eureka

Undergraduate Science Journal

FEATURING
Dr. Torah Kachur
Finding your path

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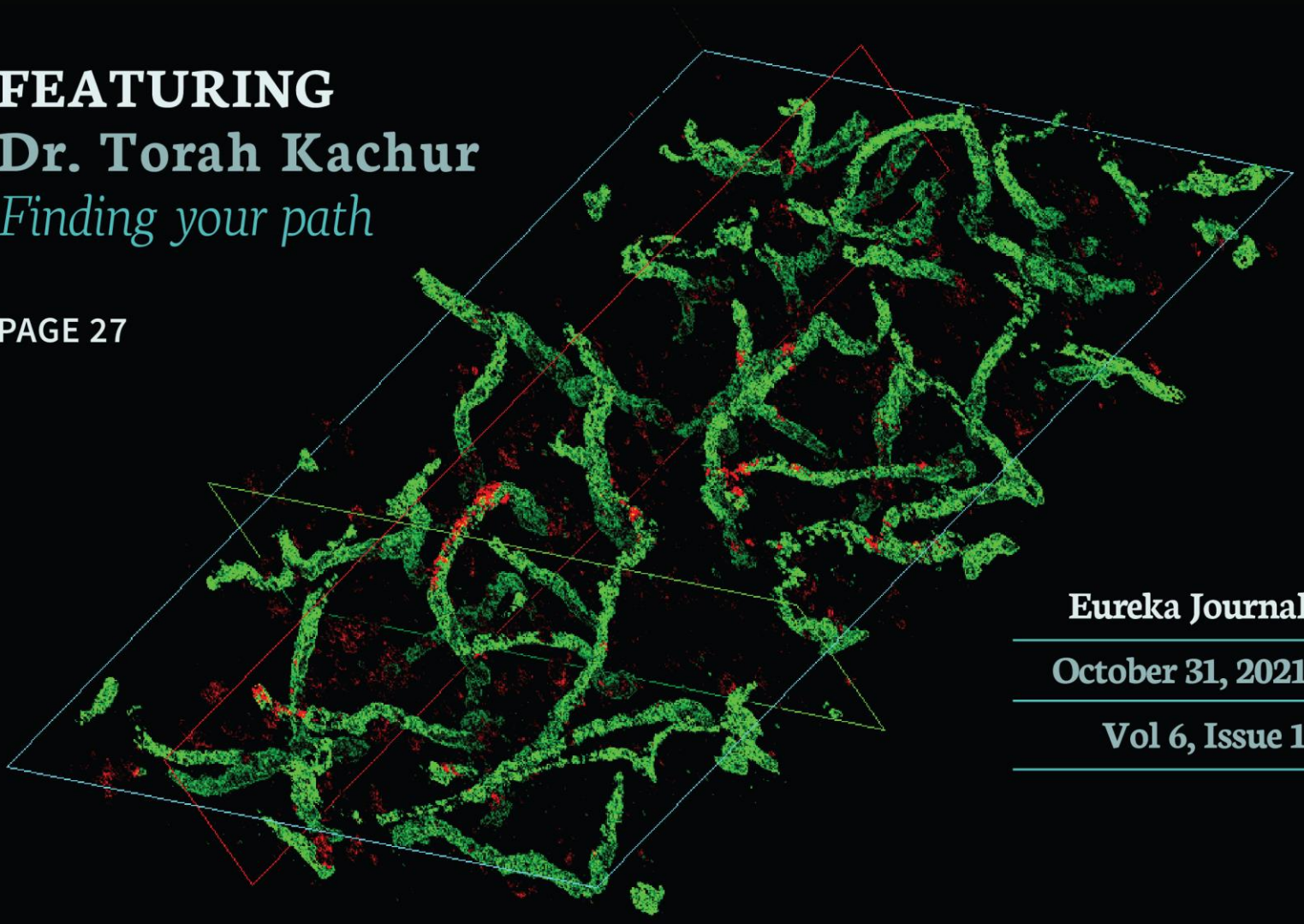

Eureka Journal
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Eureka is a student-founded and student-run initiative. This journal offers undergraduate scientists the unique opportunity to share their discoveries with the scientific world through the peer review process. *Eureka* is an educational institution, with a diverse team of reviewers from many scientific backgrounds, exposing undergraduate students to the publication process often excluded from their traditional scientific education. Through clear and effective scientific writing, students will be able to work together with faculty members to contribute original research and promote science.

Our mission is to support and promote quality peer-reviewed research by undergraduate scientists.

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From the Eureka Editorial Team

October 2021

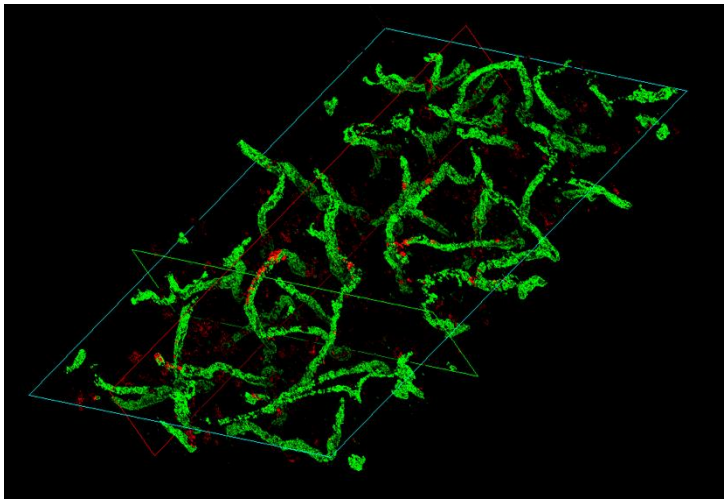
As Eureka Undergraduate Science Journal grows as a multidisciplinary publication and resource for undergraduate students, we continue to collect unique perspectives on what journeys through science can look like. We have had the pleasure of interviewing a range of scientists, from science communicators to summer researchers to Nobel laureates. Each of these featured scientists has pioneered their own unique path. Their experiences tell that science isn't made up of isolated disciplines; each discipline exists in a continuum with the others. This collection of Eureka's recently published articles reflects the diversity of research undertaken by undergraduate students in neuroscience, psychology, and microbiology.

The editors would like to invite you to read this issue across the boundaries of discipline, drawing from the experiences and findings of adjacent fields to inform your own thinking. Eureka's virtual Undergraduate Research Symposium in June 2021 was an exciting forum of discussion and showcase of undergraduate enthusiasm for science with this multidisciplinary spirit. Attendees learned about approaches to questions in neurophysiology, science ethics, oncology, mathematics, and beyond.

The next generation of scientists brings hope and excitement to the research stage. Eureka is pleased to support and train these trailblazers as they make discoveries and build bridges between schools of thought. Not everyone has the same path, but we want to support you in yours. As this issue's featured scientist Dr. Torah Kachur advised, "Say yes to opportunities that come along… you never know what they're going to be". Dr. Kachur's words on finding your passion and trying new things resonates with the editorial team and our passion for bolstering and building the undergraduate science community. Thanks to your support as readers and authors, Eureka was able to publish our first issue since 2014 last December. After all, science doesn't stop - even for a global pandemic.

Mary, Daniela, Sherry, Paul, Noam, and Shaina

Eureka Editorial Team



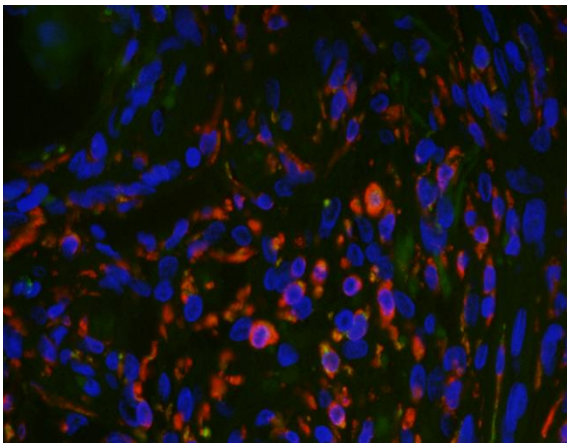
Neutrophils block brain capillaries in the penumbra region of the ischemic mouse model with a 60-min transient middle cerebral artery occlusion

Created by: An Bui
and colleagues
supervised by Dr. Ian Winship, Winship Lab
University of Alberta

This is a confocal image of the capillary segments in the penumbra region, an area surrounding the stroke core, of a mouse that has received a transient 60-minute middle cerebral artery occlusion in the left hemisphere, mimicking an ischemic event. Upon euthanasia, the brain was frozen and sectioned to obtained 40µm-thick tissues. Using immunohistochemistry, the tissues were stained with the primary antibodies CD31, which marks the endothelial cells lining the inside of the blood vessels, and Ly-6G, which is highly expressed by neutrophils. Secondary antibodies with the corresponding fluorophores were used to detect CD31 (green) and Ly-6G (red). The 3D model was constructed in Fiji, showing neutrophils (red) densely aggregate inside the capillaries (green). This obstruction of blood flow could lead to tissue hypoxia and further severe damages. This image shows how the rapid adherence of neutrophils to capillaries upon ischemia could explain why many stroke patients cannot fully recover, even after receiving timely treatments. The image is a part of my honours research project, in which I investigate the effects of a phosphodiesterase inhibitor on improving stroke outcomes by reducing neutrophil adherence and increasing blood flow.

A consortium of types of macrophages in brain tissue of a meningioma patient

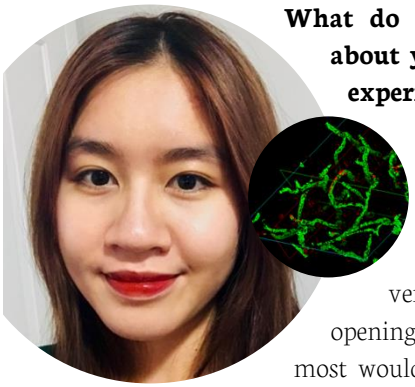
Shown here is a fluorescent stain for bodies attempting to elucidate the meningioma cancer and its this stain from the brain of a patient CD163 antibodies are lit up, different types of macrophages: M1, which are pro-tumoral. The blue which outlines the cell nucleus. In encircled in an orange stain, Occasionally, green light is apparent see how the population of M1 to M2 important to more accurately predict more favourable to anti-cancer



macrophages, with two anti-connection between grade of underlying immune response. In with meningioma, both CD68 and correlating to the presence of two aka anti-tumour cells, and M2, circles are cells stained with DAPI this case, there are more cells indicative of a M2 macrophage. for M1 macrophages. Being able to macrophages compares is patient prognosis, since M1 is responses.

Created by: Corrina Fowlow
supervised by Dr. Sanju Lama, Dr. Dustin Proctor,
and Dr. Garnette Sutherland, University of Calgary

An Bui is an undergraduate student in the Faculty of Science who is researching inflammation in stroke. Her image titled Neutrophils block brain capillaries in the penumbra region of the ischemic mouse model with a 60-min transient middle cerebral artery occlusion is featured on the front cover of this issue.



"I'm in the final year of my Neuroscience degree. I was born and raised in Hanoi, Vietnam, where I had a lot of amazing memories and found my love for health science. I came to Toronto in 2017 to finish my high school and eventually here for university. I work as a freelance illustrator and enjoy a variety of arts and crafts in my free time. I paint in different mediums and am trying to improve my sewing and sculpting skills. These are extremely therapeutic activities and a great way to discover some creative sides of mine that I've never known of. I'm also working on learning a new language!

How did you get your start in research? Can you share any advice about that first step?

Having learned that research was one of the university's strongest suits, I was already interested in getting involved in it when I started my first year. After my first semester, I had a grasp of what my study schedule would be and started browsing research labs within the Faculty of Medicine and Dentistry. Knowing that I wanted to be in a lab that focused on health research, I read about the faculty members' research topics and emailed them to ask to join their labs. I think after about 10 interviews, I finally got accepted for a summer student research position! My advice would be: know what interests you, read about the lab's work thoroughly, and actively look for opportunities in advance instead of waiting for them to come to you.

"Know what interests you, read about the lab's work thoroughly, and actively look for opportunities in advance instead of waiting for them to come to you."

What was the first thing that made you want to be involved in your current field of research?

I found the guest lectures on stroke in NEURO 210 to be remarkable and inspiring. I was really impressed with the studies that have been done in the field and the content was genuinely fascinating to me.

What do you love the most about your research or lab experience now?

It's difficult to identify a singular thing. The whole experience has been very enriching and eye-opening, but what I love the most would be how I always got help and assistance in the lab, which allowed me to learn so much along the way. My colleagues and mentors are amazing people who have a great deal of expertise in their respective fields. They are the reason why I have grown and overcome all the challenges in my project. I cannot thank them enough and I'm very fortunate to be able to work alongside them.

What do you consider your role to be as a scientist in the public eye?

I still see myself more as a science student rather than a scientist. With what happened in the pandemic, we could now understand better and appreciate the important role of scientists in our community, as well as their responsibilities. Scientists inform the public of the most recent knowledge in their field, and in many ways, protect the public with their body of knowledge. With so much more to learn, I could not yet measure myself to such a powerful and influential figure.

Where do you see yourself in 5 years?

Like many students who are graduating soon, I think we all see many different paths opening in front of us. We never know where the future will take us but for me, I hope that is somewhere in the field of healthcare and medicine. I tend not

to picture the end results as much as the journey, and I imagine that it would be a very fulfilling one, both academically and personally.

What was your favorite undergraduate experience (academic related or not!)?

Research is definitely my favorite undergraduate experience. When I wasn't studying, I spent most of my time in the lab. Research brought me the opportunities to learn different skills, to meet new friends, and to build my professional network. Other than research, my favorite undergraduate experience has to be volunteering with the Gateway as an illustrator. I never thought that after starting a science degree, I could still stay in touch with my creative side!

"Research brought me the opportunities to learn different skills, to meet new friends, and to build my professional network."

Student researchers get a lot of advice; what is a piece of advice that you've received as a student that has stuck with you?

Experiments fail a lot but stay calm, find the problems, keep going, and you will not make the same mistakes again. Ask a lot of questions and never be afraid to seek help. Even if things ended up not working out perfectly the way you wanted them to, you would still learn a great deal along the way and you might get many pleasant surprises.

eureka Research article

Replication: Self-Compassion & Health-Promoting Lifestyle Behaviours in Albertan Post-Secondary Students During the COVID-19 Pandemic

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ABSTRACT

Self-compassion as a predictor for health-promoting behaviours has been the subject of several research studies. Self-kindness, common humanity, and mindfulness have been repeatedly positively correlated with health-promoting behaviours in individuals, such as eating well and doing physical activity (Gedik, 2019; Holden et al., 2020). We hypothesized that the positive components of self-compassion (self-kindness, common humanity, and mindfulness) would positively correlate with health-promoting behaviours. In an attempt to replicate Gedik's (2019) study, researchers recruited 294 Albertan post-secondary students to respond to an online-based questionnaire. Participants filled out both the Self-Compassion Scale (SCS) (Neff, 2003b) and the Health Promoting Lifestyle Profile II (Walker et al., 1995). Findings revealed that, unlike Gedik (2019), isolating behaviours such as feeling cut-off from the world are indicative of improved stress management. Therefore, Gedik's (2019) results were not replicated. This research's implications are essential when considering the factorial breakdown of self-compassion and how the factorial relationships to health behaviours are affected by varying populations and contexts. Specifically, the occurrence of the COVID-19 pandemic and its resulting restrictions must be considered when interpreting the results presented in this current study.

KEY WORDS: Self-compassion, health, health-behaviours, COVID-19, university students

1 | Replication: Self-Compassion and Health-Behaviours in Albertan University Students

Based on Buddhist philosophy, Neff (2003a) describes self-compassion as an attitude characterized by positivity, in which individuals recognize imperfection as a universal human condition and approach mistakes and failures without judgement. The concept of self-compassion is composed of three interrelated components: (a) self-kindness vs. self-judgment, (b) common humanity vs. isolation, and (c) mindfulness vs. over-identification. Research suggests that there is a positive association between elements of self-compassion and psychological well-being, such as positive affect (Barnard & Curry, 2011; Leary et al. 2007; Neff & Vonk, 2009), and protection against anxiety and depression (Neff et al., 2007; Zessin et al., 2015). This study will replicate previous research by Gedik (2019) and examine the association between self-compassion and health-promoting behaviours in Albertan post-secondary students.

Neff's later work led to the creation of the Self-Compassion Scale (SCS) (Neff, 2003b), which was designed to measure the three core dimensions of self-compassion and their roles. The scale measures six concepts: self-kindness vs self-judgement, common humanity vs isolation, and mindfulness vs over-identification. The self-kindness and self-judgement dimensions measure an individual's tendency to respond to personal failures with compassion and forgiveness rather than self-criticism and judgement. Individuals scoring high in self-kindness are tolerant of aspects of their personality they may not like, while low scorers are critical of perceived flaws. The dimension of common humanity measures an understanding of one's experiences as being part of a larger human experience. Those scoring high in common humanity acknowledge that they are not the only to experience a problem and that others are also capable of failure. In comparison, individuals measuring high in isolation tend to see their experiences as unique to them and highly isolating. Finally, mindfulness and isolation dimensions score an individual's ability to keep perspective while thinking of problems. Mindful individuals

approach painful thoughts with understanding and a balanced mindset, while individuals scoring high in over-identification tend to fixate and obsess over a perceived mistake.

Previous research shows that the SCS is a psychometrically valid measure of self-compassion indicated by its strong positive correlation with happiness and life satisfaction (Neff, 2016; Pommier et al., 2019). Furthermore, the SCS has been shown to maintain high levels of validity across populations, language, and gender (Tóth-Király & Neff, 2021). With time and research, self-compassion and the SCS have both emerged as valid predictors of mental and physical health outcomes (MacBeth & Gumley; Kotara et al., 2019; Dunne et al., 2018).

Multiple studies have explored the relationship between self-compassion as a six-factor variable and physical health behaviours. Participants with higher self-compassion engaged in more health-promoting behaviours, which was associated with better physical health (Dunne et al., 2018). This pattern of results suggests the relationship between self-compassion and physical health is partially mediated by health-promoting behaviours (Dunne et al., 2018). Self-compassion has also been positively correlated with factors such as immunity, sleep, and global physical health in a recent meta-analysis (Phillips & Hine, 2019). Additionally, people aged 20 years or older demonstrate the relationship between self-compassion and improved physical health (Phillips & Hine, 2019).

Self-compassion can be implicated in mental health as well. Participants with high levels of self-compassion, specifically self-kindness, have higher emotional, social, and psychological well-being (Shin & Lim, 2019). Higher self-compassion has been associated with fewer mental health problems compared to students with lower self-compassion (Kotera et al., 2019). Additionally, self-compassion was more strongly associated with mental health than resilience (Kotera et al., 2019). The connection between self-compassion and mental health may be influenced by health behaviour intentions, which have yet to be examined.

These implications of self-compassion on physical and mental health may be described as the result of self-compassion's effect on health behaviour intentions. Individuals with higher self-compassion were found to have stronger intentions to participate in health-promoting behaviours (Sirois, 2015). High self-efficacy, low negative affect, and high positive affect mediate the relationship between self-compassion and health-behaviour intentions, suggesting that self-compassion can be utilized as a variable to influence individuals to make healthier choices overall (Sirois, 2015). Due to the established impact that self-compassion has on both mental and physical health and health behaviour intentions, exploring this relationship within the context of COVID-19 may lead to interesting results.

Studies conducted during the COVID-19 pandemic show that people have been experiencing more negative emotions such as anxiety, depression, and anger since the beginning of the pandemic (Mohammadpour et al., 2020). A local survey has also reported a decline in the emotional, physical and mental well-being of individuals in Edmonton, Alberta, over the past few months (City of Edmonton, 2021). When survivors of a previous pandemic, SARS, were studied, it was found that this type of pandemic-induced anxiety persists even after the outbreak (Kavakl**ı** et al., 2020). When studying the relationship between self-compassion and health-promoting behaviours, it is vital to acknowledge the higher-than-normal levels of stress and anxiety that people are experiencing during the current COVID-19 pandemic.

Self-compassion has been widely reported to help people navigate unpredictable and stressful situations (Biber & Ellis, 2017; Mohammadpour et al., 2020; Kavakl**ı** et al., 2020). The existing literature suggests that self-compassion mediates the relationship between perceived COVID threat and death anxiety (Kavakl**ı** et al., 2020). Death anxiety refers to the psychological pain and discomfort that stems from the subjective awareness of death. People who have higher self-compassion levels will be less anxious about death and feel less threatened by the COVID pandemic (Kavakl**ı** et al., 2020). These findings could be attributed to self-compassion encouraging a realistic evaluation of worries, as well as better emotional and behavioural regulation in the face of death anxiety and threat triggers, such as exposure to a number of deaths and misinformation (Biber & Ellis, 2017; Kavakl**ı** et al., 2020).

To limit the spread of COVID, specific health and safety behaviours such as handwashing and observing social distance are encouraged. This social expectation puts a greater health responsibility on the individuals. However, it is important to highlight that some people are inherently more likely to follow these health practices than others. A cross-sectional study conducted by Mohammadpour et al. (2020) suggested that people who exhibited a higher level of self-kindness were more likely to engage in handwashing and social distancing practices than people with a high level of self-judgement.

This current study was designed to replicate a study conducted by Gedik (2019), which reported the effect of self-compassion

on health-promoting behaviours in Turkish university students in the pre-COVID era. It was found that the constructs of self-compassion (ie. self-kindness, common humanity, and mindfulness) had significant positive associations with health-promoting behaviours. Contrasting variables on the SCS such as self-judgement, isolation, and over-identification were positively associated with spiritual growth, stress management, and interpersonal relationships. Overall, self-compassion was suggested to be correlated with more health-promoting behaviours (Gedik, 2019).

This study aims to test this relationship in an Albertan post-secondary student population. It is essential to acknowledge that the inclusion of previously uncommon practices such as increased sanitation and social distancing imposes an increased level of health responsibility on an individual. The relationship between self-compassion and health-promoting behaviours can fluctuate due to different pandemic-related stress levels and an increased need for health responsibility since these factors were absent when the original study was conducted (Gedik, 2019). This study is significant as it examines the relationship between self-compassion and health-promoting behaviours in a population that is different from the population examined by Gedik (2019). Therefore, our results will demonstrate how previous research findings can or cannot be generalized. This current study will allow us to examine relationships within the specific context of COVID-19, helping us better understand how correlations between self-compassion and health-promoting behaviours may change in specific contexts, starting with a global pandemic.

Results revealed in this study may have implications in the promotion of positive mental health for individuals in certain populations. For example, suppose relationships between certain aspects of self-compassion and health-promoting behaviours are discovered, this could suggest ways to provide the most effective support to post-secondary students to encourage health promotion. This would allow major provincial institutions, such as universities and colleges, to provide more targeted support for students, demonstrating to them which factors of self-compassion could be focused on to most efficiently benefit the health of individuals. The specific context of this study enables our results to help institutions better understand the effects that COVID-19 had on individuals and clarify how to enhance health promotion both during and after the global pandemic.

The goal of our study was to replicate research conducted by Gedik (2019) on the association between self-compassion and health-promoting behaviours in post-secondary students. The following research questions will be addressed: (1) Are components of self-compassion such as self-kindness, mindfulness and common humanity positively correlated with health-promoting behaviours? (2) Is negative self-compassion such as self-judgement, isolation and over-identification negatively correlated with health-promoting behaviours? We hypothesize that as self-compassion has consistently been linked to mental (Kotera et al., 2019; Shin & Lim, 2019) and physical health (Dunne et al., 2018; Phillips & Hine, 2019), self-compassion (specifically self-kindness, common humanity, and mindfulness) will be positively correlated with health behaviours. Furthermore, we expect to find similar results as Gedik (2019).

2 | METHODS

Participants

The data for this replication study was collected between January 2021 and March 2021. 294 students from varying colleges and universities across Alberta, Canada, participated in our study. Permission to conduct the study was obtained through the Research Ethics Board at the University of Alberta. To ensure the students were from post-secondary schools in Alberta, we requested that they use their school email to sign into the Google form containing the questionnaires. We removed this personal information from the data once we closed the survey and began our analysis. Before beginning the study, students were given informed consent and the opportunity to withdraw their consent, knowing that the information gathered was only to be used for the purpose of this study. In an effort to maintain anonymity, we did not ask the participants for their gender or age. Participation in our study was voluntary, and no compensation was given to participants. The data analysis was finalized with 279 participants, with 15 removed because of
incomplete data, non-school associated email, consent not provided, etc.

Materials

The participants accessed the surveys through a Google form that required logging into a post-secondary email account on their laptop or computer. They first filled out Neff's (2003b) Self-Compassion Scale (SCS), containing 26 5-point Likert scale questions with responses ranging from Never to Always (Fig. A1). Each question was related to one of 6 different factors (3 pairs). These factors include (a) self-kindness vs self-judgement, (b) common humanity vs isolation and (c) mindfulness vs. over-identification. One example of a question regarding self-kindness is, "I try to be understanding and patient towards

those aspects of my personality I don't like." A complete list of questions for each of the factors is provided in Figure A1.

The questions were ordered in such a way that the factors were spread out evenly throughout the SCS. The participants then filled out the Health Promoting Lifestyle Profile 2 (HPLP-II), a questionnaire created by Walker et al., (1995). The HPLP-II contains 52 questions relating to six categories: Health Responsibility, Physical Activity, Nutrition, Spiritual Growth, Interpersonal Relations, and Stress Management. Each question asked participants how often they engage in healthy behaviours related to the six categories listed previously. Participants responded with Never, Sometimes, Often, or Routinely. These questions were also ordered in such a way that the categories were spread out evenly throughout the HPLP-II.

Procedure

Participant recruitment occurred entirely online through social media sites, the University of Alberta's Student Digest emails, as well as through emails distributed to University of Alberta professors requesting class participation. Participants were mainly recruited from the University of Alberta, but recruitment was extended to other post-secondary students across Alberta. Responses were only received from those at the University of Alberta, The Northern Alberta Institute of Technology (NAIT), the University of Calgary, and MacEwan University. To confirm post-secondary enrollment, students signed in to the study using their school-provided email address. All participant data attached to an email address that did not belong to an Albertan post-secondary institution were omitted from the analysis. The questionnaires were done entirely on the Google form after the participants read and provided informed consent. Their responses were then automatically transferred to a spreadsheet where they were averaged and prepared for data analysis.

Analyses

The relationships between the HPLP-II and the SCS dimensions were examined through Microsoft Excel. The analysis included Pearson correlations as well as a hierarchical multiple linear regression (MLR) model. Pearson correlations were carried out between all 12 dimensions (6 from SCS and 6 HPLP-II) and total mean SCS and HPLP-II scores. The correlation matrix was used to assess the significance of the HPLP-II's relationship with all SCS dimensions. The goal was to replicate the strong positive correlations between HPLP-II and self-kindness, common humanity, and mindfulness as revealed in Gedik's (2019) earlier study, "Self-Compassion and Health-Promoting Lifestyle Behaviors in College Students.". The MLR model includes all six factors of the SCS and each of their relationships with total mean HPLP-II scores. The MLR model was used to assess which SCS dimensions explain most of the variability in total mean HPLP-II scores. Significant correlations and linear regressions that were significant at the $p < 0.10$ and $p < 0.05$ level are indicated individually in Table B1 and Table B2 below. All six factors of the Self-Compassion Scale (SCS) (self-kindness, self-judgment, common humanity, isolation, mindfulness, and overidentification) were entered as predictor variables for the multiple linear regression (MLR).

3 | RESULTS

Unlike the original study by Gedik (2019), healthy behaviours were not found to be correlated with self-kindness, self-judgment, common humanity or mindfulness. Instead, the multiple regression in Table B1 indicates that overidentification explains most of the variability in total HPLP-II scores (β = 0.114, SE = 0.056). Specifically, overidentification was found to be significantly positively correlated with stress management ($r = 0.120$, $p < 0.05$), which might indicate that isolating when stressed leads to better management of the stress or stressors. The correlation between overidentification and overall healthy behaviours was not significant at the $p< 0.05$ level. Even though overidentification was found to be a significant predictor of healthy behaviours (Table C2), the multiple regression model only explains 1.9% ($R2 = 0.019$) of the variability in HPLP-II scores. This could be because gender and age were not included in the model or because the questionnaires did not entirely capture the intended parameters. Even if overidentification was a significant predictor, there is much more that explains the variation in the HPLP-II that is not captured here. Overall, healthy behaviour amid a pandemic seems to be correlated with isolating oneself. This finding could be related to the participants' willingness to follow local COVID-19 restrictions, although we did not explicitly test for this.

It was hypothesized that different positive components of self-compassion (self-kindness, common humanity, and mindfulness) would have a significant positive association with health-promoting behaviours. This was based on the findings of several research studies that have attempted to correlate the same variables. The findings of our study were inconsistent with the hypothesis..

4 | DISCUSSION

The results in this study were inconsistent with Gedik's (2019) findings. We found no relationship between health-promoting behaviours and the positive aspects of self-compassion. Over-identification was found to be significantly positively

correlated with stress management at the $p < 0.05$ level. Over-identification was associated with increased physical activity and better nutrition, but these relationships were not found to be significant at the $p < 0.05$ level. Additionally, isolation was linked with increased health responsibility, but again, this relationship was not significant at the $p < 0.05$ level. Apart from the association between over-identification and stress management, this study's findings directly contrast with the original study by Gedik (2019).

However, caution must be exercised while drawing conclusions from the findings of this study due to the following factors that limited the scope of our results. Firstly, it's a cross-sectional study, and the surveys were posted on various social media websites. Our study used a convenience sample. Because of this, there is no way to identify or account for systematic differences between the people who responded to the survey versus those who did not. The findings are correlational in nature, and it is not possible to estimate which construct caused the other. Secondly, for complete anonymity, the study did not control for meaningful confounds such as age, post-secondary institution, and gender. Therefore, potential differences among these variables must be considered. Post-secondary students can fall within a wide age interval; thus, age could act as a meaningful confound, potentially affecting the relationships examined. Age and gender were not found to have a significant effect on the relationship between self-compassion and health behaviours in Gedik's (2019) original study. However, the absence of this data categorization must still be considered when interpreting the results of this current study. Additionally, the eligibility criteria were modified from students of a single post-secondary institution to students of several post-secondary institutions in the province. This increased the study's relative external validity but at the cost of internal validity since the influence of specific post-secondary institutions and respective cities was not controlled. Finally, and most importantly, it is reasonable to expect that different self-compassion and health behaviour measures could vary systematically because of the global pandemic. The study did not adjust for these potential variations, so the study results are not generalizable in a non-pandemic world.

Our main findings contrast with studies that have examined the relationship between self-compassion and health-promoting behaviours. Dunne et al. (2018) found that higher self-compassion was consistently related to engaging in more healthy behaviours, and promoting lower physical symptoms. Further, when considering the factorial structure of self-compassion, self-kindness, common humanity, and mindfulness have been found to correlate positively with all tested domains of health-promoting behaviours (Holden et al., 2020). Holden et al. (2020) also found negative correlations between negative self-compassion domains (overidentification, isolation, and self-judgment) and three domains of health-promoting behaviours (nutrition, spiritual growth, and health responsibility). The significant findings of these studies contrast sharply with our results. This suggests a potential measurement error or the possibility of confounding variables that must be considered. These differing findings may prompt consideration of the factorial breakdown of self-compassion, or how self-compassion factors may interact with health-promoting behaviours differently in various contexts or with different populations.

Expanding on the issue of contrasting results leads us to consider Gedik's (2019) study, which was the replication basis for the current research. Overall, the present study failed to replicate Gedik's (2019) findings, and in some cases, found contradicting correlations. Gedik (2019) emphasized the positive correlation between self-kindness, common humanity, and mindfulness and health-promoting behaviours. He also found that self-judgment, isolation, and overidentification were not associated with health-promoting behaviours (Gedik, 2019). When comparing our results to Gedik's (2019), we are prompted to consider possible explanations for the opposition.

We speculate that the context in which this current study was conducted – during the COVID-19 pandemic – may serve as a partial explanation for these results. For example, the positive correlation between health responsibility and isolation was found to be significant only at the $p < 0.10$ level, but can still be explained by the context of the pandemic, as individuals accept imposed COVID-19 regulations to take responsibility for themselves and others' health. The significant relationship between stress management and over-identification may also be explained by the current context in which individuals are forced to isolate themselves, and they consider the stressors in their immediate environment. However, these are only speculations to explain the contrasting results compared to Gedik's (2019) findings. More research would have to be conducted to further explore the multiple components of self-compassion and their relationship to health-promoting behaviours in various contexts.

The findings of this research have implications for how healthcare practitioners might approach mental and physical health during the COVID-19 pandemic and in future global

emergencies. The relationship between self-compassion and self-isolation is particularly relevant. Perhaps when social experiences expose individuals to potential health risks, self-isolation acts as a form of self-compassion and self-care. Furthermore, our research suggests that the only significant predictor of health-promoting behaviours was students' stress management skills. These findings may help school administrators and mental health practitioners at universities implement more effective techniques to bolster student health, such as online workshops and classes targeted at improving students' stress management skills. This may be especially relevant for students experiencing high levels of isolation, such as international students and physically disabled students who may be less able to participate in traditional campus activities.

5 | CONCLUSIONS

This research aimed to conduct a replication of research conducted by Gedik (2019) on the association between components of self-compassion and health-promoting behaviours in post-secondary students. As in the original study, students who scored high on self-compassion measures would also score higher on health-promoting behaviours. As previously mentioned, other studies have found that participants with higher self-compassion engaged in more health-promoting behaviours, which was associated with better physical health. According to Dunne et al. (2018), these results suggest that health-promoting behaviours at least partially mediate the relationship between self-compassion and physical health. Ultimately, we were unable to replicate Gedik's (2019) findings. Instead, our results suggested that negative self-compassion elements such as over-identification and isolation were related to health-promoting behaviours. While no definite conclusion can be made, it is a strong possibility that the presence of the COVID-19 pandemic in Canada may have influenced the relationship between self-compassion and health behaviours. Future research needs to be conducted to understand how global threats such as COVID-19 affect the nature of self-compassion and its relation to health.

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eureka **Research article**

Bacterial Signal Peptides: Structure, Optimization, and Applications

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ABSTRACT

Bacterial signal peptides are N-terminal tags that direct proteins for export through one of various transport pathways. These signal peptides are highly important as they are the key determinants of transport, ensuring that the correct protein arrives at the correct pathway. While these peptides consist of three domains with well conserved biochemical properties, there remains a large amount of diversity between the signal sequences for different proteins, transport pathways, and bacterial species. Recent advancements have allowed for the prediction and manipulation of signal sequences to optimize protein export efficiency. This knowledge can then be exploited in the field of recombinant protein production wherein bacterial species are used to produce and secrete proteins of interest. By fusing the protein with an optimized signal peptide, the yield or rate of export can be improved. This review focuses on signal peptides for two primary transport pathways (Sec and Tat) in Escherichia coli specifically, with an emphasis on applications and the production of recombinant proteins.

KEY WORDS: Signal sequence, signal peptide, recombinant proteins, Sec, Tat

1 | INTRODUCTION

For bacterial proteins to be transported to different cellular compartments or secreted from the cell, they must navigate through various transport pathways (Green & Mecsas, 2016). Faithful targeting to these pathways is paramount as it ensures that proteins arrive in the right place at the right time. With more than a dozen secretion systems and a vast exportome portfolio, strict organization must be maintained for bacteria to remain viable and functional. This protein transport specificity is provided by short N-terminal tags dubbed "signal peptides" (Emr et al., 1978; Freudl, 2018). These signal peptides have a tripartite structure with conserved motifs and are typically 16-30 amino acids in length (Freudl, 2018; Peng et al., 2019). Distinct biochemical properties of each domain or motif allow the protein to be transported by one specific pathway while avoiding others. Once the signal peptide has directed the protein to the correct transport pathway, it is cleaved off by signal peptidases (Peng et al., 2019).

This review will outline common features of signal peptides for two main transport pathways—Sec and Tat—in Escherichia coli (*E . coli*). The general secretion (Sec) and twin-arginine translocation (Tat) pathways are the primary transport systems in bacteria, with Sec predicted to transport over 90% of all secreted proteins (Georgiou & Segatori, 2005; Green & Mecsas, 2016). In brief, both pathways are composed of multiple protein components and involve transport across the cytoplasmic (inner) membrane (Green & Mecsas, 2016). However, while Sec only transports proteins in an unfolded state, Tat has the unique ability to transport folded proteins.

In addition to discussing key components of signal peptides, this review will also explore practical applications. For example, signal peptides can be exploited in the bacterial production of recombinant proteins (Freudl, 2018). This is highly relevant to many industries such as biopharmaceuticals, food production, and scientific research. By expanding upon the knowledge surrounding signal peptides and optimizing their sequence for maximum efficiency, the yield and purity of recombinant proteins can be improved.

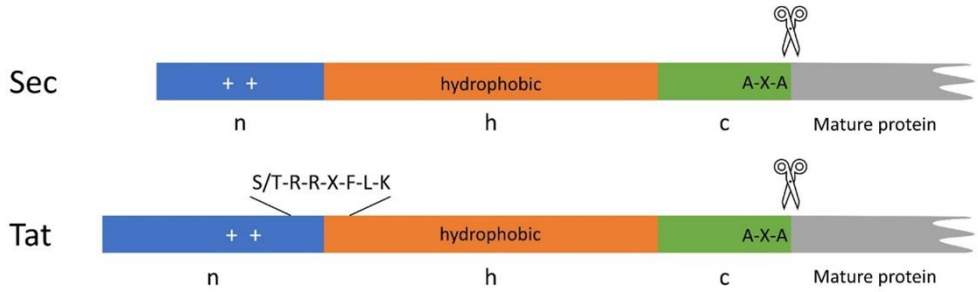

Figure 1. Schematic of the general tripartite structure of signal peptides. Both Sec- and Tat-specific signal peptides possess a positively charged n-region, a hydrophobic h-region, and a polar c-region. This polar c-region also contains the A-X-A motif, which is a signal peptidase cleavage site as indicated by the scissors. Tat-specific signal peptides also contain a twin-arginine motif (S/T-R-R-X-F-L-K) at the border between the n- and h-regions.

2 | SIGNAL SEQUENCE DIVERSITY – SEC VS. TAT

A specific signal sequence exhibits some variation, both within and between bacterial species. This variation allows for certain proteins to be targeted to different transport pathways for export, thus arriving at different final destinations (Freudl, 2018; Green & Mecsas, 2016). Despite this diversity, there are three typical domains: a positively charged amino-terminal region (n-region), a central hydrophobic region (h-region), and a polar carboxyl-terminal region (c-region) (Freudl, 2018; Peng et al., 2019) (Figure 1). It is within this c-region that the cleavage site for signal peptidases resides, with an A-X-A motif where A is alanine, and X is any amino acid (Rusch & Kendall, 2007) (Figure 1). Due to recurring motifs, bioinformatics tools may be used to predict putative signal sequences in silico (Peng et al., 2019). For example, SignalP (Petersen et al., 2011), Phobius (Käll et al., 2007), and PrediSi (Hiller et al., 2004) can be used to predict signal peptides for the Sec pathway, while TatP (Bendtsen et al., 2005), Tatfind (Rose et al., 2002), and PRED-TAT (Bagos et al., 2010) may be used for the Tat pathway. The following subsections will outline common motifs seen in signal peptide sequences, with a focus on E . coli. Additionally, differences in the signal peptide that discern Sec vs. Tat transport will be highlighted.

2.1 Sec-specific signal

Signal sequences which target proteins to the Sec pathway in *E.* coli follow the general tripartite structure as outlined previously (Rusch & Kendall, 2007) (Figure 1). As evidenced in Table 1, the positively charged n-region typically contains multiple basic amino acid residues such as lysine (K) and arginine (R). Next, the longer h-region consists primarily of hydrophobic amino acid residues (Table 1). Finally, the short c-region contains some polar uncharged amino acid residues such as serine (S) and threonine (T), as well as the signal peptide cleavage motif (A-X-A) (Lüke et al., 2009; Palmer & Berks, 2012). While the biochemical properties within each domain are well conserved, the individual amino acids themselves exhibit large variation between different proteins (Table 1). Because the amino acid substitutions are often within the same biochemical "family" (e.g., hydrophobic alanine vs. leucine), this diversity is well tolerated and does not affect the overall function of the signal peptide. However, these slight changes can lead to differences in export efficiency, as discussed further in subsection 2.3.

Table 1. Multiple sequence alignment (MSA) of exemplar Sec signal peptide sequences in *E. coli*. Sample Sec substrates were selected (Crane & Randall, 2017; Molloy et al., 2000) and signal peptide sequences were collected from the UniProt database (UniProt Consortium, 2019), then a ClustalW MSA was performed via MEGA software (Kumar et al., 2018). Colour key for biochemical properties: yellow=hydrophobic; blue=basic; red=acidic; green=hydroxyl, amine; pink=glycine, indigo=proline, brown=sulfhydryl, aqua=histidine.

Protein	Signal Peptide Sequence		
	n-region (positively charged)	h-region (hydrophobic)	c-region (polar , with A-X-A site)
1. LamB	MMITLRKL-	PLAV-AVAAGVMSAQAMA-	
2. MalE	MKIKTGAR-	ILALSALTTMMFSASALA-	
3. OmpA	----MKKT-	AIAI-AVALAGFATVAQA-	
4. OmpF	---MMKRN-	ILAV-IVPALLVAGTANA-	
5. OmpN	----MKSK-	VLAL-LIPALLAAGAAHA-	
6. OmpC	----MKVK-	VLSL-LVPALLVAGAANA-	
7. OmpP	----MQTK-	LLAIMLAAPVVFSSQEASA	
8. OmpX	---MKKIA-	CLSA-LAAVLAFTAGTSVA	
9. PhoA	----MKQS-	TIAL-ALLPLLFTPVTKA-	
10. PhoE	----MKKS-	TLAL-VVMGIVASASVQA-	
11. GBP	--MNKKVL-	TLSA-VMASMLFGAAAHA-	
12. TolC	----MKKLLP	ILI-GLSLSGFSSLSQA-	
13. TolB	----MKQA-	-LRVAFGFLILWASVLHA-	
14. CirA	---MFRLNPF	VRVGLCLSAISCAWPVLA	

2.2 Tat-specific signal peptides

Similar to Sec-specific signal peptides, Tat-specific signal peptides follow the same three-domain pattern (Freudl, 2018) (Figure 1). However, there are a few differences that distinguish Tat signal sequences from those destined for the Sec pathway, as depicted in Table 2. Most notable is the presence of the conserved Tat-specific motif at the boundary between the n- and h-regions: S/T-R-R-X-F-L-K, where X is usually a polar amino acid (Freudl, 2018). The twin arginines (R-R) are invariant in all Tat signal peptides, as this motif plays a key role in the binding of the Tat substrate to the export system (Cristóbal et al., 1999; Palmer & Berks, 2012) (Table 2). Additionally, the +2 and +3 amino acid residues (with respect to the twin arginines) are consistently hydrophobic, as evidenced in Table 2 (Brink et al., 1998; Cristóbal et al., 1999). Another distinction is that Tat signal peptides tend to be less hydrophobic than Sec signal peptides, particularly within the h-region (Cristóbal et al., 1999). This is in part due to comparatively more glycine (G) and less leucine (L) residues in the h-region of Tat signal peptides (Table 2). Additionally, Tat signal peptides are relatively longer than those for the Sec pathway, at average lengths of 38 and 24 amino acids respectively (Cristóbal et al., 1999; Freudl, 2018). A longer n-region in Tat signal peptides contributes to this increased length. The n-region also contains more negatively charged residues than what is seen in Sec signal peptides (Table 2). This may be attributed to the compensatory ability of the large positive charge from the twin-arginine motif (Cristóbal et al., 1999). Finally, Tat signal peptides tend to contain a few positively charged amino acid residues (R, K) within the c-region, termed the "Sec-avoidance" signal (Bogsch et al., 1997; Freudl, 2018) (Table 2). It is important to note that while the twin-arginine motif is ubiquitous in all Tat-specific signal peptides, it alone is insufficient to direct proteins to the Tat pathway (Bogsch et al., 1997; Chaddock et al., 1995; Palmer & Berks, 2012). Instead, a combination of these key distinguishing features is required.

2.3 Signal sequence optimization

Specific modifications to the signal sequence can be made to optimize the export of a given protein. For example, a higher charge to length ratio in the n-region typically leads to higher secretion efficiency in the Sec pathway (Peng et al., 2019). This was confirmed experimentally in *E. coli*, by substituting positively charged residues with either neutral or negatively charged residues in the n-region, thereby reducing the positive charge (Inouye et al., 1982; Nesmeyanova et al., 1997). As a result of these substitutions, the rate of transport decreased and the accumulation of protein aggregates in the cytoplasm increased. Conversely, increasing the net charge of the n-region generally improved export efficiency as demonstrated by Ismail et al. (2011) in *E. coli*, although this may be protein-specific. This effect is likely due to the electrostatic interactions that are present between the positively charged n-region and negatively charged residues found near the binding groove of the Sec translocase motor protein (SecA) (Chou & Gierasch, 2005; Gelis et al., 2007; Low et al., 2013). Altogether, increasing the charge to length ratio of the n-region generally increases transport efficiency for Sec substrates. Manipulating the charge of the n-region may also influence transport efficiency via the Tat

Protein	Signal Peptide Sequence			
	n-region (positively charged)	Tat motif S/T-R-R-X-F-L-K	h-region (hydrophobic)	c-region (polar, with A-X-A site)
1. TorA	---MNNNDLFQASRRRFLAQLG-	GLTVAGMLGPSLLTPRRA		TA
2. TorZ	---------MTLTRREFIKHS-	-GIAAGALVVTSAA-	PLPAWA	
3. AmiA	--MSTFKPLKTLTSRRQVLKAG-	---LAALTLSGMS-	--QAIA	
4. AmiC	MS----GSNTAISRRRLLQGAG-	----AMWLLSVSQV-	----SLA	
5. FtsP	---------MSLSRRQFIQAS-	-GIA----LCAGAV-	PLKASA	
6. EfeB	MQYKDENGVNEPSRRRLLKVIG-	----ALALAGSCPV-	----AHA	
7. FdnG	---------MDVSRRQFFKICAGG	MAGTTVAALGFA-	PKQALA	
8. FdoG	---------MQVSRRQFFKICAGG	MAGTTAAALGFA-	PSVALA	
9. YcbK	---MDK---FDANRRKLLA-	LG-G--VA--LGAAIL-	PTPAFA	
10. NrfC	---------MTWSRRQFLTGVGV	LAAVSGTAG-------	RVVA	
11. WcaM	------MPFKKLSRRTFLTASS-	----ALAFLHTPFA-	----RA	
12. YahJ	-------MKESNSRREFLSQSGK	MVTAAALFGTSVP---	LAHA	
13. MdoD	---------MD--RRRFIKGSMA	MAAVCGTSGIASLFSQAAFA		
14. FhuD	MS-----GLPLISRRRLLTAMA-	----LSPLLWQMNT-	----AHA	

Table 2. Multiple sequence alignment (MSA) of exemplar Tat signal peptide sequences in *E. coli*. Sample Tat substrates were selected (Tullman-Ercek et al., 2007) and signal peptide sequences were collected from the UniProt database (UniProt Consortium, 2019), then a ClustalW MSA was performed via MEGA software (Kumar et al., 2018). Colour key for biochemical properties: yellow = hydrophobic; blue = basic; red = acidic; green = hydroxyl, amine; pink = glycine, indigo = proline, brown = sulfhydryl, aqua = histidine.

pathway (Freudl, 2018). Notably, Li et al. (2006) found that charge distribution in the n-region was more significant than net charge when determining Tat-specific protein secretion rates. This further supports the notion that while there may be general trends, signal peptide optimization is still unique to a given signal sequence and protein.

The h-region can also be modified, as the degree of hydrophobicity appears to be pivotal when determining export efficiency. For example, increasing the hydrophobicity of this region can increase efficiency of export via the Sec pathway, as demonstrated by Chen et al. (1996) in *E. coli.* This is likely because the h-region adopts an α-helical conformation, which is able to form hydrophobic interactions with the SecA motor component of the Sec transport system (Chou & Gierasch, 2005; Gelis et al., 2007; Mori et al., 1997). By increasing the hydrophobicity and thereby strengthening this interaction, the efficiency of transport may increase as SecA is more readily able to recognize Sec-specific signal peptides (Chou & Gierasch, 2005; Low et al., 2013). Besides affecting the levels of secreted protein, manipulating the hydrophobicity of the h-region can also change the targeted transport pathway. Natural Tat substrates can be redirected to the Sec pathway by increasing the hydrophobicity of the signal peptide (Cristóbal et al., 1999). This aligns with evidence that Sec signal peptides tend to be comparatively more hydrophobic, as discussed in subsection 2.2. Additionally, further increasing the hydrophobicity of a signal peptide destined for the SecB pathway can redirect it to the SRP-dependent pathway, which is an alternate branch of the Sec transport pathway (Bowers et al., 2003; Low et al., 2013). This is due to SRP preferentially binding longer, more hydrophobic α-helices (Low et al., 2013). In general, by manipulating the hydrophobicity of the h-region, both export efficiency and the targeted transport pathway can be altered.

Some current advancements are being made in an attempt to streamline signal peptide optimization. For instance, the generation and screening of "signal peptide libraries" have been performed for a few bacterial species to predict the optimal signal sequence (Brockmeier et al., 2006; Degering et al., 2010; Peng et al., 2019). However, it is important to note that while a given signal peptide may be optimal for one specific protein, it may perform poorly if fused to a different protein. As such, there is no "universally optimal" signal peptide. These signal peptide libraries and other publicly available databases may be beneficial as initial screening tools, and their utility will increase as they include more species or substrates (Goudenège et al., 2010; Low et al., 2013).

Overall, targeted modifications to the signal peptide can alter the protein's export efficiency. This has beneficial implications for the design and production of recombinant proteins in bacteria, as discussed in the following section.

3 | APPLICATIONS

Recombinant proteins such as growth factors and antibodies can be produced in bacteria for use in research, food production, or the pharmaceutical industry (Freudl, 2018). Bacterial systems boast many advantages such as low cost, high yield, short production time, and relatively easy genetic manipulation (Terpe, 2006). Despite drawbacks, such as differing codon biases and a lack of post-translational modification, bacterial systems remain a popular choice for the production of recombinant proteins. Notably, 30% of protein-based pharmaceuticals were produced by E . coli in 2009 (Ferrer-Miralles et al., 2009). To produce a desired recombinant protein in bacteria, a cleavable signal peptide can be fused at the N-terminus to allow for export out of the cytoplasm (Terpe, 2006). The specific sequence can be modified to optimize export efficiency, while still bearing in mind the limitations of the endogenous transport machinery (Freudl, 2018).

While recombinant proteins can be expressed and collected from the cytoplasm directly, there are many advantages to exporting the protein to the periplasm prior to collection via the incorporation of a signal peptide (Choi et al., 2000; Guerrero Montero et al., 2019). For example, as the periplasm is the only oxidizing compartment of the cell, it is the only location wherein disulfide bond formation will occur (Guerrero Montero et al., 2019; Pooley et al., 1996). As a result, any recombinant protein with disulfide bonds must be exported to the periplasm to exhibit proper tertiary structure. Additionally, exporting to the periplasm allows for easier downstream processing, as there are fewer contaminants such as DNA in the periplasm when compared to the cytoplasm (Balasundaram et al., 2009; Guerrero Montero et al., 2019). Furthermore, protein aggregation can be prevented by exporting the recombinant protein out of the cytoplasm (Guerrero Montero et al., 2019). This is significant as cytoplasmic aggregates can be toxic to the bacterial cell, in addition to making downstream processing increasingly difficult. Finally, fewer proteases are present in the periplasm, leading to less turnover of the recombinant protein (Gottesman, 1996; Mergulhão et al., 2005). On the whole, exporting the desired protein to the periplasm via a carefully designed signal peptide is a key step in recombinant protein production.

Currently, many different recombinant proteins are being produced in bacterial systems and exported via the Sec or Tat pathway. For example, human growth hormone (Guerrero Montero et al., 2019), interferon α2b (Alanen et al., 2015), and human antibody fragments (Alanen et al., 2015) can be exported via Tat. Meanwhile, the Sec pathway has been utilized for the export of insulin-like growth factor 1 (Joly et al., 1998), human epidermal growth factor (Wong & Sutherland, 1993), parathyroid hormone (Wong & Sutherland, 1993), and alkaline phosphatase (Choi et al., 2000). In either case, the specific targeting of the recombinant protein to an export pathway is achieved by the fusing of a signal peptide to the N-terminal region. In the following subsections, the production of two key biopharmaceuticals in *E.* coli will be discussed in further depth.

3.1 Human growth hormone

One specific example of a biopharmaceutical which can be produced and exported by bacteria is human growth hormone (hGH) (Guerrero Montero et al., 2019). hGH can aid in the treatment of hypopituitarism, obesity, and burn/wound healing (Isaksson et al., 1985). Bacterial systems are a good candidate for producing hGH as it has few disulfide bonds and no glycosylation (Guerrero Montero et al., 2019; Ultsch et al., 1994). However, hGH adopts a complex, folded conformation prior to transport out of the cytoplasm. As such, it is unable to be secreted by the Sec pathway, and the Tat pathway must therefore be used instead. Montero et al. (2019) demonstrated this by fusing the TorA Tat signal peptide to hGH (TorA-hGH). This fusion protein was expressed in *E.* coli "TatExpress" strains, which have elevated levels of Tat proteins to circumvent the slower transport typically associated with the Tat pathway. Mature hGH was found to be readily abundant in the periplasm, with proper disulfide bond formation and correct cleavage of the TorA signal peptide. Additionally, the yield was quite high at 2.39-5.4 g/L of culture, which far exceeds the yield expectations for bacterial recombinant proteins (typically 0.5-0.8 g/L) (Georgiou & Segatori, 2005). This is likely due to the use of the "TatExpress" strain, allowing for increased export of hGH to the periplasm. The increased export capacity also aids in reducing insoluble hGH aggregates in the cytoplasm, which is a prevalent issue in this field (Guerrero Montero et al., 2019; Patra et al., 2000). Overall, bacterial secretion of TorA-hGH via the Tat pathway appears to be a viable option for the mass production of recombinant hGH.

3.2 Full-length monoclonal antibodies

Another specific example of exploiting bacterial signal peptides is the production and secretion of human monoclonal antibodies in *E.* coli (Zhou et al., 2016). Monoclonal antibodies are incredibly valuable to the pharmaceutical industry, as they can be used to treat a wide variety of human diseases such as breast cancer, lung cancer, rheumatoid arthritis, ulcerative colitis, and psoriasis (Spadiut et al., 2014). However, secretion efficiency remains a limitation for monoclonal antibody production in bacteria. In an attempt to circumvent this drawback, Zhou et al. (2016) tested four Sec-specific signal peptides (STII, DsbA, PhoA, MalE) fused to the N-terminus of the heavy chain (HC) antibody component. Of these, the signal peptide from DsbA was deemed to be the most efficient. Zhou et al. (2016) speculated that this was due to the high hydrophobicity of the h-region when compared to the other signal peptides tested. Accordingly, they attempted to further improve secretion efficiency by increasing the hydrophobicity of the h-region for two signal peptides (STII, DsbA). In doing so, Zhou et al. (2016) found that the proportion of mature, secreted HC could be doubled with only a single amino acid substitution in the h-region, wherein a polar amino acid residue was mutated to a more hydrophobic one. Altogether, this demonstrates that the optimization principles discussed in subsection 2.3 can be applied to recombinant protein production in bacterial systems.

4 | FUTURE DIRECTIONS

The expanding knowledge of signal peptides and their key features has proven to be incredibly valuable when applied to recombinant protein production in bacterial systems. In the future, natural signal peptides could be modified to improve secretion efficiency, such as increasing the charge of the n-region or increasing the length and hydrophobicity of the h-region (Low et al., 2013). While there have already been examples of this in the literature (as discussed in subsection 2.3), the optimization of signal peptides is an ongoing process due to their protein- and species-specific nature. As such, each signal peptide must be optimized anew when considering a novel protein or substrate. These optimization efforts may be further aided by the expansion of current signal peptide libraries to include a wider variety of bacterial species and proteins, as well as the development of high-throughput signal peptide screening methods (Goudenège et al., 2010). Another future direction may involve designing signal peptides de novo, as demonstrated by Mhiri et al. (2000) in Streptomyces. After comparing the most efficient natural signal peptides, they designed a synthetic signal peptide which then outperformed the natural signal peptide up to six-fold. This remarkable proof of concept opens the door for designing synthetic signal peptides. Finally, much is still unknown about the specific interactions between portions of the signal peptide and the transport machinery at the residue level (Blaudeck et al., 2003; Gelis et al., 2007). As such, improving our understanding of the structural and functional roles of each motif may further

advance attempts to optimize natural signal sequences or design them de novo.

5 | CONCLUSION

The importance of bacterial signal peptides cannot be understated, given that they appear to be the sole determiner of transport pathway targeting (Bogsch et al., 1997). Through modifying pre-existing signal peptides or designing them de novo, transport efficiency can be optimized (Low et al., 2013). At the same time, identifying conserved regions, such as the twin-arginine motif or the A-X-A signal peptidase site, provides boundaries for regions which should not be perturbed. These optimized signal sequences can then be exploited for the production of desired proteins, such as human hormones or antibodies, in bacterial systems (Freudl, 2018). Overall, the expanding knowledge of signal peptides is a promising step forward when applied to the field of recombinant protein production

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eureka Research article

The Neuroregenerative Effects of Intraspinal Microstimulation (ISMS) Following Spinal Cord Injury (SCI)

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ABSTRACT

Background Intraspinal microstimulation (ISMS) is a novel electrical stimulation technique that has demonstrated mobility restoration in animals with spinal cord injury (SCI). This project investigated: 1) the capacity of ISMS to restore functional walking in rats with SCI through 4 weeks of stimulation, and 2) the degree of walking deficit caused by ISMS surgery.

Methods Thirteen Sprague Dawley rats were divided into three groups: 1) rats with hemi-section SCI (hSCI) and no implants (control group), 2) rats with hSCI and passive ISMS implants (ISMS sham group), and 3) rats with hSCI and implants with active electrical stimulation (ISMS group). All groups were trained to walk on a horizontal ladder and their performance was quantified pre- and post-surgery.

Results We hypothesized that the rats with active ISMS implants would demonstrate the greatest improvement in functional walking compared to both control groups, and that the ISMS sham group would underperform the most. The preoperative functional walking scores of control, sham and ISMS rats were 5.7±0.2, 5.5±0.3 and 5.7±0.1, respectively (7-point scale; mean ± standard error). The post-surgery scores were 3.2±0.9, 2.6±0.6 and 3.3±0.8 for control, sham, and ISMS rats, respectively.

Conclusions As the difference between the post-surgery functional walking scores of ISMS and control rats was not statistically significant, this may indicate that four weeks of ISMS stimulation is not enough to cause rehabilitative effects. Additionally, the ISMS sham group demonstrated impaired functional walking compared to the hSCI control group as predicted. Future studies will employ a larger sample size to fully elucidate this trend and utilize thinner microwires to mitigate cellular damage.

KEY WORDS: Rehabilitation Technology, Intraspinal Microstimulation, Mobility Recovery, Rehabilitation Neuroscience, Paraplegia, Spinal Cord Injury

1 | INTRODUCTION

Spinal cord injury (SCI) is a debilitating neurological trauma that commonly results in paralysis and secondary complications including spasticity, bladder dysfunction, respiratory complications, and muscular atrophy (Bamford et al., 2017). Upwards of 500,000 individuals worldwide experience an SCI each year, presenting a plethora of complications to the quality of life of those affected and placing a substantial financial burden on healthcare systems (New & Marshall, 2013). To put this into perspective, in Canada, depending on whether the patient is living with paraplegia (paralysis of the legs) or tetraplegia (paralysis of the legs and arms), the lifetime cost of care of a 25-year-old can range from $1.5 million to $3.0 million (Krueger et al., 2013). This is an especially challenging circumstance as anyone can suddenly end up with this condition; the most common causes of spinal cord injury are automobile crashes and traumatic falls (Chen et al., 2013).

Much of the sensory information perceived by the body, whether it is touch, pain, heat, or pressure, goes through sensory neurons to the spinal cord and then to the brain. Similarly, all the motor neurons that control our limbs, digits, and core traverse through the spinal cord. In effect, an injury at any level of the spinal cord can sever the transmission of this information that is so critical to daily living. The most difficult aspect of SCI is that damaged neurons often do not regenerate; this is because neurons are terminally differentiated cells, which are incapable of growing new cellular populations (Frade & Ovejero-Benito, 2015).

At a cellular level, Jara and colleagues (2020) have demonstrated that the mammalian nervous system has an innate capacity to functionally reorganize its neuron in response to pathological injuries. The specific cellular mechanism behind this is still being studied, but it has been theorized that a lesion in the central nervous system transiently increases the level of secondary messenger cyclic adenosine monophosphate (cAMP). Increasing cAMP can further catalyze the upregulation of protein kinase A (PKA), cAMP response element-binding protein (CREB), and arginase 1 (ARG1), which are implicated to support the intrinsic regeneration of neurons (Cai et al., 2001; Jara et al., 2020). Importantly, these mechanisms only facilitate a limited recovery following neural injury. However, a recent breakthrough in neuroscience discovered that central nervous system circuitry can be strengthened with continuous activation of the neural connections (Jara et al., 2020). In effect, introducing electrical stimulation to activate the desired circuitry may strengthen neurological recovery, or in some cases, cause axonal growth. Further studies have also demonstrated that electrical stimulation not only activates the aforementioned growth-promoting molecular pathways but may also speed up the intrinsic recovery process following neural injury by modulating levels of transcription factors (Zareen et al., 2018).

Intraspinal microstimulation (ISMS) provides external electrical stimulation to the spinal cord by implanting hair-thin platinum-iridium wires directly below the injury site (Moritz, 2018). By doing this, special patterns of electrical stimulation can provoke a natural gait cycle (i.e., standing, stepping, walking) to help rats with paraplegia walk again. For a constant natural gait cycle to occur, this precise electrical stimulation must be present at all times to facilitate the contraction of precise motor pools. Further beneficial effects of ISMS are well documented in research conducted on rats, cats, and pigs (Holinski et al., 2016; Kasten et al., 2013). ISMS is advantageous because it offers the greatest selectivity of neurons and precise control of targeted motor pools vital for functional walking (Giszter, 2015). Furthermore, ISMS allows for specific stimulation and strengthening of the central nervous system circuitry that has been injured. Other methods of electrical stimulation often broadly target the general area of motor neuron pools, which often results in less effective motor movement and circuitry activation. However, many unanswered questions surrounding the translation of ISMS from animal subjects to human patients remain. Specifically, the impact of temporary, non-continuous ISMS on mobility restoration remains unknown. Additionally, the extent of cellular damage caused by microelectrode implantation in the spinal cord and the impact this may have on lasting mobility following SCI remains unknown. These are important questions to investigate as human patients with SCI may decide to cease ISMS following a temporary period of active stimulation or utilize ISMS in an intermittent fashion.

In effect, this project investigated: 1) the capacity of ISMS to restore functional walking in rats with SCI through four weeks of stimulation, and 2) the degree of walking deficit caused by ISMS surgery. The first objective sheds light on what might happen to patients with ISMS who stop receiving stimulation after four weeks, and the second objective investigates how mobility restoration is impacted by the ISMS surgery alone (no stimulation).

We hypothesized that, compared to spinal cord injured rats without ISMS, SCI rats that receive active ISMS for four weeks would demonstrate improved functional walking scores as a result of enhanced neural reorganization due to electrical stimulation. Additionally, compared to SCI rats without ISMS surgery, we believed that SCI rats that receive ISMS surgery alone (no active stimulation) would have decreased levels of mobility, as some tissue damage may occur as a result of the implantation surgery.

2 | METHODS

Pre-Operational

Thirteen Sprague Dawley rats were randomly divided into three groups: 1) rats with hemi-section SCI (hSCI) and no implants (control group), 2) rats with hSCI and passive ISMS implants (ISMS sham group), and 3) rats with hSCI and active ISMS implants (ISMS group). Originally, five rats were included in each group. However, one rat from the sham group and one rat from the ISMS group had to be euthanized due to toe-biting following the hSCI surgery. As a result, there were five rats in the control group, and four rats each in the sham and ISMS groups. The group assignment process was randomized by generating a number from 1-13. If a number was already assigned, it was rolled again. Rats #1-5 became group 1, rats #6-9 became group 2, and rats #10-13 became group 3. All rats were housed in a standardized living environment to ensure there was no confounding variable present. To ensure the age of the rat did not have an impact on their walking performance, the deliverer made sure they were all the same age.

The experimenter was blinded to the groups when taking recordings or measurements, and during data analysis. All groups were first trained to walk on a horizontal ladder with randomly placed rungs prior to their surgery, and gait cycle performance was quantified.

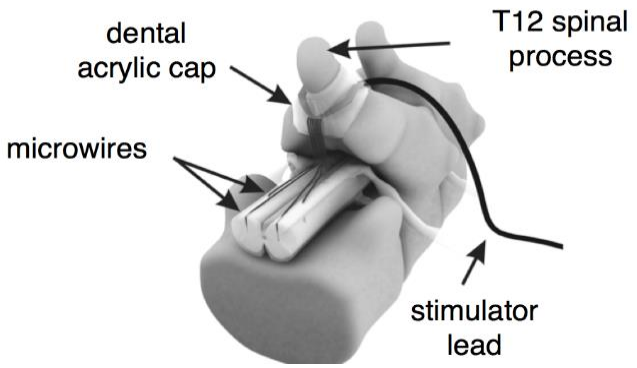

Figure 1. A model of the ISMS device. For this experiment, two microelectrodes were implanted at the T13 and L1 segments of the spinal cord, below the level of the hSCI lesion. The acrylic cap at the T12 spinal process secures the microwires in place to prevent manual disturbances to the microelectrodes. Microelectrodes are 30μm in diameter and connect to the electrical stimulator. Image is reproduced by authors from Bamford et al. (2010).

Surgery

Following hSCI surgery at the T8 segment of the spinal cord (performed by an experienced lab technician), and a two-week recovery period, the sham and ISMS group rats received an ISMS implant composed of an array of two 30 μm wires implanted in the ventral gray matter of the T13 and L1 segments of the spinal cord known to house the hind limb motor neuron pools of rats (Holinski et al., 2016). This implant was done unilaterally on the right side, below the level of the hSCI lesion. After the surgery, a threshold stimulation was introduced to make sure the wire was inserted correctly in the appropriate motor pools.

One-week post-implant, low-level, tonic sub-motor threshold stimulation through the ISMS implant was initiated in the rats in ISMS group rats. The stimulation parameter followed the standard technique described by Mushahwar and colleagues (Bamford et al., 2017). Stimulus amplitude was set at 25 μA to prevent tissue damage. This amplitude was delivered in a 1 second train at a rate of 25 pulses per second through the microwires. Stimulation was delivered for 1 hour/day for four weeks, coupled with walking on the horizontal ladder. This was done to incorporate the common practice done in the previous ISMS procedures (Bamford & Mushahwar, 2011). This is often the optimal amount of stimulation for rats as it allows neurons to further strengthen their connections by continuously being activated whilst preventing muscle fatigue or atrophy.

Operationalization of Walking Performance

The walking performance of all rats on the horizontal ladder was video recorded twice per week and analyzed by a blinded experimenter. The horizontal ladder had two layouts: regular and irregular. The regular layout had a constant spacing of 2 cm between the rungs, and the irregular layout had varied spacing of 1-3 cm between the rungs. This variety of spacing demands the rats employ different types of weight-bearing movements to walk functionally across the ladder without slipping. Their movements were captured through VHS tape for further analysis. At first, all rats were trained on a regular layout to get accustomed to the ladder, and then switched to an irregular layout. All rats traversed the irregular ladder layout five times to ensure they understood how to perform on this layout. This same paradigm was applied following surgery. The functional walking scoring system was based on previous research by Whishaw and colleagues where scoring ranged from 0 to 6, with (0) representing a total miss of a ladder rung, (1) representing a deep slip of a ladder rung, (2) representing a slight slip of a ladder rung, (3) representing replacement, where a leg is placed then lifted quickly, (4) representing correction, where a leg is aiming for one rung but is placed on another rung instead, (5) representing partial placement and (6) representing correct placement where all limbs with weight are supported (Metz & Whishaw, 2009). All of the scoring data was recorded on Microsoft Excel where means and standard errors were calculated. This data was then imported into SPSS software to elucidate any statistically significant differences that existed between treatment groups and before and after the surgery. To ensure the maximum congruence among tests, we employed Tukey HSD, Bonferroni, and Sidak post-hoc tests.

Post-Operational

At the end of the experiment, all rats were euthanized, and the spinal cord was extracted and perfused for histochemistry purposes. Hematoxylin and eosin (H & E) stain was used to evaluate the extent of injury apparent in the extracted spinal cord. All of the following methods were approved by the University of Alberta research ethics committee.

Figure 2. Rats engaging in the horizontal ladder task. The left photo demonstrates a rat with a score of (6) in which the rat has perfect weight-bearing in all 4 feet on the rungs. The right photo demonstrates a rat with a score of (0) where both hindlegs completely missed the ladder rungs

3 | RESULTS

The pre-SCI functional walking score of control, sham and ISMS rats were 5.7±0.2, 5.5±0.3 and 5.7±0.1, respectively.

After the hSCI was performed, the functional walking scores of the rats in all groups significantly decreased, as expected. Following hSCI and tonic stimulation of the spinal cord (in the ISMS group), the postoperative functional walking scores of control, sham and ISMS rats were 3.2±0.9, 2.6±0.6 and 3.3±0.8, respectively (Figure 4).

4 | DISCUSSION

In this project, we attempted to investigate 1) ISMS's capacity to restore functional walking in rats with SCI following four weeks of active ISMS, and 2) the degree of walking deficit caused by ISMS surgery alone. Our results have shed light on the effects of hSCI, ISMS surgery, and active ISMS stimulation.

Firstly, statistical analysis (one-way ANOVA) demonstrated significantly reduced functional walking scores across all rat groups following the hSCI surgery relative to their pre- injury scores ($p<0.05$). This highlights that the hSCI itself significantly disturbed all the rats' ability to walk.

Secondly, while the active ISMS group demonstrated slightly better functional walking following surgery and stimulation compared to the hSCI and sham controls groups, this outcome was not statistically significant (one-way ANOVA; $p>0.05$). This was confirmed after doing three different sets of post-hoc tests. At this time, it is difficult to conclude that four weeks of active ISMS had a strong enough impact to facilitate an improvement in the gait cycle of the rats following SCI. This finding was contrary to our original hypothesis and may indicate that four weeks of ISMS stimulation is not enough to promote rehabilitative neuroregeneration at the cellular level. In previous studies, researchers have used twelve weeks of stimulation to parallel a common intervention time frame used in rehabilitative research (Kasten et al., 2013). One of the reasons why four weeks was chosen for this experiment is because in previous research it has been shown that neurons adapt to an external factor such as electrical stimulation over 30 days (Bamford & Mushahwar, 2011). This outcome may also be a result of our sample size across all three groups. Nevertheless, the four-week stimulation time frame and small sample size are admittedly limitations of the present study and an area we hope to address with future research.

In addition to the aforementioned finding, statistical analyses comparing all groups post- injury and ISMS (one-way ANOVA followed by Tukey HSD post-hoc analysis) demonstrated that the functional walking scores of the rats in the sham group were significantly lower than those in the control and ISMS groups ($p<0.05$). This finding confirmed our original hypothesis that spinal cord injured rats that receive ISMS surgery alone (no active stimulation) will have decreased levels of mobility as some tissue damage may occur as a result of implantation surgery. In previous studies, it has been shown that the thinness of wires is positively associated with fewer physical side effects following ISMS surgery (Kasten et al., 2013). Even though 30μm thick microwires are typical for larger animals (e.g., cats, pigs), we suggest that future research in rats employ thinner, 25μm wires to mitigate tissue injury.

In future ISMS studies, an area to explore with this experiment is 3D spinal cord imaging to measure injury volume. Injury volume can function as an objective way to quantify the degree of injury incurred in the spinal cord and would allow us to explore whether performance may be attributable to the size of

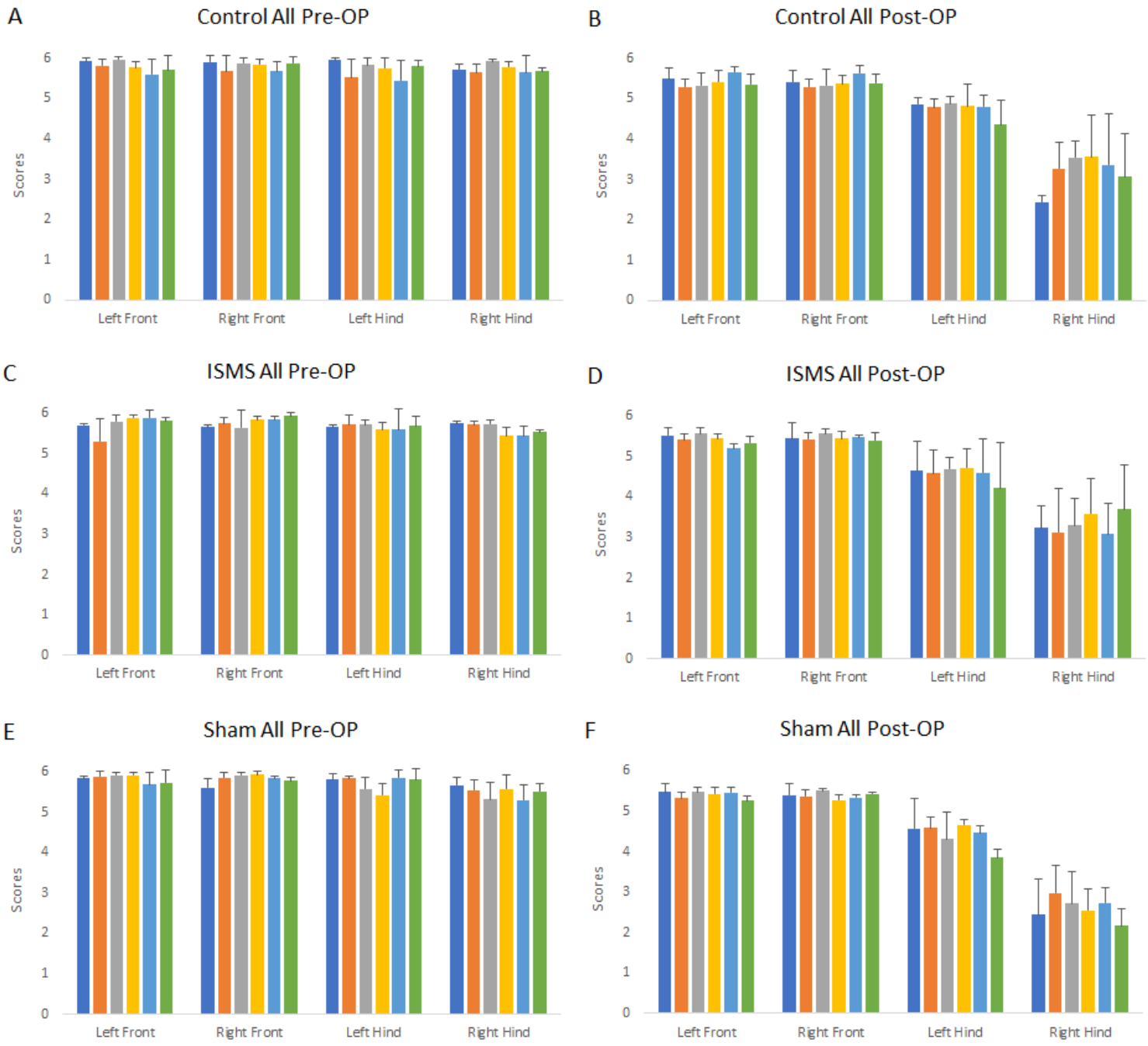

Figure 4. Pre- and post-operative functional walking scores for all groups. Ladder walking scores pre- and post-operatively of the rats in the control group (n=5), ISMS group (n=4), and sham group (n=4). 1st regular data represents a trial where rats walked on a 2 cm equally spaced ladder. Irregular trials represent walks done on a ladder with randomly placed rungs with varying separation between 1-3 cm. All scored trials were collected 6 days apart. All scores are reported as mean ± standard error.

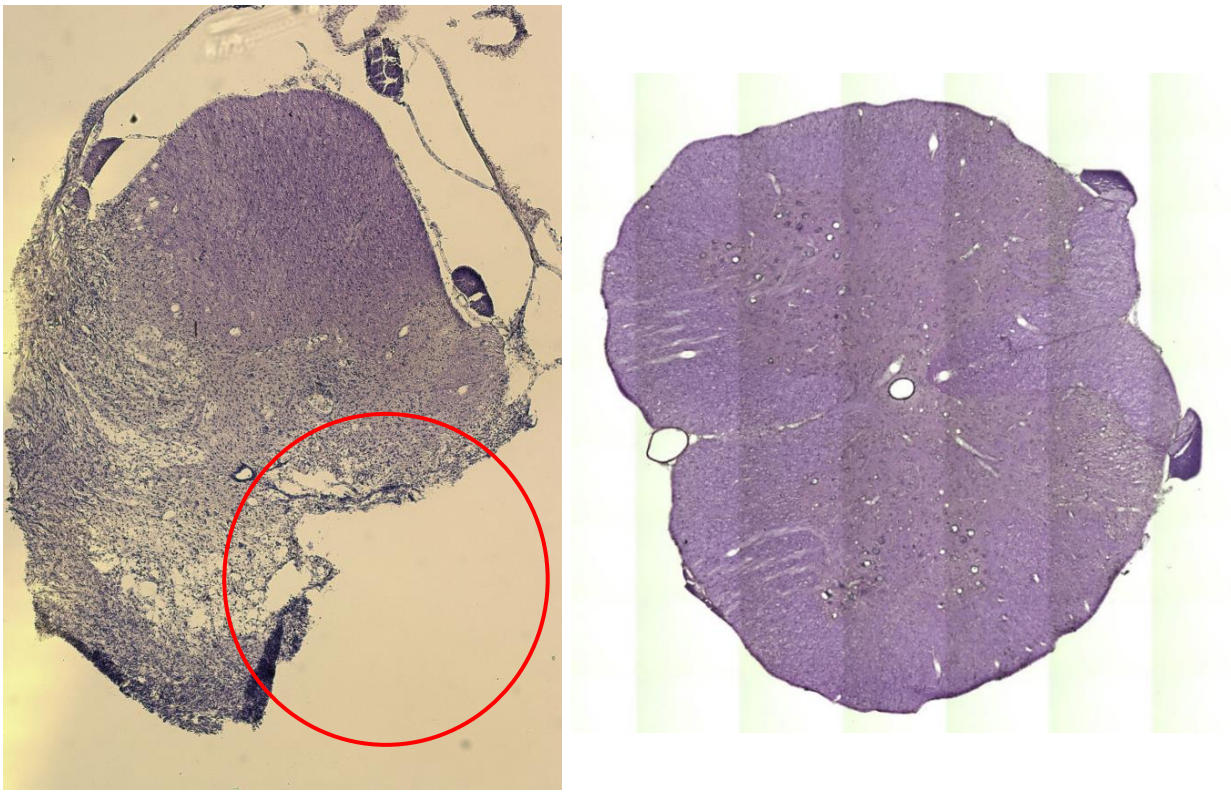

Figure 5. H & E staining was used to demonstrate the disruption of the spinal cord at the site of hemi-section injury (left) relative to an intact cord (right). The red circle shows that the hemi-section has removed a section of spinal cord, indicating that spinal cord injury was the cause of the mobility deficit.

the injury as opposed to the treatment. Similarly, injury volume could allow us to explore whether ISMS (with and without stimulation) modulate the size of the lesion. In addition to this, immunohistochemistry could also be utilized to identify levels of inflammation in response to the ISMS implant, as well as any neuroregeneration promoted by ISMS through the visualization of synaptic density in the spinal cord.

ETHICS

All the procedures have been approved by the ethics committee at the University of Alberta, AUP 302. All the scientists involved in the study have been trained to handle animals and procedures were proceeded under supervision.

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Dr. Torah Kachur is a passionate science communicator, as a science columnist for CBC Radio and co-creator of scienceinseconds.com, and a lecturer at the University of Alberta and MacEwan University.

Can you tell us a little bit about yourself as an undergraduate student? Would you have predicted that you'd be doing what you do now?

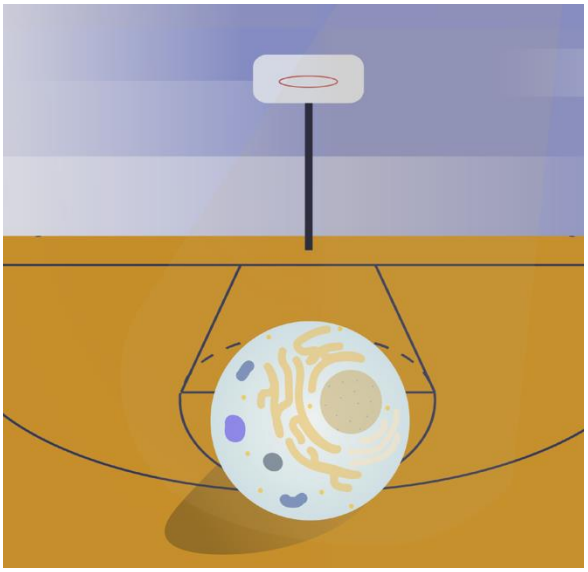
"No, but I really didn't have any idea what I wanted to do. I graduated from U of A in honors molecular biology and genetics. It was kind of a funny story how I ended up there because I was your typical undergrad where after my first semester first year – I was actually at MacEwan because back in the day there was a university transfer program, and I was playing basketball for MacEwan.

I sort of put basketball aside and I didn't know what I wanted to do. I just knew I wanted to do science. So, when I was going into second year, I filled out the transfer form at the U of A admin office, and it was like, "Put your major and your minor down." I hadn't really thought about it and then I was like, okay, well, what are my favorite subjects? Obviously, biology – I've always been bio-minded. I put in microbiology as my major, and genetics as my minor. And then I got my acceptance letter, and the letter said genetics major and microbiology minor. They'd swapped them. So, I called the admin office and they're like, "Yeah, we made an administrative error, it's going to be $40 if you want to change your major." So, I was like, oh, I guess I'm in genetics. And that's how I got into my field.

> **"I kind of accidentally fell into all the various now-strengths of mine. It was all definitely by accident."**

Many things happened to me in undergrad that were so ridiculous that kind of ended up placing me where I am. One of the things that happened after second year is that back in the day – I'm dating myself a bit – when you signed up for physiology and biochemistry, you couldn't do it on Beartracks. You had to go into their office and sign a form to enroll. So, I'm sitting there in the Biochem office and registering for the next semester and she just kind of offhandedly says, "Hey, do you want me to print off your schedule of your courses?" I'm like, "Yeah sure, that's nice, thank you." I get this printout – this is end of September of second year, a quarter of the way through the semester – and I see that I have been enrolled in Physiology 210 for a month. Nobody told me that actually they enrolled me in this 6-credit course, of which I'd missed a month, and I'm already in five classes and three labs, and then I added a sixth class because it was already past the drop deadline. So, I went to the office, and I was like, "You guys enrolled me?" and they're like, "Oh, yeah. Looks like no one ever called you." I'm like, "Yeah, can I drop then?" "No, sorry, it's past the add-drop deadline, you'll have to take a 50% refund and a W". So, all of a sudden, I was taking six classes and three labs in second semester.

I did okay because I kind of figured out what this whole university business was about. So, I kind of accidentally fell into all the various now strengths of mine, which was kind of funny, and then I decided to finish an honors project to get my honors degree in genetics. So, it was all definitely by accident.



What did you do after undergrad?

I finished my undergrad and then decided to just go off and go traveling for the summer. When I came home, I didn't really even know how to look for jobs in science. So, I went back to one of my undergrad profs and set up a meeting and said, "Well, what's going on? Can you help me, mentor me a little bit?" And he said, "Why don't you come to grad school?" At that time, I'd already decided I was going to continue traveling, at least for some larger chunk of time. So I was talking to my future supervisor – Dave Pilgrim – and I said, "I don't really

know what to do, I really like science and blah blah but I'm still not ready – I want kind of a little job, like a tech job, so I just make enough money to go traveling". He's like, "Will you come to grad school?" And it's like "No, I'm going away. I want to go away away." He's like "You can do both". And I'm like "I mean, I want to go for four months at least – away". He's like, "Yeah, that's no problem. I'll just take you off payroll for those four months". So, then I was like, this is a great opportunity for me to balance what I want to do – travel – and what I want to do – which is grad school. So, I picked my grad school lab because of that opportunity, which was awesome.

"I just wanted to share the information and get it out there."

I had worked with flies in my undergraduate honors project, and I really knew I really didn't want to work with flies, and I really liked the worm model system that we covered in my previous developmental genetics classes, and Dave Pilgrim worked in worms. So, I was like, okay well I want to work in worms, he's giving me this great opportunity to travel and balance going to grad school, and one of the major motivations was I get to wear sweatpants every day for the foreseeable future. I thought that was a great combination, so I decided to go into grad school. I think from an undergraduate audience, everyone who's an undergrad assumes that people who go and get higher level degrees – jobs that they're proud of – were super laser focused on what they wanted to do, and so much of me was just bumbling around and eventually landing on something that I enjoy.

I remember that same experience — there was a real defining moment for me where I realized, *oh, you don't have to be a genius to be a scientist. They teach you how to do it.* That wasn't until the last term of my degree that I realized this was an option and it's actually what I want.

Did you find that the academic pressure to publish was any different for you, knowing that you had other goals you wanted to accomplish?

I didn't feel the pressure to publish partly because I published in my first year. I got lucky in that I took on a project, which I asked to do – unbeknownst to me, [it] was a largely complete story from a previous graduate student and they needed a rework. We needed a more in-depth set of experiments. So I came into a really good background of a project, and then was able to put the final touches on it. My experiments ended up being the linchpin, it wasn't something like repeating a western and then it was done. There was all this background, hypotheses, and we didn't know what it meant. My set of first real experiments were like, this is what it means, it's a really big deal, so that was really lucky as I published right away. I think I was in my first year and a half by the time it was actually out. That probably took a lot of pressure off, and because I did a rework of the whole paper, I ended up with first author even though I hadn't done most of the physical experiments. I basically started and rewrote the whole thing, so that was good. But we didn't have a lab that had publishing pressure. It was really 'Just do good work and we'll find a place to publish it'.

My first one was Journal of Cell Science. So, I got a decent publication, and some decent conference publications early on, and communication awards. I sort of got the ball rolling really quickly just out of pure luck. I never really felt a pressure to publish because I didn't see myself ever as being a PI. I already knew then – I mean the job world has gotten even tougher and even 10 years ago when I finished my PhD the job market was really really really tough. I watched some of the postdocs in our lab and I was like, I don't want to be 45 and still doing a postdoc for a real job. I'm probably too ambitious to become a PI because there was too much of a delay – of a lag – and so the publishing pressure was more my pride. I just wanted to share the information and get it out there.

How do you think the public opinion of science has evolved over your career, and do you think that we as scientists have a responsibility to guide the conversation, whether it's our field or with respect to climate change or public health?

There are two sides to that. I think obviously with the Covid pandemic the reception of science information is that in a lot of ways, people are more willing. We hear about the Covid hoaxers and anti-vaxxers and those are really, really a small minority. The vast majority of people out there have learned a lot about how science is done, have accepted that early on we didn't think it was airborne and that's why masks weren't required and now they are, and I think most people understand that in an emerging pandemic the science is catching up. I think that in a lot of ways the public trust in science has gone up. But again, we don't hear about those people. We only hear about the squeaky wheels and the people who are going to be anti-science no matter what. The people who aren't getting vaccines are also the climate change deniers, and there's a lot of correlation between all those different things. So they're anti-science people – and they're very loud, but they're not the

majority. I think that, in general, people are more trusting in science than they were before.

I think the other thing is that throughout my career, scientists have gotten so much better at communicating. I remember early on even I had this attitude that the data should speak for itself, no one needs to speak for the data. That's changed significantly. I get requests all the time to run science communications workshops amongst the Canadian Forestry Services, and all these different organizations that help mentor other scientists. There's an appetite for learning how to communicate science that there hasn't been before. I think scientists do have a duty to speak for the data. I think it's too nuanced for the public to truly understand and, first of all, everything's behind a paywall so you can't get access to the primary literature anyways. I think there's a duty but also a responsibility on the scientists to really be okay with saying, "I don't know", and recognize that members of the public will hate you for it. They'll say, "Well, you're supposed to know everything, you're a scientist". But what makes me a good scientist is admitting that there are things I don't know, and that I can't speak on. And I think that's really fair. Again, we're not trying to appeal to the people who will never believe anything that comes out of my mouth. I can't care about them. I can care about the other 90 or 80% of the population who want to trust a scientist. Maybe they don't know how or why, but they want to trust that what the science is doing is real.

"What makes me a good scientist is admitting that there are things I don't know."

You mentioned that you got some communication awards at conferences early on – did that sow the seeds for you to then dive headfirst into this science communication realm? It seems like you have a lot of fingers in pies.

I do, yeah, I'm all over the place. You know, I think it was partly my first major international conference. I won a poster award, largely because of pure luck because the stuff I was interested in, unbeknownst to me, was really hot in the worm field with this embryo polarity stuff. So I kind of landed perfectly where everyone was curious about my work, and I do like talking. So it was suitable. I didn't really get it at the time. I was like, Oh, yeah, I got a poster award, I got a free book that I never read. But then people were like, "You got a poster award at the International Worm Meeting?" And I'm like, "Yeah, first year", and they're like, "That's a big deal".

But it was really teaching – being a TA – that made me realize that I liked sharing science as much as I liked doing science. I did like doing science, but I like talking about science more. I loved to TA, and I realized that that communication side is valuable. When I was finishing grad school, I realized that if you go on to do a postdoc there's almost no teaching, it's just pure research and a lot of admin. I'm really bad at admin, like I hate it with a passion. I just hate meetings, every fabric of me hates anything to do with admin. I looked at PIs a lot and it's just sitting through boring meetings. A lot of it is just managing personalities, I don't want any of that.

"I think that, in general, people are more trusting in science than they were before."

What do I like to do? I like to teach. I like to talk about science. I like to talk about my work, fine, but it is really boring. I also remember late grad school wishing I had the opportunity to learn more about other fields of science. I was getting really really narrow. My TAships were narrow. I asked to do a French course or a History of Science course and my committee was like, "You can't take any more extra courses". I wanted to still keep learning, and doing more, but I wasn't allowed so I had to figure out a way to be motivated to learn about other subjects and science in my own free time and that kind of started this science communications concept.

What actually started this transition into science communications was when I volunteered as a WISEST Grade 6 team leader for their conference. It's super fun, you just extract DNA out of bananas with these Grade 6 girls. My colleague and I were both doing a PhD at the same time and she just goes, "You're so good at just talking about science, you remind me of Jay Ingram from The Daily Planet". It was just a throwaway nice compliment, she's a wonderful person who gives all these compliments. But it just happens to be – all this luck – that Jay Ingram was giving a public lecture at the U of A a week later. So Kathy had said, "You'd be good on the Daily Planet with Jay Ingram", and I was like, Huh, probably would be, then this poster shows up in Biosci that he's giving a talk. So I went to the talk, and then I waited around and met him after and I was telling a little about me and stuff and he's like, "You should join my science communications immersive workshop in Banff this summer, I think you'd be a really good fit". So I did that for two weeks and that's kind of all she wrote.

Do you have any advice for undergrad students who are stuck on this traditional academic pursuit and maybe haven't considered a path like yours?

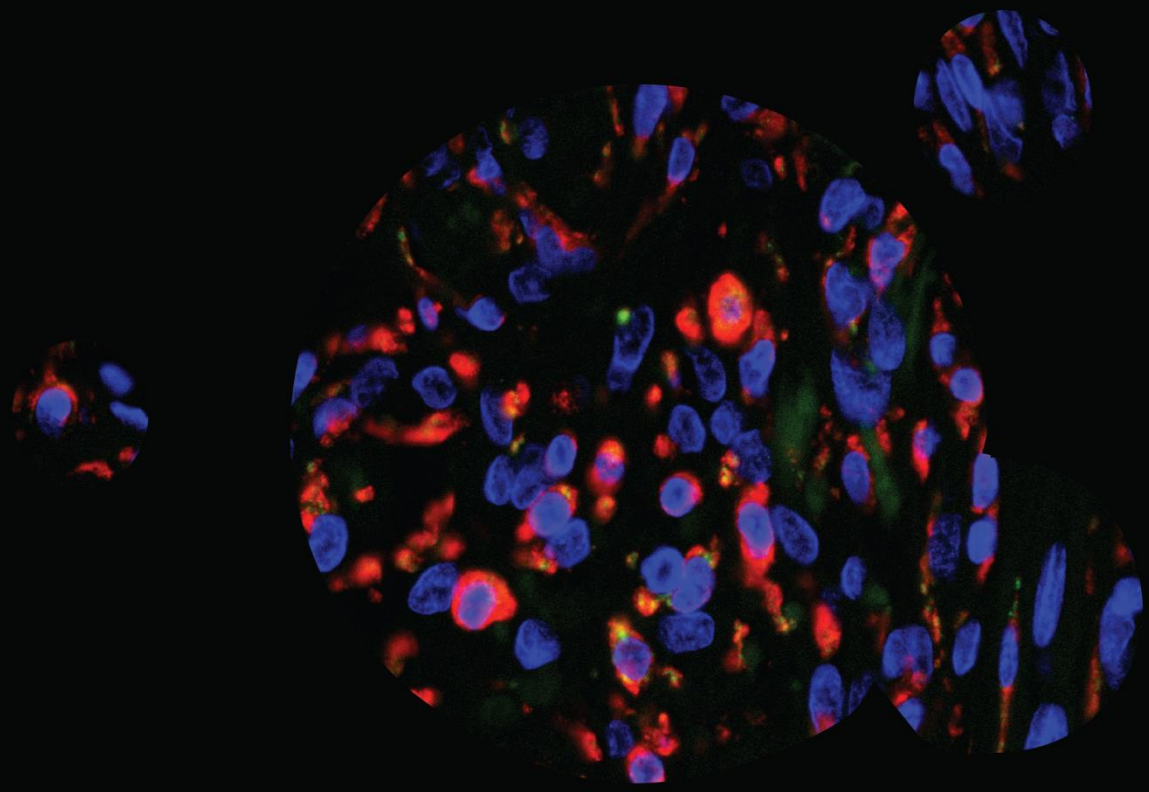
I would say, say yes. Say yes to opportunities that come along. You never know what they're going to be, whether that's presenting at a WISEST conference and you're nervous to do it, or presenting in the undergraduate conference with a poster, and you say, Well I don't really have any data – present a project poster. Do it, get yourself out there and just say yes to those opportunities that arise. Take courses that aren't part of your degree but you just really want to. Take five years to do your degree if that means you get to take courses that you love..

"Say yes to opportunities that come along."

It's a little overwhelming when you talk to undergrads and they ask, "What do I do? There are so many choices". You're going to have to accidentally find it, to stumble into this opportunity and you'll take some weird class and the prof will be really cool and you'll ask if they taught something else, and whatever the path is, it's not linear.

Right! You might really love ethnobotany, do you even know what that is? No, you won't, till you take it. I've always thrived in uncertainty. I think most people don't, but I don't mind a little chaos. I think high achieving science students really want certainty and they really want to know what to do, and it's really stressful if they don't. I totally understand that mentality, but at the same time don't let that paralyze you into not trying new things and finding what you might want to do.

It's hard to already know what you're going to be passionate about for the next 50 years of your life when you're an undergrad, that's not fair. There's a huge pressure that students feel, that they have to know what they want to do and if it's not medicine, then what are you doing in science? There's this ridiculous notion that everyone in science wants to become a doctor, particularly in my field of biology. Admitting that you don't want to do that, and you also don't know what you want to do, but you just are open to the opportunities that present themselves, is probably the biggest thing. The University has so many things to offer, and so many random courses. Maybe take biomedical engineering, maybe take basket weaving. I don't know. Just take them, right? And just say yes to those opportunities. Go to that intramural game and you never know what's gonna come out. I think so many people are obviously scared to take advantage of these opportunities, to put themselves out there. I get that. But academically, say yes. Just go for it.



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