Student Responses to RENEWAL Questions

The following apply only for renewal applications. Please consider these responses in making your assessment of the application.

1. Please describe how successful you were in achieving the intended outcomes of and adhering to the plan/timeline of your original proposal.

The goal of my Fall 2019 UROP proposal was to quantify neovessel regression during angiogenesis. The original proposal included three vessel isolations and three time-series acquisitions. So far, two isolations have been performed. Technical difficulties with the 2-photon laser have been challenging to our experimental timeline, although I have already collected a substantial amount of data. This unique culturing model necessitates comparison to control cultures, grown in standard incubators, fixed and imaged. For this, I have raw data for a negative control and one time-series experiment. The negative control consists of time-series data, during which no growth took place, for a fixed vascular gel that was cultured using previously established protocols. The time-series experiments consist of a growing microvessel culture embedded in type I collagengel that were collected hourly for a 24-hour period.

Additionally, we have collected time-series image data with growing microvessel cultures that will serve as pilot data for quantification of vessel growth parameters, including average segment length, branching, and total vascular length. The protocols in the [redacted] lab for this quantification have been developed for a different imaging modality, so I will compare the known morphometric values from previous experiments to our modified imaging modality. My intention is to demonstrate that the morphometric data resulting from the two methods are not statistically different. Once this is completed, I will be able to apply these processing protocols to the raw data sets and quantify vessel morphometrics for each timepoint.

2. Please describe how successful you think your relationship with your mentor was during your first semester of UROP.

My relationship with my mentor has been successful through this semester of UROP. We have met, and continue to meet weekly throughout the semester to discuss tasks, priorities, challenges, and ideas relating to this project. A number of unexpected issues and challenges have arisen regarding the protocol for this research, but [redacted] and I have been able to work together to come up with viable solutions to these challenges. [redacted] has been very available to me and has helped me facilitate my goals in relation to this project.

3. Please explain what you think the impact of an additional semester in UROP would be to your educational and career goals.

Thus far I have gained valuable skills in the wet lab, in microscopy, in writing code, and in image processing. In the remaining portion of this UROP period, I will be focusing on validating the image processing pipeline we have developed, and extracting biologically meaningful data from our large-scale imaging to serve in future publications. Over the course of this UROP period, the major focus of my time has been in establishing and validating the protocols necessary to gather time-series data and analyze the raw data to yield accurate vessel morphometrics. While I do have data, an additional semester in UROP will provide me with valuable time to apply the protocols established this semester to additional experiments. By the end of the Spring 2020 semester, I hope to have research that is ready to be presented at various conferences. Presenting at conferences and working on publications is a huge part of academic research, and having my research supported through UROP for another semester would greatly aid these endeavors.

UROP Proposal

Title of Proposal

Quantification of Neovessel Regression in Angiogenesis via Time-Series Imaging

Problem/Topic of Research or Creative Work

Angiogenesis is the growth of new vessels from existing vessels to form a functioning vasculature. It is prevalent in embryonic development [1, 2], wound healing [2], and tumor growth [3], and an understanding of this process is fundamental to tissue engineering [1]. In vitro models of angiogenesis enable researchers to better understand this process, but in most cases, images are taken from fixed, mature vascular networks. This process yields insights into the final product of angiogenesis but is insufficient to understand the angiogenic process. In order to understand the mechanisms of angiogenesis, direct observation of the time-dependent phenomena is required [1, 2]. Recent time-series data showed that angiogenesis is a dynamic process, with periods of neovessel growth and regression [1], behavior that cannot be captured using fixed time imaging. The purpose of this project is to quantify neovessel regression in angiogenesis using in vitro time-series imaging.

The sprouting vessels, termed "neovessels" navigate surrounding tissues as they form. The tissue is composed of fibrous protein structures, stromal cells, and other chemical products and is referred to as the extracellular matrix (ECM). Studies have shown that this growth relies on a number of chemical and mechanical factors of the ECM [1, 3, 4]. Chemical and biological factors include growth factors and macrophages [2-5]. Important mechanical properties include the density of the matrix as well as the orientation and stiffness of the protein fibrils [1].

From a mechanical perspective, properties of the ECM change during angiogenesis. Growing neovessel respond to and exert forces on the matrix, deforming local fibrils [1]. Furthermore, neovessel growth is a nonlinear process; neovessels grow in a dynamic fashion, alternating between periods of elongation and retraction [1]. Regression is observed during the maturation of vascular networks, in which entire vessel segments may regress [1, 2].

Specific Activities to be Undertaken and Timeframe for Each Activity

This project will employ a 3D in vitro model of angiogenesis by suspending isolated microvessels in a collagen matrix. In order to quantify the regression of vessels, time-series images will be taken as neovessels sprout from the suspended microvessels. The multiphoton microscope at the University of Utah will be used to take "mosaic images" of the gels, allowing for microscopic resolution over a macroscopic area. Time-series images will be taken and analyzed in order to quantify vessel morphometrics during each timepoint. Each trial for this project will include three major components: vessel isolation, imaging, and data processing for quantification.

Task 1 - Isolations and Early Incubation

Microvessels, isolated from rat epididymal fat pads, will be suspended in type I collagen and incubated to allow for neovessel growth. The isolation procedure must be completed about a week before any other tasks. During the first three days of incubation, the gels will be imaged every 24 hours on a light microscope. This imaging will document the robustness of sprouting

microvessels prior to time-series imaging. After the first three days of growth, the culture media is replaced, and a cultures is transferred to a stage-top incubator on the multiphoton microscope where time-series imaging will take place.

Task 2 - Time-series Imaging
The multiphoton microscope is capable of taking "mosaic" images. This will yield images which cover macroscopic areas of the gel with microscopic resolution. The microscope will be programmed to take a mosaic image at the same location every hour for 48 hours. Images will be acquired with two channels: second harmonic generation (SHG) and a fluorescent channel, corresponding to the collagen matrix and vessels respectively.

Task 3 - Quantification
The images will be pre-processed to enhance contrast and reduce noise using custom code we developed in Matlab, and then processed with AMIRA, a commercial software, to quantify vessel morphometrics pertaining to growth. First, mosaic images for each timepoint are stitched together and converted to a 16-bit format. Then the images are filtered and undergo contrast enhancement. The fluorescent channel images are then processed with Amira to threshold the images and create "skeletons" of the microvessel network. This allows us to quantify the vessel growth and regression over the course of the data acquisition.

Tentative Timeline
January - February
Finalize time-series acquisitions.
Finalize image processing protocols.
This includes having all protocols validated and documented.

March
Complete all image processing
protocols. o Preprocessing in
Matlab.
Processing in Amira.
Analyze data for trends over time.
Statistical analysis

April
Prepare formal reports.
Throughout the spring semester, I will attend weekly mentorship meetings with [redacted] and biweekly lab meetings with [redacted] Professor [redacted], and other collaborators.

Relationship of the Proposed Work to the Expertise of the Faculty Mentor

This project will be conducted in the Musculoskeletal Research Lab (MRL) under the direction of Professor [redacted] and PhD student [redacted] Professor [redacted] is an expert in the field of biomechanics and has been involved in similar studies, including a collaborative effort to apply time-series imaging to the research of angiogenesis. He is closely involved with the FEBio and AngioFE programs at the University of Utah. [redacted]'s research focusses on using time-series microscopy to better understand the mechanical aspects of angiogenesis. [redacted] is heavily involved in image processing and data analysis, working to extend current 2D techniques to 3D

space.

Relationship of the Proposed Work to Student's Future Goals

I am working towards a bachelor's degree in biomedical engineering and intend to pursue either a degree in medicine or a graduate degree in engineering. In either case, I intend to continue researching beyond my undergraduate years. I have gained many skills in this last semester, particularly in relation to Matlab and various applications used for image processing. My hope is to continue working on this project through the Spring 2020 semester and be prepared to present this research at various conferences next year. By continuing this research, I will gain valuable time to work on additional experiments and present this research in a professional manner. Doing this project with UROP would enhance the experience by allowing me the chance to more formally present my findings. As this project incorporates wet lab techniques, such as maintaining cell cultures and microscopy, with computational analysis (Matlab) and image processing (Amira), the skills I will gain will apply to a range of biomedical disciplines. The knowledge I gain in Matlab will be directly applicable to my coursework as Matlab is the program used by the department. Furthermore, this opportunity will provide a great foundation as I move forward in biomedical engineering and continue researching beyond my undergraduate years.

References

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