SEMESTER/EXPLORATORY GRANT APPLICATION
Cover Sheet

Amount Requested: $993.84

Project Information

Friedel, Kelton
Student Participant (Last, First)

The role of metalloproteases in retinal regeneration in zebrafish
Project Title (10 words or less)

Sandquist, Elizabeth | 2505
Faculty Mentor Name (last, first) | Mail Code

Science | Zoology
College (Weber State is the University, NOT college) | Department

This project X DOES require review by the WSU Institutional Review Board for Human Subjects or the WSU Animal Care and Use Committee.

Kelton Friedel — Student Signature — 4/1/2019 — Date

Elizabeth Sandquist — Project Mentor Signature — 3/20/19 — Date Received by Mentor. Must be 10 business days before final deadline.

2505 — Campus Mail

6139 — Phone Ext.

Tracy Covey — Undergraduate Research Committee Representative — 3-27-19 — Date Received by URC Rep. Must be 5 business days before final deadline.

Faculty Mentor Department Chair

4-1-19

Date

Please check if attended Research Proposal Workshop:

☑ Date Workshop attended 3-13-2019

(Please fill in the date of attendance)

Kelton Friedel — 4/1/2019

Student Signature — Date

Student Signature — Date

Student Signature — Date

Please make additional copies of this form for additional students.

SEMESTER/EXPLORATORY GRANT APPLICATION
Budget Worksheet

BUDGET ITEM	Department or College Funds	Outside Agency Funds	Personal Funds	Undergrad. Research Funds	GRAND TOTAL
Materials			$2,100.00	$993.84	$3093.83
Equipment					
Mileage to gather Data (.38 per mile)					
GRAND TOTAL			$2,100.00	$993.84	$3093.84

NOTES:

- Maximum request not to exceed $1000 and may not include a Research Scholarship.
- Equipment and left-over materials purchased with this grant will remain the property of WSU.
- You may not request money for gas purchases for travel. WSU reimburses travel expenses at a set mileage rate only.
- Grant money cannot be used retroactively on previously existing expenses. Requests for reimbursements will be denied. All purchases must be made after receiving funding and clearance from the OUR office.

Project Description

Description: Loss of vision due to damage, malfunction, or deterioration of neural cells within the retina is a health problem requiring far more than eyeglasses to be fixed. In mammals, neural stem cells are not present within the retina, and therefore the retina has no neural replacement capabilities. Loss of neural cells within the retina causes permanent damage (as no replacement cells can be made). Past studies have implanted neural stem cells into the retina of both mice and human subjects and have noted improvements in retinal function (Öner, 2018). While stem cell injection strategies have been successful, there are many factors that affect the outcome that have not been intensively studied. Examples include how the implanted cells survive, where they migrate to, and migration patterns of the implanted cells; all of which are not well understood.

Zebrafish (*Danio rerio*) have been chosen as a model to study these factors due to unique biology. Zebrafish have retinas with relatively similar cell types as most mammals, and function in a similar manner. A key difference between mammalian retinas and retinas of zebrafish, is a zebrafish's ability to regenerate neurons after an injury to the retina in adulthood (Martins et al., 2019). Zebrafish embryos are transparent during the early stages of development, which allows for observation of cell proliferation, cell migration, and upregulation of proteins that may promote neural repair.

Matrix metalloproteinases, or MMPs, are proteins that have been noted to be involved in both neural development and neural repair in zebrafish. These proteins are believed to be involved in the formation of cell migration pathways by cleaving extracellular matrix, and therefore may be used to assist neural stem cell migration (Steen et al., 2002). For my research project, three MMPs will be the focus of my study: MMP-2, MMP-9, and MMP-14a. For example, MMP-2 has been shown to have an effect on the success of neural stem cell implantation due to its shaping of the extracellular environment

(Suzuki et al., 2006). Early studies of MMP-9 have shown that it may play a crucial role in long term recovery after a neural injury such as a stroke (Zhao, Tejima, & Lo, 2007). Loss of function of MMP-14a has been shown to lead to developmental defects in the retinas of zebrafish (Janssens et al., 2013). These are just a few examples of what prompted the use of the three selected MMPs.

This preliminary research has two main goals. One, identify which, if any of our three MMPs selected show upregulation after injury by using *in situ* hybridization on larval zebrafish. Two, validate the larval zebrafish model of retinal injury using immunohistochemistry. This will permit future confocal live imaging experiments, in which we will view migration patterns of neural stem cells in live larval zebrafish after injury. The funds requested will be used to purchase reagents required for *in situ* hybridization and immunohistochemistry experiments.

Role: In my research project, my role would be working on my own to assist in gathering data for our labs long-term goals. I will be testing and improving *in situ* probes and running *in situ* experiments on embryos provided by Dr. Sandquist. I will also be performing immunohistochemistry on samples provided by Dr. Sandquist. I will be training other students in these experimental techniques, as they will be helping me during these multi-day experiments. I will also image zebrafish with a microscope and create figures for posters and presentations.

Dependent _____X_ Independent

(student helping faculty do research) (student doing own research)

Previous training and experience: I started doing research with Dr. Sandquist in the Fall semester of 2018. Since that time, I have learned multiple laboratory skills such as: proper micropipette technique, running polymerase chain reactions, running gel electrophoresis, and *in situ* hybridization. Initially, I helped Dr. Sandquist start up her lab by setting up our fish tank and preparing supplies for experiments.

Once everything was set up, I began to work in the lab under Dr. Sandquist and began to feed our zebrafish school. I still work with other students to take care of our zebrafish, but I am now advancing to an independent research project.

I am currently pursuing a Bachelor's Degree of Science in Zoology with a Minor in Chemistry. I am also actively working to be accepted into medical school upon completion of my bachelor's degree. One of the benefits of being a student at Weber State University is the ability to enroll in courses that prepare you for undergraduate research. Several of my classes within the college of science have prepared me with skills and knowledge required by my research project. Multiple courses in zoology, microbiology, and chemistry have taught me basic introduction to stem cells, proper laboratory techniques, and usage and disposal of chemicals.

I am currently enrolled in Advanced Human Anatomy with Dr. Meyers. I have been a Human Anatomy Lab Instructor for the past three semesters which has given me multiple experiences that have prepared me for undergraduate research. This program has enabled me to become improve my public speaking skills by teaching lessons to groups of students. It has also helped me to develop confidence as a leader, by leading dissection groups and organizing events.

Product: I plan to have enough data to present at the WSU OUR Symposium 2020. Data from this project will also be used by Dr. Sandquist for a presentation at the Gordon Research Conference on metalloproteases in Italy 2019. In addition to these presentations, I plan to submit my data to a scientific journal such as *Molecular and Cellular Neuroscience*.

Project Methods & Timeline

Methods: *In situ* hybridization will be used to identify and detect the expression of matrix metalloproteinases at a series of times after retinal injury in larval zebrafish. Probes for MMP-2, MMP-

9, and MMP-14a will be used for *in situ* hybridization, as well as control tests with no probe. Zebrafish embryos used for *in situ* hybridization were obtained from Iowa State University. Immunohistochemistry will be performed on zebrafish grown in the Sandquist lab at Weber State University. Antibodies will be used to stain for Mueller glial cells, as well as stain for cells that are actively proliferating. IACUC approval for immunohistochemistry is pending (April review).

Timeline: Currently, probes for *in situ* hybridization are being tested. I will begin *in situ* hybridization experiments at the end of April and continue throughout the summer. Final results for *in situ* hybridization will be concluded by June. I will begin preparing tissues and reagents for immunohistochemistry in May and begin actual immunohistochemistry experiments in June. Immunohistochemistry experiments will continue for the rest of the summer. I plan to begin to write a manuscript during the Fall 2019 semester.

Budget Explanation

The Sandquist lab start up fund has contributed over $800.00 in preparation for *in situ* hybridization and plans to contribute $1300.00 towards immunohistochemistry experiments. The supplies I am requesting from OUR are as follows: Proteinase K (1 vial at $143.00), sheep serum (100mL at $86.75), 5' and 3' target primers (2 primers at $11.00 for each primer), eliminase (1 vial at $32.09), diamond tipped cutter (1 at $31.00), GFP antibody (1 vial at $279.00), and PCNA antibody (1 vial at $360). I am also requesting $40.00 in anticipated shipping costs. This adds up to a total of $993.84 that I am requesting from the Office of Undergraduate Research.

References:

Janssens, E., Gaublomme, D., De Groef L., Darras, V. M., Arckens, L., Delorme, N.,... Moons, L. (2013). Matrix Metalloproteinase 14 in the Zebrafish: An Eye on Retinal and Retinotectal Development. *PLoS ONE, 8*(1), e52915. doi: 10.1371/journal.pone.0052915.

Martins, R. R., Ellis, P. S., MacDonald, R. B., Richardson, R. J., Henriques, C. M. (2019). Resident Immunity in Tissue Repair and Maintenance: The Zebrafish Model Coming of Age. *Frontiers in Cell and Developmental Biology, 7*. doi: 10.3389/fcell.2019.00012.

Öner, A. (2018). Stem Cell Treatment in Retinal Diseases: Recent Developments. *Turkish Journal of Ophthalmology, 48*. 33-38. doi: 10.4274/tjo.89972.

Steen, P. E., Dubois, B., Nelissen, I., Rudd, P. M, Dwek, R. A., Opdenakker, G. (2002). Biochemistry and Molecular Biology of Gelatinase B or Matrix Metalloproteinase-9 (MMP-9). Critical Review in Biochemistry and *Molecular Biology, 37*(6), 375-536. doi: 10.1080/10409230290771546.

Suzuki, T., Mandai, M., Akimoto, M., Yoshimura, N., Takahashi, M. (2006). The Simultaneous Treatment of MMP-2 Stimulants in Retinal Transplantation Enhances Grafted Cell Migration into the Host Retina. *Stem Cells, 24*(11), 2406-2411. doi: 10.1634/stemcells.2005-0587.

Zhao, B. Q., Tejima, E., Lo, E. H. (2007). Neurovascular Proteases in Brain Injury, Hemorrhage and Remodeling After Stroke. *Stroke, 38*(2), 748-752. doi: 10.1161/01.STR.0000253500.32979.d1.

SEMESTER/EXPLORATORY GRANT APPLICATION
Additional Questions

1. What funding have you received from OUR in the past, Where has your previous project been disseminated.

   I have not received funding form OUR in the past.

2. Is this project part of a required course? If so, please indicate the support (monetary and in-kind) provided for this project by the academic department.

   No. This study is independent of a course.

3. What additional sources of funding have been solicited? Is your department willing/able to fund any equipment they will be retaining?

The Sandquist Lab startup funds have been used. The fund will also be used to pay for supplies not listed in this grant such as secondary antibodies.

4. Where do you plan to disseminate the results of this project?

   I plan to disseminate the results of this project at the 2020 OUR Symposium.

5. If you are requesting a stipend, please list all significant time commitments (5+ hours per week) that you expect to maintain over the duration of your project including, for example, class and work schedules.

   I am not requesting a stipend.

SEMESTER/EXPLORATORY GRANT APPLICATION
Faculty Recommendation Form

Student Name (last, first): Kelton Friedel

Project Title: The role of metalloproteases in retinal regeneration in zebrafish

Mentor Directions: After carefully reviewing the proposal and assessing both the viability of this project and the qualifications of the student requesting funding, answer the questions found below. Please expand the sections as necessary (**do not attach separate letter**). If the project involves the use of human subjects or protected animals, be sure the student secures IRB or ACUC approval. If the project receives funding, it is your responsibility to work closely with the student, monitor the ongoing progress of the project and budget, and evaluate the project's results. Failure to do so will jeopardize funding for this project and any future projects.

1. How long and in what capacity have you known this student?

Kelton has volunteered in my lab as a research assistant since September 2018.

2. Briefly describe the proposed project. Is this part of a larger research project? Is this part of a course? If so, how is the project apart from the nature and scope of activities normally taken for the course (Please attach a copy of your course syllabus)?

Kelton's research is part of a larger project. Researchers are on the cusp of making neuroregenerative stem cell therapy a reality. Cell transplantation therapy has potential to remedy a wide range of presently incurable neurodegenerative diseases and injuries, including traumatic brain and spinal cord injury, stroke, Parkinson's and Huntington's disease, and blindness. However, low cell survival and retention is observed following transplantation to the central nervous system. Replicating regenerative stem cell niches may improve cell survival and retention following transplant. Lower vertebrates, including zebrafish, demonstrate extensive neural repair.

The long-term goal of this research is to explore how neural stem cells interact with the environment during regeneration in zebrafish for the application of improved stem cell therapies. The objective of Kelton's proposal is to determine how matrix metalloproteinases alter the microenvironment to promote stem cell migration and integration to heal retinal injuries. His research will also provide a foundation for imaging of stem cell behavior in life zebrafish as they regenerate.

3. Give an assessment of the project's significance to the student's discipline and of the project's educational and/or professional benefit to the student.

Kelton is majoring in Zoology, which encompasses animal and cell biology. This research will provide a critical foundation for the enhancement of stem cell therapies in the future. He is also a pre-med student with a fascination for research. During this project, will learn common cellular and molecular techniques, as well as research animal husbandry. This opportunity will provide him with a glimpse into the basic research behind the innovative medicine he may one day practice. His research will culminate in the presentation of a poster and contribution to a scientific paper, of which he will likely be an author. This research experience, as well as authorship of a publication, will place him in a competitive position for medical school.

4. Comment on the qualifications of the student to successfully complete this project, both in terms of the project's scope and its time frame.

Kelton has been volunteering 10 hours/week since Fall 2019, providing a good foundation in the technical skills required for this research. In fact, he helped create the probes which we will be using for our *in situ* labeling experiments. Kelton has shown himself to be dependable, takes direction well, works independently and performs experiments meticulously.

5. Comment on the justification and appropriateness of the project budget, including the necessity of a stipend (if requesting one).

The funds requested for this proposal are for materials to complete *in situ* labeling experiments, of which I have already contributed the majority of funds. The labeling experiments cannot be completed without these remaining reagents. The funds will also provide key reagents for Kelton's immunohistochemistry experiments. I will provide the materials for the majority of the immunohistochemistry techniques; however, antibodies are the most cost-prohibitive reagent. Funds for these antibodies will be used for the proposed and future experiments.

6. Describe your role in the project.

I am responsible for experiment design and scheduling. I will induce the retinal injuries required for studying zebrafish regeneration. I will also collect and prepare the samples for labeling, which includes sectioning tissue. I will teach Kelton the *in situ* and immunohistochemistry techniques, providing assistance as needed since these are long, multi-day experiments. I will instruct Kelton in proper microscopy and image analysis techniques. I will write the manuscript in Fall 2019, with Kelton's assistance on figures and data analysis.

7. Include anything else that you think will be helpful to the committee in evaluating this application.

Kelton is an exceptional student both in class and the lab. He maintains a positive attitude is trustworthy. I have experience mentoring undergraduates in the lab, personally advising over 20 students. I have also taught an undergraduate research course, which has provided essential experience in undergraduate research mentorship. Provided our experiments are successful, I intend to submit the findings of this research as a manuscript in Spring 2020, in which Kelton will be an author.

This project x DOES _____ DOES NOT require review by the WSU Institutional Review Board for Human Subjects or the WSU Animal Care and Use Committee.

IACUC pending (April review).

Elizabeth Sandquist
4/1/19

Project Mentor Signature Date

2050 6139

Campus Mail Code Phone Extension