THE UNIVERSITY OF HONG KONG

香 港 大 學

Laidlaw Undergraduate Research and Leadership Programme
Report Form

Please complete and return this form, together with a research attachment report, to the Horizons Office (laidlaw@hku.hk).
The report must be endorsed by your HKU supervisor.

Name:	Chen Ka Yee
Curriculum:	MBBS
Year of Study:	5
Research Attachment Details:	
Institution:	The University of Hong Kong
HKU Supervisor:	Dr. Amy Lo
Research Topic:	Preclinical Evaluation of Extra Virgin Olive Oil and Lutein in Extra Virgin Olive Oil Supplementation as a Treatment to Retinopathy of Prematurity.
Attachment Period:	27/5/2024-08/07/2024

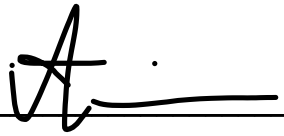
Report

Please provide two narrative reports of **1000 to 3,000 words or two videos of 3-5 minutes (with sub-titles)** describing the research activities undertaken during your Laidlaw Scholarship and your leadership development journey, which may include but need not be limited to the following:

RESEARCH REPORT (due by September 16, 2024)(extended to September 21)

Research

- Brief description summarizing the purpose of the project, hypothesis, methodology, procedures, principal results, and conclusions
- Difficulties encountered and how they were resolved
- Improvements that could be made if the project were to be repeated
- Impacts of the research beyond the classroom
- Suggestions and extensions for further study

Signature:  Date: 12 September 2024

REPORT 1 RESEARCH ACTIVITIES

Retinopathy of Prematurity (ROP) is a leading cause of childhood blindness worldwide. Its prevalence has risen due to the use of supplementary oxygen therapy in neonatal care for premature infants. This therapy creates a relatively hyperoxic environment, halting vessel growth in their retinas. Once other organs mature and therapy ceases, the resulting hypoxic environment promotes retinal neovascularization, potentially leading to blindness. While the dangers of such a treatment are manifest, it remains one of the main ways by which one can ensure the survival of premature infants. The reason for this is that a premature infant's lungs are severely underdeveloped, making breathing difficult and, as a consequence, reducing oxygen uptake and putting the infant's life at risk. Therefore, it is of the utmost importance that capital be dedicated to therapies that can work in conjunction with supplementary oxygen therapy so that both the infant's life and eyes are maintained. This study seeks to take a step in that direction by introducing a novel, noninvasive treatment that could save many children from premature blindness.

Current ROP treatments have various adverse effects, making nutraceuticals a promising alternative due to their relative safety and non-invasiveness. Nutraceuticals- any product derived from food that contains some form of extra health benefits in addition to the basic nutritional value found in said food- can manage reactive oxygen species generated during the hyperoxic stage, encouraging normal vessel growth. Lutein is a particularly promising option because of its neuroprotective and anti-inflammatory properties, and is approved by the FDA. Studies have found that lutein's bioavailability improves with olive oil (OO). Given the benefits of extra virgin olive oil (EVOO), such as its antioxidants and support for immune function, it is used instead of OO. It is for this reason that this study is of such import, as EVOO could be the silver bullet for blindness in premature infants globally.

Through the Laidlaw scholarship, I am privileged to research this topic under the guidance of Dr. Amy Lo and Miss Xiaoyuan Ye. The oxygen-induced retinopathy (OIR) model is an experimental research tool used to study the mechanisms of abnormal retinal blood vessel growth and degeneration and is well-established for studying retinal vascular pathology. Mice (wild-type C57B/6J) are born with immature retinas, similar to premature human infants. Their retinal vessels develop post-birth, allowing researchers to replicate conditions that lead to human retinal neovascularization. In the experiment, mouse neonates and nursing mothers remain in room air from day 0 to day 7, then are placed in an incubator with 75% oxygen from postnatal day 7 to day 12. They return to room air from day 12 to day 16. The high-oxygen environment mimics supplementary oxygen therapy, creating hyperoxia and vessel loss. The subsequent lower oxygen levels simulate conditions after ending oxygen therapy, leading to retinal vessel proliferation.

To investigate the effect of lutein-in-extra-virgin-olive-oil (LEVOO) in preventing retinal neovascularization in OIR, lutein doses of 2 μL/g, 4 μL/g, and 2x4 μL/g are administered daily to mice from day 12 to day 16. EVOO serves as the primary solvent, with comparison groups using OO and water. For OO, the control ensures no additional weight gain in mice. Water acts as a volume control. Mice are sacrificed on day 17 for retinal flat mounts, which are stained and imaged using fluorescent microscopy (Eclipse 80i, Nikon, Japan). Images are randomly taken from 8 quadrants. To quantify neovascularization, images are analyzed using ImageJ. Tip cells, indicative of the condition, are marked based on criteria such as proximity to avascular areas, pointed ends, and the tip of vessel sprouts. Adjustments in brightness, contrast, and color on ImageJ help identify tip cells. The number of tip cells per mouse retina is averaged from the 8 photos. Data analysis uses mean ± SEM and is presented in a bar graph. Version 4.4.0 is used for tip cell data analysis. Previous analyses employed unpaired t-tests or one-way ANOVA followed by Dunnett's post hoc test (SPSS).

Results indicate that peroral LEVOO consumption does not affect body weight in the OIR model. An increase in body weight would be a sign of confounding factors that could affect the study's validity. That is to say, maintaining a stable body weight helps isolate the effects of LEVOO on the targeted physiological processes targeted in this study. Earlier analyses suggested that peroral LEVOO improved retinal function, with dosage positively correlating with function, quantified by b-wave amplitude in flat mount images. The optimal EVOO dosage was determined to be 4 μL/g/day. However, when the experiment was repeated with LEVOO concentrations of 0.4 μL/g, 1.8 μL/g, and 3.6 μL/g, and retinal function quantified by tip cell number, no significant differences were observed between EVOO and the comparison groups. There are two conclusions which can be drawn from this discrepancy. Firstly, the discrepancy between the two data sets may be attributed to data collection errors, as precise tip cell marking demands highly skilled researchers. Secondly, it is possible that LEVOO does not produce a statistically significant improvement in retinal function. Both points highlight the need for repeated experiments to accurately determine the correct conclusion. Further discussion on data collection errors will be addressed in another report.

This investigation underscores both the potential and challenges of using LEVOO for ROP treatment. Developing better ROP treatments is crucial, especially in regions with high premature birth rates. Traditional treatments like laser therapy, cryotherapy, anti-VEGF medications, and vitrectomy can adversely affect the eye and body, imposing financial and physical stress on children and families. Alternatives such as laser therapy and cryotherapy, while effective in halting abnormal blood vessel growth, often cause collateral damage to healthy retinal tissue, potentially leading to vision loss or other complications. Anti-VEGF medications, although less invasive, may require multiple injections directly into the eye, which can be both physically uncomfortable and carry risks of infection, inflammation, or retinal detachment. Vitrectomy, a surgical procedure to

remove the vitreous gel, is invasive and carries its own risks, including bleeding, infection, and even blindness in severe cases. Thus, LEVOO provides hope for families seeking alternative treatments to alleviate children's suffering. Reducing childhood blindness due to ROP enhances the quality of life globally. This study exemplifies the integration of nutraceuticals and supplements into medical treatments, fostering innovation in medical research and neonatal care.

REPORT 2 LEADERSHIP DEVELOPMENT

Spending my summer on Sassoon Road to acquire laboratory, digital and presentation skills has been an excellent experience that is highly beneficial to my future. Within these 4 weeks, I learned microscopic dissection skills by performing retinal flat mounts on mouse eyes, skills as a researcher in the laboratory including imaging, IHC staining, and paraffin sectioning, as well as presentation skills in multiple presentation sessions. My acquisition goes beyond skills per se by thinking through and reflecting upon all the techniques I was taught in the laboratory. I realized that as a researcher, one must maintain curiosity, as well as perseverance not only in the process of carrying out experiments but also in critical thinking. Many mistakes were made during my attachment, either taking bad images or cutting the paraffin sections too thin. However, the difficulties I encountered helped me sharpen my skills and pushed me to take initiative to search for solutions. The passion to do better in research drives me to resolve all the problems I encountered in the journey, and thus I understand what "leadership" means in a research context: to be able to motivate oneself in finding answers to every question in mind, while possessing the ability to organize and communicate for the purpose.

Day 1 on retinal flat mount
My work in the first week was to practice my dissection skills under a microscope by preparing retinal flat mounts. The technique is performed by removing other ocular structures in mouse eyes while keeping the retina. The retina is diagonally incised into 4 quadrants to allow it to be flattened and mounted on glass slides for the following procedures. A mouse eye is about 3.0 to 3.5 mm, and thus flat mount must be performed under a microscope. I encountered one significant problem during my initial attempt, which was the problem of light reflection on solutions. Eyes are put in PBS for dissection, but as the source of light comes from above, intense reflections distorted my view. I took this chance as an opportunity to learn and seek advice from my supervisor, and the problem was resolved by turning down the light intensity, as well as using light from below.

Separating retina from other tissues is another difficulty. Due to the difference in storage conditions and physical variations of mice, eyes have varied consistency, making them prone to breakage when the eye is too soft or too hard. Retina is also an exceptionally fragile structure, from which I would need to separate the choroid, and thus I had to be exceptionally careful. However, I proactively sought advice from my colleague who was in the same program and had better microscopic skills, as well as guidance from my supervisor, and obtained both on-site guidance and online teaching materials. I ended up succeeding on day 22.

While difficulties with IHC staining and paraffin sectioning existed, they were mostly resolved by the clear guidance from my supervisor. However, I encountered a completely new challenge when I was doing imaging and data collection, which were my main roles in assisting Miss Xiaoyuan's study. To briefly introduce the

research, it is investigating whether lutein-in-extra-virgin-olive-oil (LEVOO) can be an alternative to traditional invasive treatment in retinopathy of prematurity (ROP) by using mice with oxygen-induced retinopathy (OIR) as a simulation of human premature babies who are given supplementary oxygen therapy after birth and suffer from ROP after the therapy. The experiment is done on mice with different concentrations of LEVOO, also with a control group which purely takes lutein in olive oil (OO) or water. Mice are put in an incubator with oxygen saturation of 75% from day 7 to day 12 after birth to mimic oxygen supplementary therapy in premature babies; they are given lutein in different solvents daily from day 12 to day 16. They are then sacrificed on day 17. Their eyes are dissected to prepare retinal flat mounts for staining and imaging. The hypothesis is that LEVOO should improve retinal function compared to lutein in other solvents. There are several ways to quantify retinal function. In the first phase of the study, Xiaoyuan, the principal investigator, used ERG to discover avascular regions and neovascularized regions on flat mounts, and the result shows that LEVOO will decrease neovascularized area and hence improve retinal function. However, we later found ERG is the most accurate in finding neovasculararеas, and thus we tried to count tip cells on retinal flat mounts directly, as increased tip cell numbers indicate neovascularization. In the project, I contribute by taking images for retinal flatmounts, then calculating and performing data analysis on tip cell numbers.

Although there are standard ways of doing image taking and calculation, critical thinking in the process is still possible and can potentially benefit future research. In the process of image taking and tip cell counting, I discovered that there are steps that are prone to errors, and initiated discussion as well as raising advice on how to do the procedures better. Firstly, for image taking, I realized that the current imaging method may not be the best, as it is not randomized. In image taking, photos at the intersection of avascular regions and neovascularizedregions from random non-overlapping 8 quadrants will be taken and the average number of tip cells in these regions will be calculated. Having participated in other research before, I realized it is difficult to randomize photo taking as image taking is inevitably biased when the procedure is done manually. Seeing the problem, I reached out to my supervisor for solutions. As historically most researchers took photos manually, I suggested demarcating intersecting regions between avascular and neovascularized regions, and potentially building a model so 8 photos can be randomly taken in the region. I then organized my idea and presented it to my colleagues who are good at building models to seek advice and provide inspiration for the work of other researchers. Secondly, for tip cell counting, there is a scientific consensus on identifying tip cells based on morphology and methodology. However, I discovered multiple problems when counting tip cells. On the one hand, shapes of tip cells are strongly affected by the quality of photos, including brightness, contrast, and colors in the camera settings. On the other hand, according to guidance, tip cells should have filopodial extensions, but in real practice, vessel sprouts without pointed ends are counted as tip cells as well because the extensions may not be well-captured in the photo. I thought the

problems through and realized others may encounter the same difficulties in counting tip cells. And these problems may ultimately lead to errors in tip cell counting. I took the initiative to ask my supervisor and try to adjust my tip cell counting method. At the end of my research attachment, I organized my questions for tip cell counting and included them as a vital part of my presentation, to raise concerns to them and help other researchers reflect upon the problems.

Leadership in research is seemingly different from the traditional definition of it: In other fields, leadership is mainly about teamwork and the interactions between people. Instead, in research, leadership is about proactively navigating through problems, communicating with more knowledgeable individuals, and if possible, organizing and taking the lead to make a change to the problems everyone is facing and waiting for betterment. The attachment provided me with a great chance to spot the difference and shape a personality in me that is not just a team worker but also a researcher who can work individually. After the attachment, I will continue to contribute to the team for data analysis and collection in retinal function, to continue the learning of research skills and leadership soon.

TO BE COMPLETED BY HKU SUPERVISOR

I have examined the research report and rate the student's performance as satisfactory / unsatisfactory (*Please circle whichever is applicable*)

Comments:

Christina has gained a lot of hands-on experience in microsurgical techniques and image analyses. Despite the short time in the laboratory, she had mastered the techniques. More importantly, she had critically assessed the methodology and provided valuable suggestions on optimizing the protocol in tip cell counting. This was a significant contribution to our research. I am very happy that Christina has been trained to be a critical researcher, a quality that is important in being a clinician-scientist in ophthalmology.

Altogether, Christina has performed well in the laboratory despite the short time. It was a pleasure to have Christina as part of my research team.

Name: Prof. Amy Lo Signature:  Date: Sep 20, 2024