



NATIONAL CENTER FOR
REGENERATIVE MEDICINE

ENGAGE 2012

National CENter for ReGenerAtive Medicine UnderGraduate StudEnt Summer Program

Funding Application for Summer 2012

Deadline: March 30, 2012

Overview

The National Center for Regenerative Medicine (NCRM) is a multi-institutional center composed of investigators from Case Western Reserve University, University Hospitals Case Medical Center, the Cleveland Clinic, Atherys, Inc., and The Ohio State University. Building on the 30 year history of adult stem cell research in northeast Ohio, the Center was created in 2003 with a \$19.4 million award from the State of Ohio as a Wright Center of Innovation. An additional \$8 million award in 2006 from the State of Ohio's Biomedical Research and Commercialization Program--and, in June 2009, a \$5 million award from Ohio's Third Frontier Commission--further validated the Center's ability to achieve its mission to utilize human stem cell and tissue engineering technologies to treat human disease. For more information please visit <http://www.thestemcellcenter.org>.

This is the fifth year this program is being offered and the purpose of ENGAGE for Summer 2012 is to promote and support undergraduate students' participation in research and creative projects within the field of stem cells and regenerative medicine. Proposed projects will be expected to match the complexity for what is accepted by SOURCE and SPUR funding.

NCRM brings together established investigators from five institutions with proven expertise in stem cell research and clinical studies. Due to the impressive breadth of investigator expertise, the scope of stem cell types under study within the Center is comprised of nine adult cell types:

CSC	Cochlear Stem Cells
CTP	Connective Tissue Progenitor Cells
HSC	Hematopoietic Stem Cells
HB1	Hemangioblast (AC133) derived from umbilical cord blood
MAPC	Multi-potent Adult Progenitor Cells
MSC	Mesenchymal Stem Cells
NSC	Neural Stem Cells/oligodendrocyte progenitors
SKMB	Skeletal Myoblasts
UCB	Umbilical Cord Blood Derived Stem Cells
ESC	Embryonic Stem Cells
iPS	Induced Pluripotent Stem Cells

The adult stem cells are being investigated for therapeutic uses in musculoskeletal, cardiovascular, hematopoietic and neurological disorders as outlined below.

Orthopedic and Musculoskeletal	CTP, MSC, SKMB	Bone and cartilage repair, MSC homing factors, myoblast homing using surface molecule “painting” - - for fractures, joint disease, muscular dystrophy.
Cardiovascular and vascular	HB1, HSC, MAPC, SKMB, UCB	Myocardial regeneration with cell homing, differentiation and regeneration, vascular remodeling -- for ischemic, congestive and dilated cardiomyopathy.
Hematopoietic and cancer	HB1, HSC, MSC, UCB, MAPC	UCB stem cell expansion and transplantation, cytokine directed differentiation for GVL (graft vs. leukemia), marrow reconstitution and stem cell selection, HSC gene transfer, MSC modulation of GVHD (graft vs. host disease), -- for curing cancer, correction of genetic and immune diseases and protection from chemotherapy.
Neurodegenerative and neurological	MAPC, NSC, iPS, ESC	Cell implantation into brain and spinal cord for neuronal or glial replacement therapy in Neurodegenerative disorders – Huntington’s disease, multiple sclerosis, amyotrophic lateral sclerosis and spinal cord injury.

Applicants should review the NCRM directory below to determine potential projects and mentors. **Applicants will need to choose a therapeutic area in Biology and Immunology, Cardiovascular, Dermatology, Imaging, Musculoskeletal, Neurological, Oncology, Reproductive or Sensory** and, if a mentor has not already accepted the student, the ENGAGE Administrator, Michael Gilkey, will assign them to a principal investigator in that area. If there is an investigator the applicant especially desires to work with please mention them on the form. As applicants are assembling their project description and objectives, please feel free to use http://www.thestemcellcenter.org/NCRM_bios/bios_main.html to investigate the NCRM membership and contact our investigators directly. Abstracts of previously funded projects by SOURCE or SPUR with participation by some of our members are listed at the end of this document.

Eligibility

All undergraduate students in Ohio currently enrolled in an accredited college Fall 2012 are eligible to apply for ENGAGE summer funding. Award recipients must be registered for fall classes prior to beginning the summer project. Projects must be completed at CWRU, Cleveland Clinic, University Hospitals Case Medical Center or Athersys. To this extent, students may apply for funding to support research and creative projects, including stipend and materials. Students who are awarded full research stipends (\$3,000) cannot enroll in more than one summer class. While students may apply for funding for different projects, students may not accept funding for 2 different projects (that is, for example, students may not be part of the SPUR program and receive ENGAGE funding for a separate project). If you are accepted into more than one project program, you must choose in which project you want to participate. Material costs of up to \$500 will be covered by ENGAGE and funds will go directly to the mentor assigned to the student.

Award Description

The NCRM office recognizes and is grateful to the following for Summer 2012 funding:

- *MSC 2011: Regenerative Medicine and Adult Stem Cell Therapy Conference*

Application Procedure

A completed application packet consists of the following:

- 1) The ENGAGE summer funding application
- 2) Official Academic Transcript (can be unofficial)
- 3) A brief (approximately 2-3 pages) proposal that addresses the following:
 - Project Title
 - Goals and Objectives
 - Project Description (please review the abstracts at the end of this document)
 - Discussion of estimated time commitment required for the project
 - Discussion of how this project is part of the student's overall educational plan and goals
- 4) Resume

Also, see the SOURCE website for SOURCE Summer Funding Hints

(<http://www.case.edu/provost/source/opp/funding.htm>)

Deadline

The deadline for submitting a completed Application Packet, which includes application form, transcript, proposal and resume is **March 30, 2012**.

Selection Criteria

Applications will be reviewed to confirm that the applicant meets the eligibility criteria. The selection committee will evaluate application files and identify recipients based on the strength and educational value of the proposal, the do-ability of the project, and ability to match with an appropriate mentor. Depending on funding, up to 10 students will be accepted into the ENGAGE 2012 program.

Please send completed form to Michael.gilkey@case.edu or campus mail to Michael Gilkey, Wolstein Research Building 2-501, no later than March 30, 2012.

Questions regarding this program can be directed to Michael Gilkey via email or phone, 216-368-2079.

Projects Previously Funded by ENGAGE

Project Title: *Maintaining embryonic stem cell self-renewal and pluripotency by inhibiting Shp-2 phosphatase function*

Goals and Objectives

- To determine if Shp-2 inhibition and subsequent reactivation allows embryonic stem cells to remain in state of self-renewal and prevent differentiation, until Shp-2 phosphatase function is restored
- To evaluate the efficacy of various pharmacological candidates in inhibiting Shp-2 phosphatase function in murine embryonic stem cells.

Project Description

Over the past decade, innovations in embryonic stem (ES) cell research have progressed rapidly. A multi-disciplinary collaboration of scientists has shed significant light on mechanisms of development and cellular differentiation. Therapeutic uses for embryonic stem cells are under development and are likely to provide treatments to patients who were otherwise untreatable.

Unfortunately, there are obstacles impeding the progress of ES cell research. The pathways of ES cell differentiation and self-renewal are not thoroughly understood. Consequently, scientists must surmount the problems presented by the tendency of ES cells to invariably differentiate on their own accord. It has been demonstrated that the enzyme Src Homology-2(SH2) domain-containing protein tyrosine phosphatase (Shp-2) plays an important role in the fate of an ES cell.

In normal stem cell development, the cytokine leukemia inhibitory factor, LIF, binds with a dimeric receptor protein, gp130, activating its JAK tyrosine kinase which phosphorylates STAT3. STAT3 dimerizes and activates transcription of its target DNA. The LIF transduction pathway favors ESCs remaining in a self-renewing state and suppresses differentiation. This process is negatively regulated by the tyrosine phosphatase Shp-2. This enzyme's regulatory role in LIF signal transduction makes it a fitting target of experimentation

Inhibition of Shp-2 phosphatase may allow a means by which to maintain ES cell self-renewal. Since Shp-2 is a negative regulator of LIF signal transduction, it correlates, and has been demonstrated that Shp-2 is a positive effector of ES cell development (Qu and Feng 1998). When the src homology2 (SH2) domain of Shp2 is disrupted, the enzyme loses its phosphatase function. Drs. Qu and Feng observed differentiation of normal ES cells and ES cells homozygous for the loss-of-function mutation, and found that the Shp2 knockout ESCs do indeed favor self-renewal and are resistant to differentiation.

To further understand LIF-stimulated self-renewal, Dr. Rebecca J. Chan et al. have examined the genes stimulated by this transduction pathway. Using Shp2 knockout ES cells, DNA microarray analysis revealed a 2.1-fold upregulation of the the gene *KLF4*, which expresses Krüppel-like factor (Klf-4), when compared to embryonic stem cells with normal Shp-2 function. The Klf-4 protein is involved in regulating histone acetylation to facilitate transcription. To further understand the impact of Klf-4 upregulation, *KLF4* cDNA was retrovirally transduced into Shp2 knockout ES cells. It was found that ES cells with elevated Klf-4 levels favored self-renewal and were more resistant to differentiation than cells without exogenous *KLF4* cDNA (Chan 2005). This evidence implicates that Klf-4 expressed in wake of Shp-2 disruption is responsible for ES cells favoring self-renewal over differentiation.

The importance of Klf-4's role in ES self-renewal is underscored by a recent innovation in which previously differentiated somatic cells were reprogrammed to a pluripotent state. A research group collected human dermal fibroblasts (HDF) and retrovirally transduced into them the cDNA for the transcription factors

Oct3/4, Sox2, c-Myc, and Klf-4. Several weeks later, the HDFs had been reprogrammed into cells which were “similar to embryonic stem cells in morphology, proliferation, surface antigens, gene expression, epigenetic status of pluripotent cell-specific genes, and telomerase activity” (Takahashi 2005). This cell type is called the *inducible pluripotent stem cell* (iPSC). Again, Klf-4 is connected to pluripotency and in this case, it is connected to virtually reversing the differentiation of HDFs.

Dr. Cheng-Kui Qu’s lab has produced multiple selective inhibitors of Shp-2 phosphatase. I will evaluate their inhibitory function of differentiation, and preservation pluripotent ES cell lines.

Experimental Design

Analysis of ES cell self-renewal levels will be conducted in two ways: first by colony count in a one-step differentiation assay in liquid medium, and secondly by a two-step embryoid body (EB) assay in semisolid medium. Murine ES (mES) cells will be grown in standard ES cell medium, Dulbecco’s Modified Eagle’s Medium (DMEM), as well as 15% fetal cow serum (FCS), amino acid supplementation, and leukemia inhibitory factor (LIF) at a concentration of 60 units LIF/ml.

One-step differentiation assay: mES cells will be plated in liquid media at a density of 2×10^3 cells/ml. LIF will not be present. If it is found that completely removing LIF from the mES cell medium results in extremely low ES colony counts, then the experiment will be conducted using a LIF concentration of 4 units/ml. The control will contain DMSO, and the experimental medium will contain 200 units/mL of Shp-2 inhibitor. After five days, I will perform a count of ES cell colonies. Colony morphology will reveal which cells have differentiated or self-renewed.

Two-step embryoid body (EB) assay: mES cells will be plated in semisolid methylcellulose media at a density of 2×10^4 cells/m for both control and experimental media, both of which will not contain LIF. After ten days, I will perform a count of primary EBs. If at this point, EB count is extremely low, the experiment will be repeated with a LIF concentration of 4 units /ml. I will then harvest EBs and replat them in fresh semisolid methylcellulose media with the control again in DMSO, and the experimental mES cells with Shp-2 inhibitor molecules. After another ten days I will count secondary EB formation, which will indicate which group’s primary EB bodies consisted of pluripotent cells versus differentiated cells.

Procedures adapted from Ding et al., 2005 and Qu et al., 1998.

Time Commitment

If accepted into the ENGAGE program, I am willing to commit ten forty-hour weeks. My starting and ending dates are flexible and will be left to the discretion of my P.I., Dr. Cheng-Kui Qu.

In relation to my overall education and goals

I have long been interested in the potential of stem cell research. When I was in high school, my interest in embryonic stem cell research was first piqued early in high school by mainstream media reports of the promise of stem cells and their use for regenerative medicine and therapeutic treatment. Having a sibling with autism, my naiveté led me to believe that therapeutic treatment for neurological disorders was just around the corner. I was fortunate to later attend a Genetic Update lecture given by Samuel Rhine of Indiana University with my AP Biology class. This gave me a realistic picture of where stem cell research was at, but at the same time made me aware of its expanding horizons.

My course work and previous research experience at Case have directed my future ambitions toward a career in biomedical research. I am presently looking at programs in biochemistry, molecular biology, and pharmacology. Last summer, I gained some perspective in colorectal cancer research when I worked with Dr. James Swain of the Nutrition Department. Under his guidance, I studied the effects of iron supplementation on intestinal tumorigenesis in APC^{min} mice. I value last summer’s experience, and I would

like to explore other areas of research. Dr. Qu's research offers me the unique advantage to participate in stem cell research, in which I have long aspired to take part, as well as the opportunity to execute an experiment testing inhibitory candidates, thus consisting of a perspective into an element of pharmacology research.

As a biochemistry major, I am required to participate in undergraduate research and ultimately to present my findings before the biochemistry department during my senior capstone seminar. I find the work of Dr. Qu's lab exciting, and I expect that my contribution would be meaningful. I feel that this experience be excellent preparation for my senior capstone, as well as provide an invaluable perspective into an area of research in which I have long been interested and may ultimately pursue professionally.

References

- Cheng-Kui Qu and Gen-Sheng Feng. Shp-2 has a positive regulatory role in ES cell differentiation and cell proliferation. *Oncogene*. 30 July 1998, Volume 17, Number 4, Pages 433-439.
- Li Y, McClintick J, Zhong L, Edenberg HJ, Yoder MC, Chan RJ. "Murine embryonic stem cell differentiation is promoted by SOCS-3 and inhibited by the zinc finger transcription factor Klf4." *Blood*, v. 105 issue 2, 2005, p. 635-7.
- Ding S, Chen S, Do JT, Zhang Q, Yao S, Yan F, Peters E, Scholer HR, Schultz PG. "Self-renewal of embryonic stem cells by a small molecule". *PNAS*. 2006 Nov 14 vol. 103 no. 46. 17266-71.
- [Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K, Takahashi S](#). Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell*. 2007 Nov 30;131(5):834-5.

***In vitro* Evaluation of Statins for Bone Regeneration -Proposal-**

Department of Biomedical Engineering

Case Western Reserve University, Cleveland, Ohio, 44106

The need for novel ways to regenerate bone in injured and diseased patients stems from the rising cost of treatment. Revenue in the orthopedics industry has been increasing at a steady 15 % over the last five years and is forecasted to continue to rise at this rate for the next decade. Much of this is due to the use of titanium as an implant device in orthopedics, dentistry, and craniofacial surgery, which has vastly improved the properties of load-bearing implants because of the mechanical strength and biocompatibility of titanium oxide. Osseointegration refers to the mechanical and biologic connection between bone and titanium implant devices and is one of the major factors that influences long-term implant success. The current method of osseointegration promotion involves administration of bone morphogenic proteins (BMPs) to the target area, in this case being the implant/bone interface. This however has proven to be very costly; the cost of BMP-7 used in spinal fusion surgery is \$5,000-\$17,000 and the estimated cost of using BMP-7 in orthopaedic revision surgery would be \$100,000 per patient (*Sandu & Lieberman, 2008*). Statins, which competitively inhibit 3-hydroxy-3-methylglutaryl coenzyme A reductase, increase the level of BMP-2 when applied directly to the area and thereby increase bone formation (*Garrett and Mundy, 2002*). This class of drugs has traditionally been used for regulating cholesterol production, and thus is primarily metabolized in the liver, when taken orally, before it could reach the bone at significant concentrations.

The primary goal of this project is to evaluate the potential use of statins as a more cost-effective treatment for increasing osseointegration of skeletal implants. *In vitro* analysis using pre-osteoblastic cells and human mesenchymal stem cells will allow us to monitor bone generation on a titanium surface. It has been shown that mesenchymal stem cells are useful in bone regeneration because of their ability to differentiate through osteogenesis into osteoblasts—the main cell involved in bone regeneration and regulation (*Caplan, 2005*). Our hypothesis is that statins will provide for new methods of tissue reconstruction in orthopedics through the use of mesenchymal stem cells. If the results of the proposed *in*

vitro studies are consistent with our hypothesis, future studies, which are beyond the time frame of this proposal, would test whether statins increase osseointegration in the *in vivo* murine model that Dr. Greenfield's laboratory has recently developed.

Human pre-osteoblastic hFOB1.19 cells and human mesenchymal stem cells (generously donated by Dr. Jim Dennis, *CWRU Dept. of Orthopaedics*) will be cultured on titanium disks. We will test different methods for statin application, including adding statin solubilized in the media, dried onto the titanium surface, dispersed in a fibrin gel, and dispersed in a gel formed from platelet-rich plasma. Platelets release growth factors which enhance the regeneration of naturally-occurring tissue. There are also several ways to characterize and quantify osteogenesis on the titanium surfaces. We will measure cell attachment and growth (DNA assays), expression of the osteoblast differentiation marker alkaline phosphatase (biochemical assays), and mineralization (xylenol orange fluorescence assays). Future studies, which are beyond the time frame of this proposal, will also measure expression of additional osteoblast differentiation markers (Runx2, PTH-receptors, and osteocalcin) by real-time RT-PCR. Dr. Greenfield's laboratory has prior experience with all of these assays.

This project will take at least ten weeks of work over the course of the summer and, depending on the findings in our data, could continue on into the Fall 2008 semester. I am looking to contribute approximately 7 hours a day for five days a week and am willing to come in on weekends if necessary to maintain cells. I have already spoken to Dr. Greenfield about this project and have been assured that it works into his and his personnel's schedules. Because my schedule is flexible and I have no other commitments during this period of time, I am willing to take on more or less hours if need be.

This project is of importance to me because it directly applies to the material that I have covered in my engineering coursework. I am currently in a Structure of Biologic Materials course (EBME 303) where we have discussed bone structure, biomimetics, tissue regeneration, and the use of stem cells for practical application in tissue engineering. My focus within in the Biomedical Engineering program is Polymer Biomaterials—a sequence that places strong emphasis on utilizing chemistry and mammalian biology to solve real-world problems. With this project, I see a chance to get involved in cutting-edge research in something that is relevant to my interests, important for the science of orthopedics, and economically viable for industry. As a third-year undergraduate student, I am wishing to pursue a PhD in Biochemistry or Polymer Biomaterials. My interest in research has led me away from my initial plans to attend medical school and in the direction of academics and/or industry, so I feel this project is specifically relevant to both my short-term and long-term goals. Last summer I interned at a pharmaceutical company in order to see how research in an industry setting is carried out. I am hoping this summer to have the opportunity to explore the similarities and differences between my industry experience and academic research.

References:

- AI Caplan, "Mesenchymal Stem Cells: Cell-Based Reconstructive Therapy in Orthopedics," *Tissue Engineering*, **11**, 1198-1211, (2005).
- R. Garrett and GR Mundy, "The role of statins as potential targets for bone formation," *Arthritis Res*, **4**, 237-240, (2002).
- HS Sandu & J. Lieberman, 2008, ORS/AAOS Combined Symposium on Biologic Strategies to Grow Bone in Difficult Clinical Situations



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Deadline: March 30, 2012

I. Identification Information

Student Name:

_____ Last First MI

_____ Email Address Phone Expected Graduation

_____ Academic Major 1 Academic Major 2/Academic Minor

Cumulative GPA _____

II. Project Information

Project Title:

Therapeutic Area: _____ Preferred Mentor: _____

Project Begin Date: ____/____/____ Project Expected End Date: ____/____/____

Total Weeks Devoted to Project: _____

Expected number of hours per week to be spent on project? _____

Budget Information

Award amounts are up to \$3500.00 for full- or part--time commitment to proposed project.

Itemized Budget:

Stipend Requested _____ (\$3000 maximum)

Project Materials Requested _____ (\$500 maximum)

Other budget sources _____

Total Budgeted Amount _____ **Total Amount Requested** _____

List all other summer funding sources and or project programs for which you have applied (e.g. SPUR; SOURCE; etc.).

III. Project Commitment

Signature below (or emailing information) documents agreement to commitment.

Student Commitment

- To participate in the proposed project
- To participate in the ENGAGE and SPUR Undergraduate Symposium & Poster Session, Fall 2011.
- To prepare final written report
- To prepare project evaluation

Mentor Commitment

- To supervise and direct the student's work
- To prepare project evaluation

Student Signature

Date

Faculty Signature

Date

(Can be signed after applicants are matched with mentors)