REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

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1. REPORT DATE *(DD-MM-YYYY)*	2. REPORT TYPE	3. DATES COVERED *(From - To)*
09/10/2010	Quarterly Technical Report	06/11/2010 - 09/11/2010

4. TITLE AND SUBTITLE

Human Neural Cell-Based Biosensor

5a. CONTRACT NUMBER

N00014-10-C-0066

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

Stice, Steven L.
Chilton, Jamie
Powe, Allan

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

ArunA Biomedical, Inc.
425 River Road
Athens, GA 30602

8. PERFORMING ORGANIZATION REPORT NUMBER

TR-03112010.3

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)

Director, Naval Research Lab
Attn: Code 5596
4555 Overlook Avenue, SW
Washington, D.C. 20375-5320

10. SPONSOR/MONITOR'S ACRONYM(S)

DoD/ONR

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

ONR 5252.235-9714

12. DISTRIBUTION/AVAILABILITY STATEMENT

Approved for public release; distribution is unlimited.

13. SUPPLEMENTARY NOTES

14. ABSTRACT

Human neural progenitor cells have a strong potential for use as cell-based biosensors for environmental toxins. The overall goal of this project is to develop a human neural cell based biosensor using ArunA's neural progenitor cell line, hNP1™. In this report, we detail the following progress toward development of a human cell-based biosensor using these neural progenitor cells: (1) we continue to develop methods for enriching neural progenitor populations using VVA lectin and antibodies against CNTFRα, (2) we have begun to develop methods for directed differentiation of neural progenitors into motoneurons and astrocytes using defined medium conditions, and (3) we have begun development of fluorescence based assays for proliferation, mitochondrial function and reactive oxygen species generation.

15. SUBJECT TERMS

neurotoxicity, biosensor, neural cell

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			Steven L. Stice
Unclassified	Unclassified	Unclassified	UU	5	19b. TELEPHONE NUMBER *(Include area code)* 706-583-0071

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Standard Form 298 (Rev. 8/98)
Prescribed by ANSI Std. Z39.18

425 River Road
Athens, GA 30605

Quarterly Report
Human Neural Cell-Based Biosensor

Date: September 10, 2010

Reporting Period: June 11, 2010 – September 11, 2010

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: N00014-10-C-0066

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: sstice@arunabiomedical.com

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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

Stice Q3 2010 DoD Progress Report final

Summary

Human neural progenitor cells have a strong potential for use as cell-based biosensors for environmental toxins. The overall goal of this project is to develop a human neural cell based biosensor using ArunA's neural progenitor cell line, hNP1™. In this report, we detail the following progress toward development of a human cell-based biosensor using these neural progenitor cells: (1) we continue to develop methods for enriching neural progenitor populations using VVA lectin and antibodies against CNTFRα, (2) we have begun to develop methods for directed differentiation of neural progenitors into motoneurons and astrocytes using defined medium conditions, and (3) we have begun development of fluorescence based assays for proliferation, mitochondrial function and reactive oxygen species generation.

Enrichment of mature neural cells via cell sorting

Our previous flow cytometric data showed that carbohydrate moieties recognized by VVA lectin are up-regulated on the surfaces of neural progenitors, but not human mesenchymal stem cells. This suggested that VVA lectin could be used a tool for enrichment of human neural progenitor cells from a mixed population of cells. Recent experiments show that mixtures of neural progenitor and mesenchymal stem cells sorted with VVA lectin produced populations that were exclusively neural progenitors (SOX2+, Nestin+) and devoid of mesenchymal markers (CD166). These results indicate the neural progenitors can be specifically isolated using VVA lectin.

In addition, our preliminary proteomic screening and flow cytometry studies identified ciliary neurotrophic factor receptor alpha (CNTFRα) as a novel cell surface marker. We found that 73% of ArunA's hNP1^{TM} cells were CNTFRα positive by flow cytometry. To corroborate this result, we examined the subcellular localization of CNTFRα by immunofluorescence. As expected, nearly all hNP1™ cells were positive for CNTFRα. Moreover, we found that CNTFRα appears to localize primarily to plasma membrane and recycling endosomes; co-labeling studies are needed to confirm this conclusion. We will next determine if CNTFRα antibodies can be used to enrich for neural progenitors, as done with VVA lectin.

Use of defined media conditions to direct differentiation and increase synaptic formation

We have begun development of methods for directed differentiation of neural progenitors into motor neurons and astrocytes. Motor neurons are the target cell type for botulinum toxin and would be a useful cell type for the detection of this potential bioterrorism agent. Astrocytes, a type of glia, can be used to promote synapse formation and to support the long-term survival of neurons in a cell-based biosensor. Using previously published protocols, we show that our neural progenitors, cultured for 21 days under conditions for motoneuron differentiation, express neuronal markers β-III-tubulin and MAP2, indicating that these cells did indeed differentiate into neurons. Further experiments using motoneuron specific markers (e.g., HB9 and ChAT) are needed to assess the efficacy of differentiation. Our preliminary experiments with astrocytic differentiation suggest the epigenetic state of the neural progenitors can affect the ability to direct maturation into astrocytes. As a way to promote neural network formation and survival, we examined the effects of growth factors, steroids and estrogens on long-term cultures of differentiated neurons by measuring levels synaptic proteins as an indicator of synapse formation. Here, we found that inclusion of either brain-dervied neurotrophic factor (BNDF) and neurotrophin-3 or cholesterol and estradiol could increase levels of the synaptic proteins syntaxin, SNAP25, and synapsin over levels seen in neural progenitors differentiated with basal differentiation medium. These results suggest that inclusion of growth factors and steroids can promote synapse formation and long-term survival.

Use of fluorescence based assays as sensor elements to monitor neuronal health and viability

Currently, we are exploring the use of high-throughput fluorescence based assays as a means to monitor neural cell health, function and viability and to screen a broad variety of environmental toxins rapidly and efficiently. To that end, we have begun adaptation and optimization of the Alamar Blue assay for use with neural progenitors and differentiated neural cells; the Alamar Blue assay measures the activity of mitochondrial reductases, providing an indicator of both mitochondrial health and cell number. In

collaboration with Platypus Technologies, we have also begun development of a cell migration assay using neural progenitors. Migration of neural progenitors to their proper location in the central nervous system is a critical process for normal development and can be affected by environmental toxins. Early results suggest the migration assay can be used to identify chemical inhibitors and stimulators of migration.

In addition, we have begun development of a reactive oxygen species (ROS) assay using novel dye chemistries; in the presence of ROS, these novel dyes become highly fluorescent. Preliminary results indicate that certain growth factors reduce cellular levels of ROS in neural progenitors, as indicated by lower levels of dye fluorescence within cells. The results suggest that the growth factors confer a neuroprotective effect. Conversely, toxins known to elevate cellular levels of ROS increase the fluorescence of dye taken up by cells. All three assay methods show much promise as potential sensor elements.