# Drexel University

# Annual Progress Report: 2010 Formula Grant

### Reporting Period

July 1, 2013 – June 30, 2014

### Formula Grant Overview

The Drexel University received $1,275,294 in formula funds for the grant award period January 1, 2011 through June 30, 2014. Accomplishments for the reporting period are described below.

### Research Project 1: Project Title and Purpose

*Conformational Signatures of Neurotransmitter-Induced Gating and Desensitization of Nicotinic Ion Channels: A Collaborative Simulation and Experimental Approach* - This project launches a new collaboration between the PI (Abrams) and Mike White (DUCOM Biochemistry) dedicated to the study of nicotinic ligand-gated ion channels (LGIC's), the key proteins that mediate synaptic transmission in higher organisms. LGIC's are targets for drugs involved in treating behavioral problems, substance addiction (certain LGIC's are the chief targets of nicotine), muscle control disorders, and pain. Our efforts here to elucidate the links between the molecular structure of LGIC's and their function form the foundation of longer-term rational drug design which would have a major, sustained impact on human health.

### Duration of Project

1/1/2011 - 6/30/2013

### Summary of Research Completed

This project ended during a prior state fiscal year. For additional information, please refer to the Commonwealth Universal Research Enhancement C.U.R.E. Annual Reports on the Department's Tobacco Settlement/Act 77 web page at http://www.health.state.pa.us/cure.

### Research Project 2: Project Title and Purpose

*Ischemic Injury and Neuroprotection in Newborn Piglet Brain* - The objective of this research plan is to develop an in-depth understanding of the events that protect neurons from perinatal hypoxia-ischemia. At present, there are no therapies available to protect the infant brain from perinatal insults. One of the strategies of neuroprotection is neuronal hypoxic preconditioning (PC). PC provides a potential route for prophylactic intervention in patients in whom ischemic events are anticipated, such as those undergoing brain and heart surgery and those with transient ischemic attacks. We will elucidate a novel signaling pathway leading to neuroprotection by PC induced vascular endothelial growth factor (VEGF) and its receptor activation. These studies will

demonstrate a key role for VEGF in facilitating neuroprotective processes and will determine the downstream signaling intermediates that suppress cell death and promote survival during hypoxia-ischemia following PC.

**Duration of Project**

1/1/2011 - 6/30/2013

**Summary of Research Completed**

This project ended during a prior state fiscal year. For additional information, please refer to the Commonwealth Universal Research Enhancement C.U.R.E. Annual Reports on the Department's Tobacco Settlement/Act 77 web page at http://www.health.state.pa.us/cure.

**Research Project 3: Project Title and Purpose**

*Targeted Gene Delivery for Treatment of Cardiomyopathy in Muscular Dystrophy* - The purpose of the project is to develop methodologies to deliver genes into the heart muscle cells safely and effectively to treat cardiomyopathy. These studies will assess the feasibility, safety and efficacy of gene delivery to restore myocardial structure and function. We also anticipate investigating the mechanisms of action and optimization of strategies of these methodologies.

**Duration of Project**

1/1/2011 - 6/30/2014

**Project Overview**

In this project, we will pursue the hypothesis that gene therapy can restore myocardial structure and function. We will test this hypothesis by 4 specific aims:

1. To develop: 1) micro-bubbles; 2) green florescence protein (GFP) reporter system; and 3) DNA plasmid for *in vitro* and *in vivo* gene delivery experiments.
2. To optimize the efficiency of delivery of GFP reporter gene complex using cultured murine myocytes and micro-bubble targeted methodologies.
3. To pursue the efficacy and safety of modified gene therapy for *in vivo* delivery using mouse models and micro-bubble targeted methodologies.
4. A final set of experiments will investigate myocardial structure and function by these methodologies and strategies to treat cardiomyopathy in mouse models.

Our experimental designs are as follows:

1. Chemistry and in vitro experiments
DNA loading onto the micro-bubbles. Optimized DNA-loaded micro-bubble will be tested in vitro by contacting them with myocytes grown to 80% confluence in Opticell cassettes. After 24

hours, cells will be checked for GFP (fluorescent microscope). Optimized procedures will be finally tested with the gene complex.

2. *In vivo* animal studies
Loaded micro-bubbles will be injected into the tail veins of anesthetized mice at 9 months. The animals will be studied by histology, molecular and cell assays for mechanism of action, safety and efficacy. Gene delivery efficiencies will be assessed by measuring GFP expression by fluorescence studies. Longitudinal follow-up studies will also be conducted by microscopy before and after treatment as well as weekly x 4 weeks for structural and functional alterations.

**Principal Investigator**

Shuping Ge, MD
Associate Professor of Pediatrics
St Christopher's Hospital for Children
3601 A Street
Philadelphia, PA 19134

**Other Participating Researchers**

Margaret A. Wheatley, PhD – employed by Drexel University
Xiongwen Chen, PhD – employed by Temple University

**Expected Research Outcomes and Benefits**

These studies will provide the first preliminary data to assess the feasibility, safety and efficacy of gene delivery to restore myocardial structure and function. We also anticipate investigating the mechanisms of action and optimization of strategies of these methodologies. These data will provide the rationale to design in depth experiments to rigorously evaluate these methodologies in *in vitro* and *in vivo* experiments by securing external funding. The ultimate goal is that scientific discoveries and opportunities from these studies will be further validated and translated into clinical trials to search for a cure for this lethal disease.

**Summary of Research Completed**

*Progress for Aim 1:* Completed last reporting period.

*Progress for Aim 2; To optimize the efficiency of delivery of GFP reporter gene complex using cultured murine myocytes and micro-bubble targeted methodologies.*

In this study, basic fibroblast growth factor (bFGF or FGF-2) - loaded nanoparticles (NP)(bFGF-NP), composed of Poloxamer 188–grafted heparin copolymer, were prepared by water-in-water emulsion technique as shown in Figure 1. Phospholipid-based microbubbles (PMBs) were prepared to construct UTMD system. Final bFGF concentration in bFGF-containing solutions was 2 mg/mL.

bFGF used in the bFGF-NP and bFGF solution was labeled with fluorescein isothiocyanate. Six groups were used to investigate bFGF cellular intake in vitro: (1) Dilated cardiomyopathy (DCM) control: LV cardiomyocytes without any treatment; (2) bFGF alone: Only bFGF solution was added in the well plate without PMB and ultrasound treatment; (3) bFGF-NP alone: Only bFGF-NP solution was added in the well plate without PMB and ultrasound treatment; (4) UTMD alone: Only PMB was added in the well plate before ultrasound treatment; (5) bFGF + UTMD: bFGF and PMB mixture were added in the well plate before ultrasound treatment; (6) bFGF-NP + UTMD: bFGF-NP and PMB mixture were added in the well plate before ultrasound treatment.

*Fluorescent label of bFGF with isothiocyanate:* One mL of fluorescein isothiocyanate (FITC) solution (100 mg/ml in dimethylsulfoxide) was added slowly in 1 mL bFGF solution (10 mg/mL in bicarbonate buffer, pH=8.8, 0.1 M). The mixture was then incubated for 3 h at room temperature away from light with gentle stirring. Residual unconjugated FITC was eliminated from FITC-bFGF with a Sephadex G-25M column. The FITC-bFGF solution was then dialyzed against ultrapure water for salt elimination. Finally, the solution was lyophilized to obtain a powder of FITC-bFGF. With a sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS–PAGE) of FITC-bFGF, it was proved that the molecular weight of bFGF was still intact after the labeling procedure.

*bFGF cellular intake in vitro:* LV cardiomyocytes from 1- to 2-day-old Sprague-Dawley (SD) rats were cultured in well plates. After 48 h culture, the growth medium was removed and replaced with fresh RPMI 1640 media containing 10% FBS. PMB (3.4 μm in diameter, final volume concentration = 5%) and bFGF-containing solution (bFGF-NP or bFGF solution, final concentration =100 μg bFGF/ml) were also added in fresh growth media. UTMD was generated by a linear array transducer (14 MHz, Acuson Sequoia 512C system, Siemens). Cultured LV cardiomyocytes on the bottom of the well plates were exposed to ultrasound radiation. The ultrasound transducer was inserted into a 37 °C water tank and directly faced the bottom of the cell plate. A spongy rubber ultrasound shield was used to focus ultrasound on the experimented cells. MBD (microbubble destruction) function key attached to the machine was used to blast microbubbles (MI=1.9). After insonation, the well plates were placed in an incubator for 4 h. The media was then removed and replaced with fresh media containing 10% FBS and antibiotics. The cells were then imaged with a Nikon fluorescence microscope to evaluate the efficiency of the technique. The efficiency of bFGF cellular intake in vitro was the ratio of the cells expressing green fluorescence to total cells, which was defined by the following equation: (green fluorescent cells / total cells) × 100. In addition, MTT-based assay was performed to evaluate the percentage of cell viability.

*Flow cytometry:* Flow cytometry was used to detect the influence of FITC-bFGF-NP or UTMD on FITC-bFGF uptake in H9c2 cells, while untreated H9c2 cells were served as the blank control. Both the control and test groups were incubated at 37℃ in an atmosphere of 5% CO2 within a humidified incubator. H9c2 cells were cultured in 6-well plates overnight and then treated with UTMD, bFGF, bFGF-NP, bFGF+ UTMD and bFGF-NP+UTMD for 24h respectively. Live cells were gated by forward/side scattering from a total of 10,000 events. bFGF or bFGF-NP were detected in the fluorescence level (FL2) channel of the flow cytometer.

The cellular intake rate of bFGF by primary cardiomyocytes in vitro was shown by green fluorescence (Fig. 2A), which was quantitatively analyzed (Fig. 2D). Significantly increased signals in the cells treated with bFGF-NP were noted compared to bFGF alone group. However, use of UTMD significantly increased the cellular intake of bFGF in both the bFGF group and the bFGF-NP group.

Flow cytometry showed that the H9c2 cells treated with only UTMD produced no shifts (right-shift and up-shift) compared with control group (Fig.2B and 2C). Meanwhile, a slight shift was observed in the cell treated with bFGF alone. Significant shifts were detected in cell treated with bFGF-NP in contrast to bFGF alone group. The flow cytometry analysis also confirmed that the use of UTMD dramatically increased the uptake of the cells in both the bFGF group and the bFGF-NP group. The effect of bFGF and bFGF-NP with and without UTMD on cell viability were examined with MTT assay in NIH-3T3 cells (Fig. 2E). Compared to a control, there was no toxic effect on the cell viability in any treatment groups.

*Progress for Aim 3: To pursue the efficacy and safety of modified gene therapy for in vivo delivery using animal models and micro-bubble targeted methodologies.*

We used the diabetic cardiomyopathy (DCM) model to test the safety and efficacy of the UTMD approach using the bFGF-NP system developed and described above.

DCM was induced in SD rats by intraperitoneal injection of STZ at 70 mg/kg after 12 hours of fasting. STZ (Sigma Corporation, USA) was prepared as 1% STZ solution in 0.1 M citrate buffer (pH 4.0-4.5). On the 3rd day, 7th day, and 2nd week after final STZ administration, the fasting blood glucose was measured from the tail tip using an autoanalyzer (Surestep, Roche, Germany). Only the rats with a fasting blood glucose stabilizing in the next two weeks and exceeding 16.7 mM after STZ treatment were selected as diabetic rats. Age and sex matched nondiabetic control rats were injected with citrate buffer.

As shown in Fig. 3, the linear array transducer was applied over the area of the heart of DCM rats. Ultrasound images were observed before the filling of microbubble agent and bFGF containing solution (Fig. 3a). With the infilling of microbubble agent via the tail vein, ultrasound imaging of heart area gradually brightened (Fig. 3b). When the video imaging detected the presence of microbubble agent to the ventricle of the heart, the MBD was used to insonicate microbubbles (Fig. 3c).

*Echocardiography evaluation* (Table 1)
Before treatments, all echocardiographic indexes (Vs, Sc and SRc) in the DCM rats were significantly lower than those in the control. Four-week treatment with various forms of bFGF treatment changed all indexes in DCM rats compared to DCM rats without bFGF treatment. As shown in Table 1, only the indexes in bFGF-NP + UTMD group showed most improvement ($P<0.05$). Compared to negative control, although some improvements on echocardiographic indexes were also observed in the bFGF-NP alone group and the bFGF + UTMD group but their differences were not statistically significant ($P>0.05$).

*Hemodynamic evaluation*
As shown in Table 2, LVESP, LVEDP, and LV +dp/dt and -dp/dt were measured to evaluate hemodynamic characteristics. Compared with control rats, DCM rats showed lower values in LVESP, LV +dp/dt, and LV -dp/dt, but a higher value in LVEDP ($P<0.05$). Compared to DCM rats, DCM rats treated with bFGF alone did not exhibit any improvement on hemodynamic characteristics ($P>0.05$). Although hemodynamic characteristics in bFGF-NP + UTMD group, bFGF-NP alone group, and bFGF-NP + UTMD group all showed a trend close to the control rats, only bFGF-NP + UTMD group displayed a significant difference from DCM rats without treatment and were closest to the control rats. This finding is coincident with the above results of bFGF cellular intake in vitro.

*Progress for Aim 4. To investigate myocardial structure and function by these methodologies and strategies to treat cardiomyopathy in mouse models.*

*Myocardial collagen volume fraction and capillary density*
Considering that cardiac dysfunction induced by diabetes is predominantly attributed to cardiac remodeling, we examined myocardial collagen volume fraction (MCVF). Masson staining and CD31 immunohistochemical staining were used to measure MCVF and capillary density respectively. MCVF was significantly increased in the heart of DCM rats compared to control rats. Although bFGF treatment slightly reduced MCVF ($p>0.05$), treatment with bFGF-NP/UTMD significantly reduced MCVF compared to DM group (data not shown).

Since one of the major functions of bFGF is its angiogenesis, we examined MCD for angiogenesis index. Quantitative analysis showed that compared with control rats, hearts from DCM rats showed low values of MCD ($P<0.05$), which were not improved by treatment with bFGF alone. In contrast, treatment with bFGF-NP/UTMD showed a significant improvement in MCD compared to DM group (data not shown).

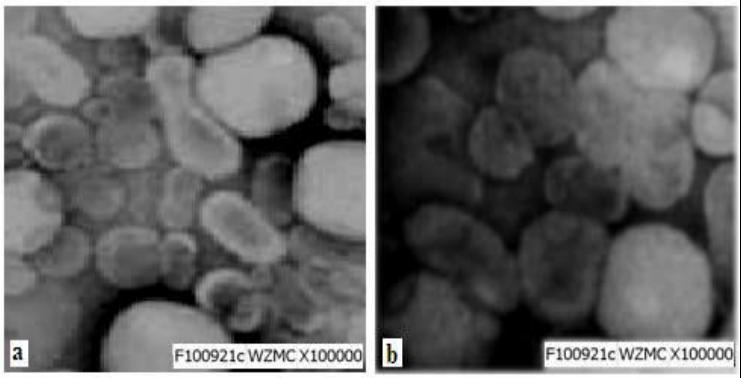
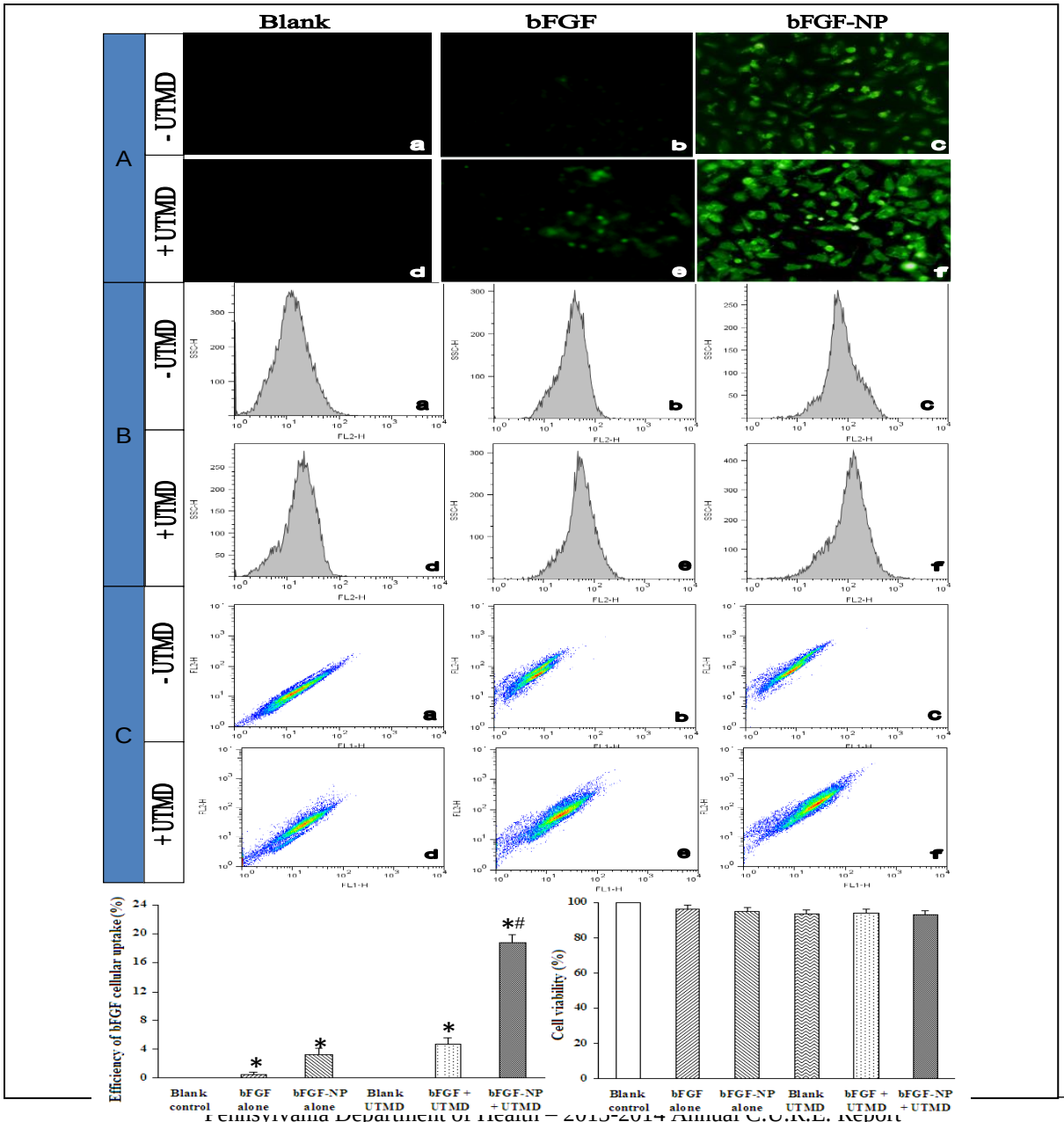



Figure 1. Representative TEM micrographs of the NP and bFGF-NP. Dynamic light scattering results demonstrated that the average particle size of blank NP and bFGF-NP were 106±1.84 nm and 128±1.65 nm, respectively. Polydispersibility index (PI) represents the distribution of particle size. Low PI values (<0.3) were observed in both blank NP and bFGF-NP, which indicated that blank NP and bFGF-NP approached a monodisperse stable system.

Figure 2 ***bFGF cellular intake***



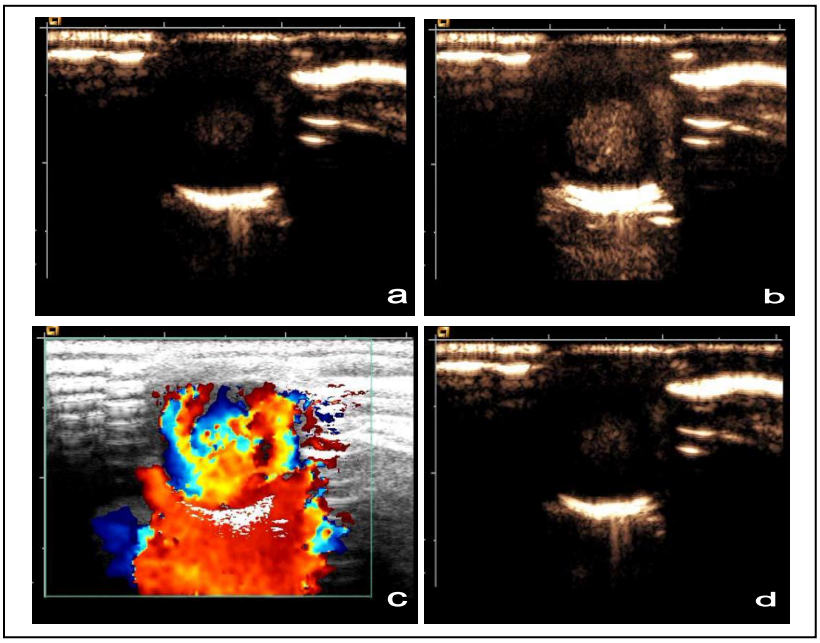



Figure 3. UTMD technique with bFGF-NP in vivo. Linear array transducer was applied over the area of the heart of DCM rats. Ultrasound images were observed before the filling of microbubble agent and bFGF containing solution (a); with the infilling of microbubble agent via the tail vein, (b) presence of microbubble agent to the ventricle of the heart, the MBD was used to insonicate microbubbles (c).

**Table 1. Results of velocity, strain and strain rate in experiment in vivo (mean+/-SD, n=10**

| ***Vs(cm/s)*** | | ***Sc(%)*** | | ***SRc(1/s)*** | |
|---|---|---|---|---|---|
| Before intervention | After intervention | Before intervention | After intervention | Before intervention | After intervention |
| 0.719±0.093 | 0.721±0.068 | -13.961±2.264 | -14.369±2.047 | -3.773±0.293 | -3.848±0.309 |
| 0.564±0.076 | 0.406±0.078 | -11.736±0.727 | -10.033±1.020 | -2.886±0.358 | -2.060±0.295 |
| 0.557±0.087 | 0.412±0.089 | -11.695±1.554 | -10.054±0.978 | -2.862±0.340 | -2.045±0.263 |
| 0.562±0.080 | 0.478±0.067 | -11.674±1.467 | -12.083±1.143 | -2.876±0.386 | -2.402±0.343 |
| 0.561±0.092 | 0.432±0.078 | -11.687±1.565 | -10.043±0.998 | -2.798±0.335 | -2.144±0.289 |
| 0.554±0.067 | 0.503±0.088 | -11.732±1.341 | -12.244±1.131 | -2.844±0.289 | -2.527±0.356 |
| 0.568±0.076 | 0.652±0.075 * | -11.804±1.475 | -13.265±1.122 * | -2.825±0.363 | -3.443±0.354 * |

Note: Vs= peak systolic velocity; Sc= peak circumferential strain; SRc= peak circumferential strain rate. Compared with DCM control group in the same stage, *$P<0.05$.

**Table 2. The hemodynamic data *in vivo* (mean± SD, n=10)**

| *Group* | ***LVESP(mmHg)*** | ***LVEDP(mmHg)*** | *LV* ***+dp/dt$_{max}$(mmHg)*** | *LV* ***-dp/dt$_{max}$(mmHg)*** |
|---|---|---|---|---|
| Normal control | 97.568±6.222 | 2.824±0.430 | 4717.011±204.734 | 4059.807±259.757 |
| DCM control | 69.546±6.317 | 3.797±0.354 | 3247.850±230.544 | 2752.232±160.066 |
| bFGF alone | 70.653±6.537 | 3.923±0.346 | 3252.435±262.655 | 2743.808±248.887 |
| bFGF-NP alone | 76.032±4.768 | 3.575±0.325 | 3542.643±234.342 | 2956.464±324.638 |
| Blank UTMD | 70.272±6.161 | 3.763±0.226 | 3332.173±217.235 | 2843.808±248.887 |
| bFGF + UTMD | 77.104±5.104 | 3.513±0.363 | 3547.347±224.624 | 3032.643±334.767 |
| bFGF-NP + UTMD | 83.424±5.243 * | 3.102±0.244 * | 4345.344±276.328 * | 3624.537±296.546 * |

Note: LVESP=left ventricular systolic pressure; LVEDP=left ventricular end diastolic pressure; ±dp/dt$_{max}$ - maximum rate of the rise and fall of left ventricular pressure, *$P<0.05$.

**Research Project 4: Project Title and Purpose**

*Improving the Epidemiology of Alcohol-Related Violence and Morbidity in the City of Philadelphia* - The goal of this project is to use geographic information systems (GIS), spatial analysis and advanced statistical techniques to improve epidemiological studies of alcohol-related violence and injuries in the city of Philadelphia.

**Duration of Project**

1/1/2011 - 6/30/2013

**Summary of Research Completed**

This project ended during a prior state fiscal year. For additional information, please refer to the Commonwealth Universal Research Enhancement C.U.R.E. Annual Reports on the Department's Tobacco Settlement/Act 77 web page at http://www.health.state.pa.us/cure.

**Research Project 5: Project Title and Purpose**

*Identification of Genetic Modifiers in a Transgenic Model of Amyotrophic Lateral Sclerosis (ALS)* - Amyotrophic Lateral Sclerosis is a neurodegenerative disease leading to death in 2-5 years. Ten percent of all cases are familial; of these, 20% are caused by a mutation in the SOD1 gene. Transgenic (Tg) mice possessing the human G93A SOD1 gene also develop ALS. However, mice of the C57Bl6 strain carrying the Tg live significantly longer than do SJL mice. We have identified a Quantitative Trait Locus on the mouse Chromosome (Chr) 17 that is strongly linked to this difference in survival. The overall goal of this project is to identify genes within Chr 17 that influence longevity in this ALS model. Identification of the responsible gene(s) will highlight cellular pathways involved in motor neuron degeneration and provide new targets for the development of therapeutics to slow or stop the progression of ALS.

**Duration of Project**

1/1/2011 - 6/30/2012

**Summary of Research Completed**

This project ended during a prior state fiscal year. For additional information, please refer to the Commonwealth Universal Research Enhancement C.U.R.E. Annual Reports on the Department's Tobacco Settlement/Act 77 web page at http://www.health.state.pa.us/cure.

**Research Project 6: Project Title and Purpose**

*Collaborative Analysis of Nuclear Pores: Protein Trafficking and Recognition* - This project will increase our understanding of the mechanisms by which the nuclear pore complex (NPC) operates. The NPC is an elaborate cellular machine that controls traffic of macromolecules between the nucleus and cytoplasm of living cells, and as such is critical for normal cellular

functions. The NPC plays key roles in the delivery of viral DNA and gene therapy reagents, and its functions are thought to be hijacked or reprogrammed in disease states such as cancer; hence, it is a prime drug target. In addition to producing fundamental biological information about NPC functioning, this project will also produce new tools that will allow for rapid screening of potential drugs that can modulate NPC activity.

**Duration of Project**

1/1/2011 - 6/30/2012

**Summary of Research Completed**

This project ended during a prior state fiscal year. For additional information, please refer to the Commonwealth Universal Research Enhancement C.U.R.E. Annual Reports on the Department's Tobacco Settlement/Act 77 web page at http://www.health.state.pa.us/cure.

**Research Project 7: Project Title and Purpose**

*Can Up-regulation of Glutamate Transporters Be Protective in Traumatic Brain Injury?* - The purpose of this project is to investigate if a glutamate transport activator, Parawixin1, has protective effects on the pathology of traumatic brain injury (TBI). This hypothesis is suitable since TBI increases extracellular levels of glutamate resulting in injured tissue, membrane depolarization and calcium influx that activates phospholipases, endonucleases and proteases that can lead to apoptosis. Rats will be subjected to moderate TBI and glutamate transport with radioactive assays will be performed in synaptosome preparations of the brains of rats injected with Parawixin1 prior and after the injury. In addition, analyses of edema, tissue loss, activation of calpain and loss of neuronal MAP-2 (markers of neurodegeneration and apoptosis) will be done. This knowledge could provide new therapeutical strategies for amelioration of the secondary outcomes of TBI.

**Duration of Project**

1/1/2011 - 6/30/2013

**Summary of Research Completed**

This project ended during a prior state fiscal year. For additional information, please refer to the Commonwealth Universal Research Enhancement C.U.R.E. Annual Reports on the Department's Tobacco Settlement/Act 77 web page at http://www.health.state.pa.us/cure.

**Research Project 8: Project Title and Purpose**

*Mechanisms of Carbon Monoxide Mediated Hypercoagulability* - Exposure to tobacco smoke has been associated with a variety of chronic and acute diseases, one of which is thrombotic disease (e.g., acute coronary syndrome, stroke). Among the many constituents of smoke, carbon monoxide (CO) has long been recognized as an important poisonous component. Critically, it

has been recently recognized that exposure of human plasma to CO concentrations well within the range encountered during smoking results in enhanced coagulation and diminished fibrinolysis *in vitro*. The purpose of this project to further define the molecular mechanisms by which this occurs, specifically focusing on the heme-mediated modulation of fibrinogen and $\alpha_2$-antiplasmin function by CO. It is anticipated that these insights will significantly impact on future diagnostic and prognostic management of patients exposed to CO.

**Duration of Project**

1/1/2011 - 6/30/2012

**Summary of Research Completed**

This project ended during a prior state fiscal year. For additional information, please refer to the Commonwealth Universal Research Enhancement C.U.R.E. Annual Reports on the Department's Tobacco Settlement/Act 77 web page at http://www.health.state.pa.us/cure.

**Research Project 9: Project Title and Purpose**

*Role of Cytoskeletal Dynamics in Radiation-Induced Breast Cancer Invasion* - The goal of the proposed research is to investigate the role of cytoskeletal-associated pathways in regulating radiation-induced invasion in ErbB2-positive breast cancers. We hypothesize that changes in cytoskeletal pathways that regulate neuronal branching may play a significant role in breast cancer cell invasion and invadopodia formation, and may offer novel therapeutic targets for treating invasive breast cancer including radiation-induced progression of pre-malignant state to invasive breast cancer. I propose to unveil new aspects of cancer invasion and to test relevant potential therapeutic targets for blocking the invasion of breast cancer cells using 3D cell culture systems developing in my laboratory.

**Duration of Project**

1/1/2011 - 6/30/2012

**Summary of Research Completed**

This project ended during a prior state fiscal year. For additional information, please refer to the Commonwealth Universal Research Enhancement C.U.R.E. Annual Reports on the Department's Tobacco Settlement/Act 77 web page at http://www.health.state.pa.us/cure.

**Research Project 10: Project Title and Purpose**

*Using Biowalls to Sustainably Reduce Human Exposure to Indoor Volatile Organic Compounds* Exposure to Volatile Organic Compounds (VOCs) has been associated with health effects that include cancer, as well as respiratory, immunological, neurological, and renal effects. This study centers on the future Drexel Biowall, an indoor, vertical wall of plants designed to remove VOCs from the indoor air by using the bio-degrading capacity of microbes that live around the plant

roots. The purpose of this work is to measure the VOC removal kinetics of microbial communities on plant roots for a common suite of eight VOCs at typical indoor concentrations while simultaneously characterizing the diversity, species, and numbers of bacteria in those root communities with both culture-independent and culture-dependent techniques. This work will allow us to identify efficient VOC degraders and clear a path for research bent on engineering effective degraders.

**Duration of Project**

1/1/2011 - 12/31/2013

**Project Overview**

The broad research objective is to understand whether VOC removal by plant roots is a function of the diversity, species, and/or numbers of bacteria in those root communities. Aims 1 and 2 relate to developing the testing apparatus and measurement techniques required to fulfill this broad research objective:

(1) Construct the chamber system used to measure VOC removal by plant roots; and
(2) Develop culture-independent and culture-dependent methods to characterize diversity, species, and numbers of VOC degrading bacteria in plant root communities.

Once the apparatus and methods to characterize bacteria are developed, we will carry out Aims 3 and 4:

(3) Measure the VOC removal kinetics by microbial communities on roots of 12 plants for a common suite of eight VOCs at indoor concentrations typical of a new academic building over 22 weeks; and
(4) Periodically use culture-independent *and* culture-dependent techniques to characterize the diversity, species, and numbers of bacteria in those root communities over the 22 weeks of testing.

The chamber system will consist of individual growth vessels for each plant, which are grown aeroponically. Into each growth vessel, air containing particular concentrations of VOCs will be steadily delivered, and the removal for each VOC by each root system will be measured with a gas chromatograph/photo-ionization detector. On the same day as the VOC removal testing, we will characterize bacteria on those roots with: (a) Terminal Restriction Fragment Length Polymorphism (T-RFLP) to study changes in the diversity and composition of microbial communities among plant roots exposed to the VOCs; (b) High throughput 454 sequencing of bacterial 16S rRNA (targeting the V1-V3 regions) to identify the species found in the roots of plants that degrade VOCs; and (c) Real-time, quantitative PCR (qPCR) to estimate the numbers of bacteria on plant roots. To determine whether VOC degrading bacteria will increase in abundance after prolonged exposure to the VOC mixture (which should consequently increase the VOC removal rates over the 22 weeks of testing), we will compare the identity of bacteria that proliferate on roots after VOC exposure with the identity of bacteria cultivated from those plant roots and characterized *in vitro* with respect to their degradation capacities. That is, we will compare root bacteria that increase in abundance to those cultivated on media consisting of the VOC as the sole carbon source (e.g., benzoate as a mimic for benzene).

**Principal Investigator**

Michael S. Waring, PhD
Assistant Professor
Drexel University
Dept. of Civil, Architectural and Environmental Eng.
3141 Chestnut St. AEL 273-B
Philadelphia, PA 19104

**Participating Researchers**

Jacob A. Russell, PhD, Shivanthi Anandan, PhD - employed by Drexel University

**Expected Research Outcomes and Benefits**

Major outcomes of this work include: (1) Development of methods to be used to characterize VOC removal by roots of plants and to characterize bacterial diversity, species composition, and number on plant roots. (2) A database of values of VOC removal kinetics as a function of bacterial diversity, species composition, and number of each type of species, for each VOC/plant combination. This database will be used to identify *associations* between the VOC removal and bacterial diversity, species composition, and number. Values in this database and these functional associations can be used in models we will develop in future work that predict VOC removal by Biowalls, which we can validate in the future Papadakis ISB (the building that will house the Drexel University Biowall). Once validated, these results can be used in VOC exposure models and risk analyses that elucidate the benefits of using Biowalls in institutional, academic, and corporate buildings, etc. Furthermore, it will be an important finding if bacterial communities are initially different on the plant roots but tend towards similar communities over time, which would imply that the types of plants present on the Biowall are much less important than the communities they harbor. Finally, identifying efficient VOC degraders will clear a path for research that attempts to engineer effective VOC-degrading bacteria for selective inoculation onto Biowalls; this inoculation will allow us to engineer Biowalls tailored to remove VOCs in particular buildings that have their own unique concentrations of health-degrading pollutants.

**Summary of Research Completed**

<u>Rationale</u>
This work falls under our broad research Aim 3, which was measuring VOC removal by microbial communities on plant roots.

<u>Methods</u>
To determine removal efficiencies, one must sample upstream and downstream of the removal device simultaneously. Sampling upstream simply means that the air in front of the wall has its concentration measured. Sampling downstream requires the use of a pre-construction-installed sampling port that accesses the ducting behind the Biowall. Then, to calculate the removal efficiency of any compound $i$, $\eta_i$ (-), one uses:

$$\eta_i = \frac{C_{\text{up},i} - C_{\text{down},i}}{C_{\text{up},i}} = \left(1 - \frac{C_{\text{down},i}}{C_{\text{up},i}}\right) \quad (1)$$

where $C_{\text{up},i}$ or $C_{\text{down},i}$ (ppb or μg/m$^3$) are the upstream or downstream concentrations of any compound *i*, respectively.

*For the VOC sampling*, we pulled air from upstream and downstream locations with vacuum pumps through sampling lines with flow rates regulated by mass flow controllers. The VOC sampling was performed by using SUMMA canisters; these are electropolished, passivated stainless steel vacuum sampling devices that start out evacuated and then are used to collect whole-air samples over a pre-determined time period, which are then used for laboratory analysis. In our case, we collected twelve 2-hr samples over four different sampling times. SUMMA canister analytical testing was provided by a third-party laboratory, which applied the EPA TO-15 method and determined the concentrations of 73 pre-selected compounds. Detection limits were 0.2 ppb.

Results and Discussion

*VOCs*. Of the twelve VOC samples attempted, one SUMMA canister malfunctioned, so eleven were taken in total. Fifteen of the tested for 73 VOCs were detected at least once during the sampling times, reflecting that most VOCs are present indoors at concentrations lower the test detection limits. Only five VOCs were detected during all eleven tests, which were ethanol, isopropyl alcohol, acetone, chloromethane, and n-butane. Table 1 lists the VOC concentrations measured during the eleven sampling times for upstream and downstream sampling points, along with the date the test was taken and the time at which it commenced.

We can only draw conclusions about VOC removal efficiencies for those that were detected repeatedly in both upstream and downstream sample. According to Table 1, the Biowall consistently reduced ethanol and acetone across samples, and to a lesser extent chloromethane. For these VOCs, the average removal efficiencies according to Equation 1 were 0.59, 0.63, and 0.070. Standard deviations are not listed for these removal efficiencies because there were only three sample points. Other VOCs, such as isopropyl alcohol, methylene chloride, and n-butane were little affected by the Biowall in this dataset. A few VOCs were detected in one downstream sample only (3Db), but we cannot draw any conclusions as to their origin with a sample size of n = 1. Finally, there was one unknown VOC in this report, which consistently appeared downstream of the Biowall, and may be generated by a biological wall process. More detailed VOC research than that allowed by the SUMMA canister measurements is clearly warranted.

Table 1. Upstream (U) and downstream (D) VOCs measured during the four sampling times. For the column IDs, the number is the sample time, the large letter signifies up or downstream, and the small letter is the replicate (i.e., 2Db is the second sample time, downstream, replicate canister for that sample).

| | 6/13 – 11:25PM | | | 6/13 – 5:04PM | | | 6/17 – 7:00PM | | 6/18 – 6:32PM | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Compound** | **1Ua** | **1Da** | **1Db** | **2Ua** | **2Ua** | **2Da** | **3Da** | **3Db** | **4Ua** | **4Ua** | **4Da** |
| Ethanol | 40 | 5.1 | 4.6 | 24 | 32 | 9.5 | 7.7 | 9.9 | 16 | 15 | 12 |
| Isopropyl alcohol | 6.1 | 3.8 | 3.5 | 4.3 | 5.6 | 5.1 | 6 | 7.5 | 5.2 | 5.3 | 10 |
| Acetaldehyde | | | | | | 1.3 | | | | | |
| Acetone | 6.6 | 1.3 | 1.4 | 8.3 | 8.3 | 3.1 | 1.8 | 2.1 | 5.8 | 5.2 | 2.9 |
| 2-Butanone | | | | | | 0.88 | | | | | 0.71 |
| Chloromethane | 0.62 | 0.61 | 0.52 | 3.1 | 3.4 | 3.2 | 0.53 | 0.56 | 0.64 | 0.65 | 0.6 |
| Methylene chloride | | | | 0.72 | 0.72 | 0.78 | | 0.65 | | | |
| Tetrachloromethane | 0.85 | | | | | | | | | | |
| n-Butane | 1.9 | 1.8 | 1.7 | 0.58 | 0.61 | 0.65 | 0.72 | 0.71 | 0.5 | 0.54 | |
| Ethane1,1-difluoro | | | | | | 1.9 | 3.7 | 2.2 | | | |
| 1,3-Butadiene | | | | | | | | 0.45 | | | |
| Acetonitrile | | | | | | | | 0.39 | | | |
| Freon 11 | | | | | | | | 0.22 | | | |
| Freon 12 | | | | | | | | 0.27 | | | |
| Unknown | | 1 | 1.3 | | | 1.9 | 1.6 | 1.9 | | | |