701	Full technical report	2	2

Summary

The purpose of this project is to develop in vitro, cell based biosensors for environmental toxins. By using ArunA's neural cell lines derived from both human embryonic stem cells (hESCs) and human induced pluripotent stem cells (hiPSCs), our goal is to provide a human neural cell based biosensor that is a more biologically relevant model of human physiology.

This report describes the progress made in these major areas: (1) directed differentiation of hESC- and hiPSC-derived neural progenitor cells into dopaminergic neurons; (2) directed differentiation of hESC- and hiPSC-derived neural progenitor cells into astrocytes; (3) cell culture medium development for the maintenance and differentiation of ArunA's neural cell lines as sensor elements for neurotoxicity; and (4) label-free, adhesive signature-based microfluidic cell separation.

We received an award letter from NIH on a \$700K grant funding the development of a label free microfluidic device for commercialization. This method is described below and the progress we have made in the present grant contributed to a successful grant application and potentially new and better ArunA product lines.

(1) Directed differentiation into dopaminergic neurons

In our previous studies we successfully differentiated hESC-derived neural progenitor cells into mature neuronal populations demonstrating positive protein expression of dopaminergic markers with reproducible results over multiple rounds of differentiation. Consequently, since our last Q2 report, we are in the process of developing a new dopaminergic progenitor cell line and dopaminergic differentiation kit for commercial release. We have completed multiple test rounds of different kit configurations, and we are currently optimizing a differentiation protocol for customer use. We have also completed preliminary HPLC studies evaluating our cultures for dopamine release with favorable results indicating dopamine biosynthesis and degradation. Work has begun to repeat our dopamine release studies, as well as to further characterize our differentiated cultures for more extensive dopaminergic marker expression via immunocytochemistry and qPCR. Preliminary work has also been conducted whereby dopaminergic neuron differentiation protocols were translated to hiPSC-derived neural progenitor cells which showed positive protein expression of dopaminergic markers.

(2) Directed differentiation into astrocytes

Since our last Q2 report, we finished initial studies on differentiating hESC-derived neural progenitor cells into astrocytes using different media and supplement combinations to improve yield and quality. From these studies we have determined the optimal conditions so far for astrocytic differentiation with our cells. Gene expression profiles (qPCR) were also repeated successfully with favorable results for increased glial specific gene expression. Also since last report, cryopreservation studies of hESC-derived astrocytic progenitor cells have been conducted with high post thaw recovery rates. In addition, post thaw astrocyte cultures were able to be successfully co-cultured with neurons. Functional network based electrophysiological studies of astrocyte/neuron co-culture systems remain in progress. Considering the successful progress made in studies with hESC-derived neural progenitor cells, we have started translating our astrocytic differentiation protocols to hiPSC-derived neural progenitor cells.

(3) Cell culture medium development

Previously, we developed a new basal medium to propagate both hESC- and hiPSC-derived neural progenitor cells and enhance their differentiation into different mature neural cell types. We have completed evaluating neural progenitor proliferation, mature neuronal differentiation and neural marker expression of cells cultured in this new medium. We are now in the process of characterizing and validating the new medium for astrocyte differentiation of both hESC- and hiPSC-derived neural progenitor cells and resulting glial marker expression.

(4) Label-free, adhesive signature-based microfluidic cell separation

We have begun collaboration with Georgia Tech to develop and establish the feasibility of using a novel microfluidic-based approach to efficiently isolate different cell populations based on their distinct 'adhesive signature' via controllable fluid forces. This label-free and non-enzymatic method is faster, simpler and higher throughput than current cell separation techniques. Preliminary data suggests that hESC- and hiPSC-derived neural cell types show distinct 'adhesive signatures' that are compatible for label-free microfluidic cell isolation and enrichment. If effective across multiple hiPSC lines (both healthy and diseased) and multiple derivations of neural cell types, this novel adhesion-based, label-free microfluidic system will shorten and streamline the scaled-up production of enriched populations of hiPSC-derived neural rosettes, neural progenitor cells and mature neuronal and glial cell types for direct use in cell-based assays.