Cell Culturing Techniques and Comparative Studies: Seeking Understanding

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Introduction

I can say with confidence that summer 2023 was by far the greatest experience of my life. After completing a course in science communication, I was enabled by the Texas A&M Science Influencers program to pursue a summer internship. Massachusetts Institute of Technology (MIT) has been a dream of mine for as long as I can remember, and I now had the opportunity to attend the institution. With help from Drs. Holli Leggette, Gary Wingenbach, and Barbara Gastel, I identified a lab that would challenge me academically as well as an individual. After multiple emails and some Zoom interviews, I was confirmed to work under Dr. Qin (Maggie) Qi in the chemical engineering department. While I had prepared myself to leave, the transition from Houston to Boston was still difficult. Across the country, 1,861 miles away from everything I've ever known, I immersed myself in the lab.

Background

Upon my arrival, we outlined our objective for the summer. They were defined as cell culturing, in vitro characterization of cells and quantitative analysis, immunofluorescence, cell functions, and theory driven predictive models. To jumpstart my cell culturing training, I began with maintaining L-Cells; L-cells are extremely resilient making them great for beginners. While training, it became clear to me how much time and dedication is necessary for success in the lab. An adjustment was learning that success presents itself differently in research than other scenarios. Maintaining healthy samples is a victory within itself, and the collaborative feel of the lab made the small victories more rewarding.

After proving my proficiency with the basic cell culturing concepts, I transitioned to more delicate cells. ARPE-19 cells (arising retinal pigment epithelia, Human Choroidal Endothelial Cells, and Human Retinal Endothelial cells were the final stage of familiarization before moving onto my final project. Learning the operations of a biology lab was one of the more difficult tasks I've completed, particularly regarding contamination. The cells are living, and therefore function in their own time. If they are not monitored/maintained correctly, they will die and render all experiments useless. There is also the risk of contamination which will hinder all experiments. To ensure proper observation, I would organize images of each sample in a comparative outline on PowerPoint. The PowerPoints would be submitted to Dr. Qi along with my observations; this was critical in my development as a researcher. PowerPoints also provided an unexpected aid in the outreach aspect of my internship. I found it was much easier to guide

someone to understanding through visual aids in which they could directly see the differences as opposed to attempting to describe the differences in very technical terms. During my attempts to describe my research it became evident how there was a preconceived notion contributing to mental isolation during the conversation. Those with no technical background seemed to mentally distance themselves during the conversation regardless of how understandable the concepts were. Curiosity is a slowly fading trait, and a challenge I decided to take on.

Methods

The main project of my internship was a collaborative study between MIT and Tufts University. All the training I experienced led up to my personal research with HBEC 5i (Human Brian Endothelial Cells) and the adherence of malaria infected red blood cells. Researchers from Tufts needed confirmation of cell adherence to brain cells through microfluidics analyses, manipulating fluids on the scale of a few to hundreds of microns. My role was maintaining the cells for experiments and analyzing the cell morphology (cell shape/structure) as they reached confluency. The main deliverable of my internship was cell culturing techniques, which I had become proficient in. Next, the best way to analyze cell morphology is through immunostaining, which is performed by first fixing the cells, permeating the cells, then adding the stain to highlight desired areas. My common immunostains highlighted the cell nucleus, and cytoskeleton. The most important stain to my experiment, however, was Tight Junction Protein Expression. Tight Junction Proteins (TJP) are present in all endothelial and epithelial cells. They regulate the passage of molecules and ions through a cell, and the 2 specific components I analyzed were Claudin-5 and Occludin. A key component in understanding TJP expression is realizing that TJP expression only occurs when cells are touching. This means that to analyze TJP, cells must be confluent before fixing (this kills the cells for staining)- even then, it is not assured that the TJP expression will be captured. The intention behind cell culturing proficiency was to prepare for immunostaining techniques that were used for a deeper understanding of the cell functions. This allowed me to perform both a qualitative and quantitative analysis on the cells when analyzing TJP.

TJP is so important to this experiment because Malaria infected red blood cells break down the TJP therefore rendering the cell helpless. The malarial parasite we were specifically studying was cerebral malaria, which is also the deadliest form of the disease. If untreated, it will result in death by herniation of the brain. Even if it doesn't result in fatality, the brain will be severely damaged therefore impacting the quality of life of the individual. The goal of this research is not to prevent malaria, but rather understand how the parasite invades the cells to develop a treatment.

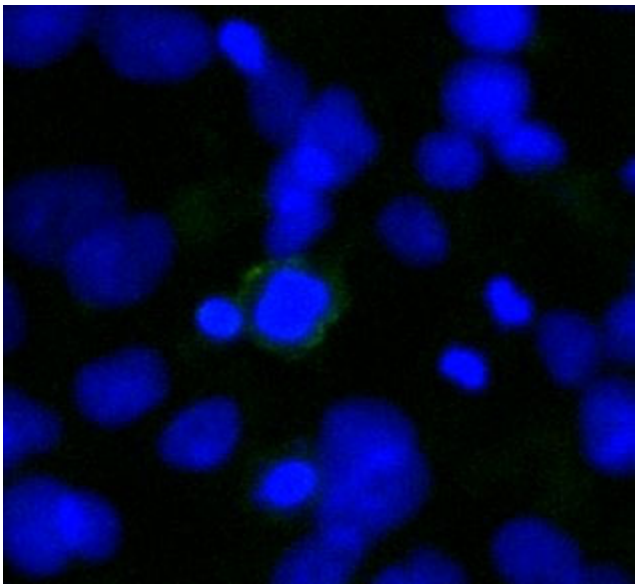

Figure 1: *Pictured is the characterization of Tight Junction Protein (TJP) in HBEC 5i cells. Blue is the cell nucleus, and green is the Occludin TJP expression.*

Results

All my training came to fruition for this project, and I was able to both quantitatively and qualitatively analyze TJP expression for the cells. I used the data I collected to develop an optimized protocol for culturing HBEC 5i cells. This was done through a comparative study in a 6-well plate using different cell medium (nutrients for the cells) and attachment factor. I organized the well plate with both independent and dependent variables with cell confluency and morphology as the basis for success. This outline was based off my previous training in cell culturing techniques and deeper understanding of cell functions. I captured images each day and compared confluency percentage for a qualitative aspect. My immunofluorescence protocol was adjusted for the brain cells and played a key role in my analysis of the cells. The images I captured were posted on the Science Influencers social media and had much more interaction when compared to previous posts. This can be attributed to the appeal of colors and initial drawing to photos. My entire model was derived from my observations of Bob Zhang and his theory driven projects.

Conclusions

I gathered all the data and pictures I had been collecting into a PowerPoint that I presented to my group at the end of my internship. A key point was 2 of my samples were contaminated leaving me with only 2 samples to compare. However, I was able to conclude that the given protocol is the optimal procedure for cell growth and Information. This conclusion is based off an analysis comparing TJP expression intensity quantified through ImageJ technology. My results verified the optimized protocol suggested in literature and will be used in future experiments in the Qi Lab. I feel such gratitude to Dr. Qi, my mentor, Bob Zhang, as well as Dr.

Gary Wingenbach, Dr. Holli Leggette, and Dr. Barbara Gastel for their unconditional support. While this was one of the most difficult experiences of my life, it has also been the most rewarding. I've been able to relate my understanding of science communication and newfound understanding of chemical engineering concepts to disseminate information in both a technical and unambiguous manner.

I made incredible connections not only with the individuals at MIT, but those in the Boston area. I was able to explore surrounding cities such as New York City, Cape Cod, Nantucket, Providence, RI, and of course, Boston. I experienced significant growth both as a researcher and an individual, and I am eternally grateful. If I could relive the experience again, I would.

Future Directions

For future experiments, I would place a higher emphasis on preventing contamination due to its effect on my data. I was able to gather enough data before the contamination to verify my hypothesis, but I hoped for more definite results. However, the basis for the research is something I am extremely passionate about and am grateful to have contributed to through the *Science Influencers* program.

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