Doctoral Dissertation Defense Announcement

“The Development and Characterization of a Primary Porcine Cell Culture Model of Dry Age-Related Macular Degeneration”

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Cell and Developmental Biology
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Committee in Charge:
Daniel Lipinski, DPhil (Mentor)
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Brian Link, PhD

Date: Thursday, April 4, 2024
Time: 1:00 PM (CST)
Defense Location: Alumni Center
Zoom: Zoom Link Available Upon Request
Graduate Studies:
Biochemistry of the Cell
Molecular and Cellular Biology
Mechanism of Cellular Signaling
Fundamentals of Neuroscience
Graduate Neuroanatomy
Neuroscience Journal Club
Advanced Systems Neuroscience
Advanced Cell Biology
The Biology of Vision
Translational Genomics
Ethics & Integrity in Science
Research Ethics Discussion Series
Reading and Research
Doctoral Dissertation
Age-related macular degeneration (AMD) is the leading cause of blindness in the elderly, affecting an estimated 8.7% of all individuals between the ages of 45 and 85. In ‘dry’ AMD, the most common form of the disease, dysfunctional transport processes due to natural aging and a combination of risk factors leads to the pathological accumulation of lipids, proteins, and cellular waste products within Bruch’s membrane, the thin layer of extracellular matrix between the retinal pigment epithelium (RPE) and the vascular choroid. These deposits, termed drusen, further exacerbate nutrient and waste product trafficking dysfunction and trigger an inflammatory response that together lead to RPE cell atrophy and subsequent photoreceptor death. This disease process, when occurring within the macula, results in a slow progressive loss of central vision. Dry AMD remains without any effective interventions until the latest stage of the disease, known as geographic atrophy, wherein significant vision loss has already occurred. The lack of effective treatments for dry AMD can partially be attributed to substantial difficulties in modeling this disease for research and drug development purposes. Due to the slowly progressing nature of the disease and critical anatomical differences between human and animal ocular anatomy, no animal model has been developed that successfully recapitulates all aspects of AMD pathology. As such, the development of a cell culture model by which to study the mechanisms of drusen formation as well as to screen novel therapeutics for dry AMD would be of great benefit to the field.

First, we contribute to mounting evidence for decreased utility of the ARPE19 cell line, commonly used for the study of RPE cell biology and some aspects of AMD pathology. While numerous studies have demonstrated that ARPE19 cells accumulate genomic and transcriptomic aberrations over time, we identify and explore the mechanisms by which ARPE19 cells become more resistant to the delivery of exogenous genetic material via transfection with increasing passage number. Utilizing mass spectrometry and immunofluorescence, we identify 18 differentially expressed proteins and
alterations in protein localization which indicate that decreased in transfection efficiency at later passages occurs as a result of dysregulation of endocytosis and intracellular endolysosomal trafficking. This study further points to the need for better cell culture model systems for studying AMD pathology and developing therapeutics.

Second, we standardize and characterize a previously described ‘drusen-in-a-dish’ primary porcine RPE model system in which drusen-like sub-RPE deposits occur spontaneously in primary porcine RPE cultured without passaging for extended periods of time on porous transwell inserts. We employ repetitive lipid staining to monitor sub-RPE deposition over time and demonstrate using a semi-automated image analysis pipeline that the total number and average size of sub-RPE deposits increases significantly over time.

Finally, we employ a battery of staining and immunofluorescence techniques in order to evaluate the protein and glycoprotein components of the observed sub-RPE deposits in ‘drusen-in-a-dish’ cell cultures as compared to drusen and subretinal deposits from human AMD donor tissues. We document encouraging similarities in primary sub-RPE deposit composition compared to both macular and peripheral human drusen which suggest physiological relevance of this cell culture model.

Together, we propose that the characterized drusen-in-a-dish cell culture model represents a high-throughput, highly manipulatable, and cost-effective alternative to animal models and human tissues in which to study the mechanism of drusen accumulation and may serve as useful tools for screening novel therapeutics aimed at treating dry AMD.
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Education

**Medical College of Wisconsin** – Milwaukee, WI  
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Expected April 2024  

**PhD Candidate in Cell and Developmental Biology**  
Cell Biology, Neurobiology, and Anatomy

**Wisconsin Lutheran College** – Milwaukee, WI  
**Wisconsin Lutheran College** – Milwaukee, WI  

May 2018  

Bachelor of Science in Biology

Research Experience

**Medical College of Wisconsin** – Milwaukee, WI  
**Medical College of Wisconsin** – Milwaukee, WI  
**Medical College of Wisconsin** – Milwaukee, WI  

January 2019 – Present  

PhD Candidate  
Mentor: Daniel Lipinski, MSc, DPhil

- Thesis work: Development and Characterization of a Primary Porcine Cell Culture Model of Dry Age-Related Macular Degeneration
- Development of a novel gene therapy approach for the treatment of dry age-related macular degeneration through selective enzymatic secretion
- Development of a novel CRISPR-Cas9 generated zebrafish model of AMD via complement factors H and I dysregulation

**Wisconsin Lutheran College** – Milwaukee, WI  
**Wisconsin Lutheran College** – Milwaukee, WI  

August 2017 – May 2018  

Undergraduate Student  

- Senior Capstone Project - Designed research to study the effects of gestational caffeine exposure on the development and myelination of the neural tube in zebrafish, *Danio rerio*. This research did not result in a publication.

Publications


- Manuscript in Submission: **Shaw EM**, Tate AJ, Lipinski DM. Longitudinal characterization of sub-retinal pigment epithelium deposit formation in a primary porcine tissue culture model of dry age-related macular degeneration.
Presentations
