Exogenous Retinal Extracellular Matrix Proteins and De-differentiation of Müller Glia Cells—Pursuing Retinal Regeneration

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Project Purpose

People around the world suffer from degenerative diseases of the retina. These conditions can eventually lead to blindness. We hope to aid in the search for a cure for these diseases by exploring the effects of exogenous retinal extracellular matrix (retinal ECM) proteins on Müller glia cells. With the potential to transform into progenitor cells which can differentiate into rod photoreceptors, Müller cells could be the solution to restore the retina to full functionality.[1, 2]

Project Importance

Damage to the human retina is often irreversible. However, in some vertebrates, "including frogs [and] fish", the regeneration of damaged retinal tissue is "robust".[1] In fish, Müller cells are a key component of spontaneous retinal regeneration. This effect is not seen in higher vertebrates, including humans, to the same extent.[2] At least one attempt at retinal regeneration has shown that it is possible to slow or halt retinal degeneration in humans.[3] Unfortunately, this is not a complete solution to the tissue damage caused by retinal degeneration. Rather, regeneration of the retina is necessary to restore sight.

Müller cells have the potential to transform into progenitor cells that can eventually differentiate into rod photoreceptors.[1, 2] This process is complex and involves multiple steps and many molecular factors.[1] Generation of retinal neurons via Müller cells consists of five processes: de-differentiation of Müller cells to "Müller glia-derived progenitor cells (MGPCs)", proliferation of MGPCs, migration of MGPCs, neural differentiation of MGPCs to retinal neurons, and integration of new retinal neurons into the existing retinal neural network.[1] We aim to test the effects of retinal extracellular matrix proteins on Müller cell de-differentiation to MGPCs.

We hypothesize that proteins in the extracellular matrix (ECM) of the retina contain signals that enhance Müller cell dedifferentiation to MGPCs. We will develop a sustainable Müller cell culture that will be involved in numerous projects in the upcoming weeks and months. These cell cultures will allow us to perform our experiments and make it possible for other researchers on campus to perform experiments on Müller cells.

Other projects that will be pursued in Dr. Cook's lab will also involve retinal regeneration via Müller cells. We will work alongside the students who are participating in those projects. These other projects include performing gel electrophoresis and mass spectrometry on decellularized retinal ECM samples and designing a kinetic model for the factors involved in Müller cell transformation. However, only our project will focus on the actual culturing of Müller cells. Also, our project focuses on just one process (de-differentiation) in the generation of retinal neurons via Müller cells.

Project Profile Body

Our project will consist of three major phases: acquisition of Müller glia and establishment of sustainable Müller glia cultures, treatment of Müller cell cultures, and analysis.

To acquire Müller glia we will seek out research teams across the country that have experience with Müller cells. We will contact them regarding the possibility of obtaining Müller cells from them for free or for purchase. We have already contacted a researcher at the University of Washington regarding the development of our Müller cell culture and we are awaiting his reply. We have also contacted a researcher at the University of Utah. If these searches prove unsuccessful we will attempt to isolate Müller cells from bovine retinas using a protocol others have used to isolate and culture Müller cells from other species.[2, 4] We have experience dissecting bovine eyes and removing the retina. We have also experimented with sodium dodecyl sulfate (SDS) as a decellularization solution.

Fig. 1: Dissected bovine eyes

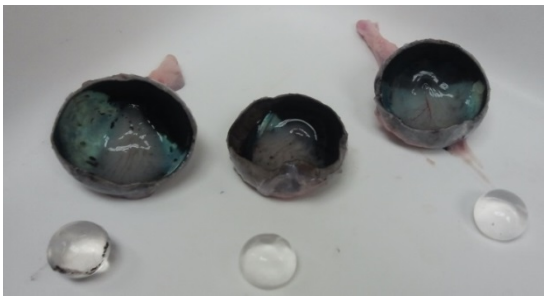
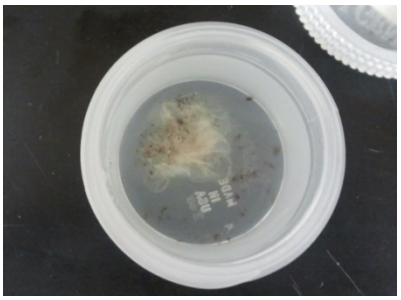


Fig. 2: Bovine retina after approx. 23 hours in 1% SDS solution



To establish Müller glia cultures, we will acquire the necessary equipment, including petri dishes, culture medium, etc. We will learn Müller cell culturing protocols, and proper sanitation/disposal techniques. Furthermore, we will be trained and become familiar with the biosafety cabinet and incubator.

During the treatment phase we will experiment with at least four aliquots of Müller cells from our established culture. We will decellularize bovine retinas using a 1% SDS solution. We will separate the remaining ECM and (in conjunction with other

members of Dr. Cook's lab) isolate the ECM proteins with which the Müller cell cultures will be treated. Each aliquot of Müller cells will be grown in individual petri dishes and subsequently treated with a unique mixture of factors: (1) control--no mixture added, (2) isolated retinal ECM proteins, (3) cocktail of factors found to be involved in Müller cell de-differentiation, and (4) mixture 3 plus isolated ECM proteins. The exact factors included in mixtures 3 and 4, their respective concentrations, and the duration of exposure (time between exposure and analysis) will be determined based on continued review of the current literature, work being done concurrently by other members of Dr. Cook's lab, and Dr. Cook's NIH R21 grant application. Also, additional mixtures, containing various combinations of factors from mixture three (or possibly individual factors) may be tested if we determine that such tests would be informative. Each of these additional mixtures would be applied to an aliquot of Müller cells, and another aliquot of Müller cells would be treated with the unique mixtures plus isolated retinal ECM proteins.

Finally, we will analyze each of the treated cultures to determine whether (and to what extent) de-differentiation of Müller cells to MGPCs has occurred. To do so we will identify an analysis to distinguish between Müller cells and MGPCs, and determine the number of Müller cells prior to and after the addition of the mixtures of factors. Ultimately, our findings could assist in the creation of a therapeutic that could be delivered via subretinal injection to regenerate retinal tissue in patients with degenerative diseases of the retina.

Anticipated Academic Outcome

We anticipate the production of an article for publication in a peer-reviewed journal, for example, *Experimental Eye Research.* Our lab group participated in the BYU Biomedical Engineering Club's *Emerging Ideas in Biomedical Research* conference this year. We plan to present at that conference again during fall semester 2014.

Qualifications

Dr. Cook has extensive experience in the tissue engineering field. He received his PhD from MIT focusing on engineering applications in medicine. Since he completed his education, he has worked in the tissue engineering and medical device industry. He specialized in cardiovascular, ophthalmic and orthopedic tissue engineering solutions. Since his arrival at BYU winter semester of 2013, he has established a lab with over five different projects that currently deal with organ regeneration.

Dr. Cook has obtained bovine eyes from a local supplier for use in the lab. Since Spring term 2013, we have spent time dissecting the eyes and decellularizing whole eyes and eye parts. Chris Burns and Chase Jackson are pursuing degrees in public health with an emphasis in health science. Both have successfully completed courses in the areas of molecular biology, inorganic and organic chemistry, human anatomy and physiology, etc. Chris and Chase have also been involved in over seven different lab classes and are adequately trained to work in a laboratory setting. Our lab equipment includes an incubator and biosafety cabinet.

Project Timetable

Task	November	December	January	February	March	April
Acquire Müller Cells	X					
Establish Cell Cultures		X				
Treat Cell Cultures with Protein Mixtures			X			
Analyze Results				X	X	X

Scholarly Sources

1. Gallina, D., et al., "A comparative analysis of Müller glia-mediated regeneration in the vertebrate retina", Experimental Eye Research (2013), http://www.sciencedirect.com/science/article/pii/S0014483513001681.

2. Giannelli, S.G., Demontis, G.C., Pertile, G., Rama, P., Broccoli, V., "Adult human Müller glia cells are a highly efficient source of rod photoreceptors", Stem Cells 29:344-356 (2011).

3. Lanza, R., "The use of pluripotent stem cells in regenerative medicine." Abstract, The Annual Hilton Head Workshop on Regenerative Medicine (2013).

4. Hicks, D., Courtois, Y., "The growth and behavior of rat retinal Müller cells in vitro 1. An improved method for isolation and growth", Exp Eye Res 51(2):119-129 (1990).