Misericordia University
Student Research Grants Program
2022-23

Student Researcher: Jane Smith
Department/College: Department of Biology, Misericordia University
Telephone: 570-123-1234 **Email smithj@misericordia.edu**
Address: (if on campus put your box #) 301 Lake Street
***Title of the Research Study*: The Effect of Creatine on LPS-Treated Schwann Cells**

Student Co-Researcher(s): B. Jones

Department(s)/College(s): Department of Biology, Misericordia University
Email Address(es): jonesb@misericordia.edu

Faculty Advisor Name: (*attach signed support letter*)

Print: Dr. Angela Asirvatham

Signature: L. A. Angela **Date: 9/28/22**

Faculty % contribution: 30%
Student % contribution: 70%

***CIRCLE ONE:* IRB approval obtained?**
YES (attach approval letter) **NO (date you plan to submit):**
Submitted (date of submission): **(NA) (Justify why approval not needed): This study does not involve human subjects.**

Month/Year of Graduation: May 2023

Please add a brief description (no more than 300 words) of your proposed research project. *(single spaced to fit on this cover sheet-any size font)*

In the peripheral nervous system, Schwann cells (SCs) are responsible for the growth, protection, and migration of neurons. SCs secrete myelin that wraps around axons to function as an insulator, which increases conduction of nerve impulses. During the inflammation process after nerve injury occurs, SCs stop myelinating and begin dividing to create an environment for axonal repair. *In vitro* studies have used lipopolysaccharides (LPS) to produce this inflammatory response in SCs. Studies conducted on oligodendrocytes, the glial cell that regulates myelination in the central nervous system, have shown that adding creatine enhances survival of cells after a demyelinating injury. However, not much is known about creatine's role and ability to aid SCs after neuronal injury. Creatine is known as an intracellular buffer for ATP (the cellular source of energy) and has protective roles against oxidative damage. Based on this, it is hypothesized that "the addition of creatine to Schwann cells stimulated with lipopolysaccharides will have higher rates of growth in comparison to control cultures incubated without creatine". To investigate this hypothesis, the optimal dose of creatine and LPS that was previously determined will be added to Schwann cells and assayed for growth and programmed cell death. The analysis of these results will be performed using the SPSS statistical software.

1) **Cover Sheet:**

Please see attached enclosure #1.

2) **Research Plan:**

a. **Introduction of the Research Problem:**

The nervous system is divided into two parts: the peripheral nervous system (PNS) and the central nervous system (CNS). The CNS includes the brain and spinal cord and the PNS includes all the nerves and supporting cells that extend from the brain and spinal cord to the extremities of the body. In the PNS, Schwann cells are the supporting cells that secrete myelin and insulate and protect neurons to increase efficiency of electrical impulses.

When myelin and neurons in the PNS are damaged, Schwann cells change their phenotype from myelinating cells to dividing cells to combat the injury (5). Schwann cell growth is regulated by heregulin, a neuronal growth factor, and forskolin, an unknown mitogen, which activates the cAMP pathway that is required for Schwann cell division (4). When Schwann cells are injured, they produce pro-inflammatory cytokines to fight the injury (3). *In vitro* studies have used lipopolysaccharides (LPS) to simulate an inflammatory model within Schwann cells. Schwann cells recognize LPS as a harmful substance, which triggers an inflammatory process within the cell to fight the invading substance (2). Similarly, other *in vitro* studies have used creatine to demonstrate a substance that increases proliferation of oligodendrocytes in the CNS. Creatine allows for rapid adenosine triphosphate (ATP) production near high usage sites, which oligodendrocytes and Schwann cells have a high capacity for (1). Because

oligodendrocytes are the Schwann cell equivalent in the CNS, it is reasonable to anticipate Schwann cells to respond to creatine and LPS similarly. The primary goal of this research project is to investigate the role of creatine on LPS-treated Schwann cells. Studying the role of creatine would allow for the future development of regenerative strategies for post-injury nerve repair.

b. Research Hypothesis:

To investigate the mechanism by which creatine enhances cell growth of LPS treated Schwann cells, the effects of creatine (2μM and 20μM) on LPS treated (500ng, 1μg, and 10μg) Schwann cells will be explored. Preliminary investigations have shown that creatine alone enhances cell growth and LPS inhibits cell growth. Therefore, the investigator hypothesized that:

The addition of creatine to Schwann cells treated with lipopolysaccharides will have higher rates of growth in comparison to cells incubated without creatine.

c. Literature Review:

Previous studies have shown that cAMP-dependent pathway activation is needed for Schwann cell proliferation. Two mitogens that do this are heregulin and forskolin and when these mitogens are combined in treatment, they increase proliferation compared to heregulin alone, forskolin alone, or an absence of mitogens (4). Additional studies focusing on proliferation have found that when treating oligodendrocytes, the Schwann cell equivalent in the PNS, with creatine, there was less cell death than in control cultures. When treating oligodendrocytes with LPS and creatine, cell death was significantly reduced compared to cells treated with only LPS. This suggests that creatine enhances oligodendrocyte

chances of survival after a demyelinating injury (1). When treating Schwann cells with LPS alone, studies have shown that pro-inflammatory cytokines, such as TNF-a, are produced. However, these cytokines are produced in a time- and concentration-dependent manner (2). Additional preliminary observations have shown that creatine enhances Schwann cell proliferation while LPS-treatment decreases Schwann cell proliferation in a dose-dependent manner.

Based on these findings, it is reasonable to hypothesize that the addition of creatine to LPS-treated Schwann cells will increase Schwann cell proliferation compared to cells treated without creatine.

d. Need for the Research:

Schwann cells myelinate and protect the neurons of the peripheral nervous system, which allows for efficient, effective nerve impulses. Interest in Schwann cell myelination has increased because of its relation to patients that suffer from diseases with degenerating nerves and nerve injury. Studies have primarily focused on oligodendrocytes in the PNS, but have produced limited data relating to Schwann cells. Investigating the role of creatine on LPS treated Schwann cells will provide a better understanding of Schwann cell recovery after injury-.

e. Experimental Design:

To test the hypotheses, an immortalized Schwann cell line is being grown on poly-L-lysine coated culture dishes in Dulbecco's modified Eagle's medium (DMEM). The cells will be transferred to a 96-well plate in colorless DMEM for 24 hours. After 24 hours, the media will be changed to serum free N2 (control) media for another 24 hours. Then, the media will then be changed to serum-free

medium containing mitogens (heregulin, forskolin, and heregulin + forskolin). The cells will be treated with creatine (2μM or 20μM), LPS (500ng, 1μg, or 10μg), or a combination of creatine and LPS. MTT viability assays will be performed to calculate proliferation after LPS-treatment.

f. Analysis of Results:

Schwann cell proliferation will be measured by calculating optical density of each well and analyzed using percent control. The percent control optical density of Schwann cells treated with a combination of growth factors, LPS, and creatine will be compared to the percent control optical density of cells treated without creatine. The analysis of means between various treatments will be performed using SPSS statistical software.

g. Contribution to the Field:

Not much is known about Schwann cell recovery after nerve injury. Understanding the response of an immortal line of Schwann cells to creatine after LPS-treatment will provide better knowledge of Schwann cell mechanisms for targeting a treatment for nerve injury.

3) Research Contribution:

The researcher has been studying the role of creatine and LPS treatment in Schwann cell proliferation under the direction of Dr. Angela Asirvatham since May 2022. Results from this study will be presented at the American Society of Cell Biology's International Conference December 3-7, 2022 (Please see attached enclosures…). The researcher, partnering with B. Jones,

intends to publish this research in a scholarly peer-reviewed journal with the assistance of Dr. Asirvatham.

4) Support:

Please see the attached letter of support from Dr. Angela Asirvatham, Associate Professor of Biology, Misericordia University, Dallas, Pennsylvania (Enclosure #2).

5) Contribution to Student Learning:

Attending the ASCB international conference will significantly increase the investigator's knowledge in the field. It will provide the investigator with an experience that cannot be gained anywhere else and will allow the investigator to engage with multiple specialties in the biological field. The investigator's future plans are to attend medical school and attending this conference would be essential to these future plans because of the ability to communicate with post-graduate and renowned researchers and professionals in the biological and medical fields. Misericordia University will receive recognition for supporting the above research and having undergraduate student representation of the biology department at the ASCB conference.

6) Timeline:

The research will be completed October 31, 2022. The investigator will be attending the ASCB Conference in Washington, DC, from December 3-7, 2022 with research poster presentations occurring on December 5 and 6.

7) Budget:

Please see enclosure #3 for budget sheet.

8) Budget Justification:

Travel – Expenses are related to travel expenses for the research presentation at the ASCB Conference in Washington, DC. The cost of 3 meals a day while in DC for 5 days is $44.00/day for a total of $220.00 (https://www.ascb.org/cellbio2022/program/). A conference registration fee is required for $95.

Supplies – A CyQUANT MTT Cell Viability ASSAY will cost $334.00. 1000mL of Hank's Balanced Salt Solution will cost $57.00. Cell scrapers will cost $280.06. Total expenditure costs will be $986.06.

References:

(1) Chamberlain K, Chapey K, Nanescu S, Huang J. 2017. Creatine enhances mitochondrial-mediated oligodendrocyte survival after demyelinating injury. J Neurosci. 37(6):1479-1492.

(2) Cheng C, Qin Y, Shao X, Wang H, Gao Y, Cheng M, Shen A. 2007. Induction of TNF-α by LPS in Schwann cell is regulated by MAPK activation signals. Cell Mol Neurobiol 27:909-921.

(3) Orr M, Gensel J. 2018. Spinal cord injury scarring and inflammation: therapies targeting glial and inflammatory responses. Neurotherapeutics. 15:541-553.

(4) Rahmatullah, M., Schroering, A., Rothblum, K., Stahl, R.C., Urban, B., Carey, D.J. 1998. Synergistic regulation of Schwann cell proliferation by heregulin and forskolin. Molecular and Cellular Biology 18(11): 6245-6252.

(5) Soto J, Monje P. 2017. Axon contact-driven Schwann cell dedifferentiation. Glia. 65(6):864-882.

Misericordia University
Student Research Grants Program
Budget Form

Your Name: Jane Smith

ITEM	ITEM CALCULATION	GRANT AMOUNT	OTHER SOURCE (if any)
Supplies and Materials	MTT Cell Viability Assay Kit (CyQUANT, catalog # V13154)	334.00	
	Hanks' Balanced Salt Solution (Sigma, catalog # 55021C-1000ML)	57.00	
	Cell Scrapers (VWR, catalog # 10062-904)	280.06	
Other	Meals ($7.00 x 5 breakfasts, $12.00 x 5 lunches, $25.00 x 5 dinners)	220.00	
	Registration Fee	95.00	
TOTAL		986.06	

Cell Bio 2022 OFFICIAL ABSTRACT Notification- Poster Selection

ascb@support.ctimeetingtech.com <ascb@support.ctimeetingtech.com>
Tue 9/27/2022 12:14 AM
To: Jane Smith<smithj@misericordia.edu>
Cc: abstracts@ascb.org <abstracts@ascb.org>

This is an official notification from the ASCB regarding your abstract submission for Cell Bio 2022-An ASCB|EMBO meeting:

Dear Jane Smith,

We are pleased to confirm that your abstract Creatine attenuates the effect of LPS treatment in S16 Schwann cells, has been selected for poster presentation at Cell Bio 2022 at the Walter E. Washington Convention Center in Washington, DC from December 3-7.

Please follow this link to view presentation information and guidelines, and meeting registration:

https://www.abstractsonline.com/notify/notifyIntro.asp?Mkey=9139b71d-5800-4838-ad9e-3037dd8b2ff4&NKey=F6374F24-BB96-41A1-B38A-ECB9FEAF7696

Your board number and unique poster presentation number will be sent to you by November 4.

Please visit https://www.ascb.org/cellbio2022/ for more information on the meeting.

If you are not the person who will be presenting this poster, please contact abstracts@ascb.org by October 15 so we can update this in our publications. If you no longer wish to present a poster, please let us know by October 22 so that we do not assign a board number or presentation number to you.

We look forward to your participation in Cell Bio 2022!

Sincerely,

Alison Harris

Alison Harris, CMP, DES | Director of Meetings
American Society for Cell Biology | www.ascb.org
6120 Executive Boulevard | Suite 750 | Rockville, MD 20852
301.347.9346
Mark your calendar for Cell Bio 2022 - an ASCB|EMBO meeting this December.

ascb
an international forum for cell biology

CELL BIO
An ASCB | EMBO Meeting
Dec. 3-7, 2022 • Washington, DC

Walter E. Washington Convention Center
#cellbio2022

Registration

Attend Cell Bio 2022 In Person - Registration Open!

Cell Bio is back in-person after a two-year hiatus! Register for Cell Bio 2022 and reconnect with your colleagues at the largest scientific exchange in cell biology organized by the American Society of Cell Biology (ASCB) and the European Molecular Biology Organisation (EMBO).

When:
December 3-7, 2022

Location:
Walter E. Washington Convention Center

Washington, DC

Join us and:

- Re-engage with colleagues and form new collaborations
- Immerse yourself in science
- Discover the latest in new tools and innovation
- Advance your career

Fees

Become an ASCB member and enjoy **the lowest rate** on meeting registration. As an ASCB member, save on registration and abstract submission fees, as well as, ASCB's many other member benefits.

Full Meeting Registration Rates

(Saturday through Wednesday)

Registration Category	Early Registration (Through Sept. 30)	Regular Registration (Beginning Oct. 1)
ASCB Members		
ASCB Regular Member	$310	$400
ASCB Postdoc Member	$285	$370
ASCB Grad Student Member	$105	$160

ASCB Undergrad Student Member	$55	$95
ASCB Educator Member*	$30	$50
ASCB Emeritus Member	$105	$160
EMBO Members/EMBO Community Members**		
EMBO Regular Member	$495	$632
EMBO Postdoc Member	$363	$467
EMBO Grad Student Member	$155	$222
EMBO Undergraduate Member	$81	$127
Nonmembers		
Regular Nonmember	$535	$690
Postdoc Nonmember	$520	$555
Grad Student Nonmember	$255	$295
Undergrad Nonmember	$105	$145

One-Day Registration Rates

(Saturday, Sunday, Monday, Tuesday, or Wednesday)

Registration Category	Early Registration (Through Sept. 30)	Regular Registration (Beginning Oct. 1)
ASCB Members		
ASCB Regular Member	$160	$215
ASCB Postdoc Member	$155	$195
ASCB Grad Student Member	$60	$85
ASCB Undergrad Student Member	$30	$55
ASCB Educator Member*	$15	$25
ASCB Emeritus Member	$60	$75
EMBO Members/EMBO Community Members**		
EMBO Regular Member	$160	$215

EMBO Postdoc Member	$155	$195
EMBO Grad Student Member	$60	$85
EMBO Undergraduate Member	$30	$55
Nonmembers		
Regular Nonmember	$300	$365
Postdoc Nonmember	$275	$310
Grad Student Nonmember	$135	$160
Undergrad Nonmember	$60	$80

Registration is nontransferable. Participants are not registered for the meeting until the fees are paid in full. ***Note: Payment for abstract submission fees is separate from meeting registration fees.***

**To receive the Educator rate, you must be an Educator member of the ASCB.*

***See "EMBO Member Rates" section below.*

ASCB Member Dues

Join or renew your ASCB membership before beginning your registration to receive discounted rates. Registrants must be in good-standing as an ASCB member or member of the EMBO community*. If you register at the nonmember rate and become a member later, ASCB will not refund the difference in registration fees. Contact ascbinfo@ascb.org for questions concerning membership. Member dues are non-refundable.

ASCB Member Dues by Category

Member Category	12-Month Membership Rates (Membership valid now through Dec 31, 2022) **(Rates through Sept. 30)**	15-Month Membership Rates (Membership valid Oct 1, 2022 - Dec 31, 2023) **(Rates begin Oct. 1)**

Regular	$185	$232
Postdoctoral	$78	$97
Educator	$55	$69
Student	$50	$62
Undergraduate	$26	$32

Registration is nontransferable. Participants are not registered for the meeting until the fees are paid in full. ***Note: Abstract submission fee payment is separate from meeting registration fee.***

EMBO Member Rates

EMBO Members and EMBO community members* will receive coupon codes from EMBO communications to register at the discounted rates, equivalent to the ASCB Member registration rate plus the rate of ASCB membership. If you missed these emails and need a coupon code, contact communications@embo.org.

**EMBO Fellows, EMBO Installation Grantees, EMBO Members, Lab Members of EMBO Members, EMBO Young Investigators, Lab Members of EMBO Young Investigators and Installation Grantees*

Need Assistance to Attend? Apply for a Travel Grant

ASCB is committed to ensuring equitable access for all scientists in the cell biology community to participate and benefit from attending Cell Bio. With the generosity of our supporters, ASCB is able to provide financial assistance through grants to our members to make this possible.

Cancellation Policy

ASCB honors requests for refunds in writing and received by November 28, 2022. Email requests to ascbinfo@ascb.org. Cancellations are subject to a $40 processing fee for regular and postdoc attendees and $20 for students.

Meeting registration is nontransferable.

Questions?

Contact us at ascbinfo@ascb.org.

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MISERICORDIA
UNIVERSITY
BIOLOGY

September 26, 2022

To
The Members of the Student Research Grants Committee
Misericordia University
Dallas PA-18612

Dear Members of the Student Research Grants Committee,

This recommendation letter is written in support of Jane Smith who is applying for funds to obtain laboratory research supplies and travel to present her findings at the Annual Meeting of the Association for Cell Biology (ASCB) in December 2022 at Washington D.C.

Jane was a recipient of the Misericordia University Summer Research Fellowship Program in summer 2022. During this period, Jane studied the effect of creatine on Schwann cells that were treated with lipopolysaccharide, a bacterial toxin. Schwann cells are required for myelination, which is necessary for fast conduction of impulses in the peripheral nervous system. During spinal cord injury, Schwann cells play a pivotal role in repair of injured neurons where they divide post-injury to grow. Since creatine has been shown to reduce neuronal loss in neurodegenerative disorders, Jane simulated an inflammatory environment by adding LPS to Schwann cells and proceeded to treat them with creatine. Jane found that adding creatine to LPS- treated Schwann cells helped them recover from LPS treatment. An abstract of these remarkable findings were accepted for presentation at the ASCB conference. To replicate the experiments, she needs funds for supplies, in addition to travel for the conference.

Jane's diligent work ethic, singular focus and intellectual acumen assisted her to obtain these exciting results. Attending and presenting at an international scientific conference is a tremendous opportunity for Jane as she will be able to interact with peers and attend scientific talks given by renowned scientists performing cutting edge research. Since Jane plans to pursue a career in medicine, I am confident that this productive laboratory experience from 2022 will help accomplish her long-term goal. As her faculty research and academic advisor, I fully support her application for funds to complete the experiments and for travel to present at ASCB in December 2022. Please do not hesitate to contact me if you have further questions.

Sincerely,

L. A. Angela

Angela Asirvatham, B.V.Sc., Ph.D.
Associate Professor, Biology