Communicating Research to the General Public

The WISL Award for Communicating PhD Research to the Public launched in 2010, and since then over 100 Ph.D. degree recipients have successfully included a chapter in their Ph.D. thesis communicating their research to non-specialists. The goal is to explain the candidate's scholarly research and its significance—as well as their excitement for and journey through their area of study—to a wider audience that includes family members, friends, civic groups, newspaper reporters, program officers at appropriate funding agencies, state legislators, and members of the U.S. Congress.

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MECHANISMS OF NEUTROPHIL MIGRATION THROUGH CELL DENSE TISSUES

By

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CHAPTER 7

WISCONSIN INITIATIVE FOR SCIENCE LITERACY PUBLIC CHAPTER

The purpose of this chapter is to convey my dissertation research to a non-scientific audience, funded through the Wisconsin Initiative for Science Literacy (WISL).

HOW OUR IMMUNE CELLS MOVE THROUGH CELL-DENSE LIVING TISSUES

PRELUDE

“So what do you do?”

When I describe the fifteen-year post-undergraduate training timeline required to become a physician scientist, it is, fairly, met with a bewildered look: one that implies that I am either mistaken, joking, or insane.

“So you’re going to be a physician?”

Me: *“Yes!”*

“So… why are you getting a PhD?”

The oversimplified simple answer of: *“To do research!”* is often met with less excitement. This is likely because, at the start of training, the fundamental purpose of a Ph.D. can be unclear to us students, limiting the ability to communicate the skillset that one truly develops during Ph.D. training to a public audience.

Broadly, the goal of the combined (M.D./Ph.D.) degree is to blend knowledge of the human body acquired in medical school with research skills developed in graduate school to uncover new treatment strategies for human diseases. In my Ph.D., I learned tangible skills of how to build tiny cell-sized (i.e. microscale) systems to study cell migration, how to perform high resolution microscopy, and how to edit the genes of living organisms. When starting as a graduate student, we often cite the development of these

sorts of skills in explaining the purpose of getting a Ph.D. However, now at the end of my graduate training, I cannot help but recall the words of a professor I had my sophomore year in undergraduate training at the University of Minnesota, Dr. Jonathan Sachs:

“Some of you will go on to graduate school, thinking that you are learning how to do science. However, a Ph.D. is not a degree in science, it is a Doctorate of Philosophy (Ph. D.). *What you are really learning is how to reason, how to think*.”

The irony is of course that us graduate students rarely come to this realization until we have finished the Ph.D. This is because all our prior schooling has been working towards a fundamentally different goal than that of a Ph.D. dissertation. As an undergraduate, we are tasked with learning a lot of new information in many different fields. Transitioning to medical school, the amount of information gets exponentially greater and the time to learn it much shorter. Throughout this time, while intellectually and emotionally challenging, the goal remains well-defined, to learn as much information as you can, and acquire as many skills as you can, so that you can one day apply that knowledge to save lives. This goal is tangible; you know whether you know enough based on your performance on tests. And it is bound; there is a fixed number of diseases one can have, and a fixed number of treatment options, both bound by our current state of medical knowledge.

The goal of a Ph.D. is very different. Instead of learning and applying existing knowledge, you are tasked with *creating new knowledge* about the world. This goal is

completely intangible; you have no idea whether you are on the path to creating new knowledge or not. And it is completely unbound, as what knowledge you create is up to you as the scientist. The nature of this goal challenges our notion of what “school” is. While you might be really good at microscopy, genetics, or anatomy, none of those skills, by themselves, will enable you to create new knowledge without learning new skills in reasoning. That is, how to narrow your thinking into very precise questions that are answerable with the right tools. Where the path for success in medical school might be long hours in the library, the path to success in graduate school is learning how to ask very clear, well-defined questions, and design experiments that definitively answer those questions. Stated simply, *where medical school teaches you how to yield current knowledge to save lives of those that will outlive us, a PhD teaches one how to yield the tenets of logic that preceded us, to uncover new truths about the world that will outlive us.*

I therefore use this chapter to not only describe what new knowledge I discovered during my Ph.D., but also to reflect upon the reasoning strategies underlying my approach. I’d like to thank the entire staff at the Wisconsin Initiative for Science Literacy (WISL) for the opportunity to draft this chapter, and specifically Elizabeth Reynolds in editorial work critical to its completion.

To communicate different scientific approaches used in my Ph.D., I begin with an analogy.

BACKGROUND

APPROACHES TO DISCOVERING NEW KNOWLEDGE: A TALE OF TWO FISHERMAN

The endless number of discoveries that can be made about nature result in many different approaches to doing science. As a Minnesota native, I cannot help but appreciate the common comparison of the scientific process to fishing. In this analogy, fish represent new discoveries. While the goal of catching bigger, or totally new fish is well-defined, the methods to accomplish this goal, for example the combination of lures, time of year, or fishing spots is unbound. This models distinct strategies employed by scientists of different backgrounds, for example engineers or biologists, to discover new knowledge about nature. Engineers design fundamentally new tools that enable new discoveries, and biologists yield established knowledge to form hypotheses that they can evaluate experimentally to methodically arrive at a new discovery.

Consider two fishermen, aiming to catch a new species of fish in the lakes of Minnesota:

Fisherman 1, Eunice, is an engineer, endowed with an in-depth understanding of how different lures work. Tasked with catching a totally new fish, she figures a need for a totally new tool. She knows, from previous fishermen, that plastic worms work wonders in

catching new fish in Canada, where plastic flukes have caught many new species in nearby waters of Wisconsin. Each of these lures behave differently in the water, depending on their material composition. She therefore designs experiments to understand the advantages and limitations of each lure, using her results to create a totally new lure maximizing the advantages of existing tools. Over years, she uses her new lure to try to catch a new species in the lakes of Minnesota.

Fisherman 2, Bob, is a biologist. He is well versed in the mating patterns and history of how different species arrived in lakes throughout the country. He knows, from previous fishermen in Canada, that new species tend to spawn in deep waters, where new species in Wisconsin tend to spawn in shallow waters. Given that Minnesota lakes share similarities with these regions, he designs a set of experiments to analyze the mating characteristics of fish in Minnesota. Over years, he wakes up at the crack of dawn to quantify mating patterns of existing species in shallow water and deep water, closing in on the specific depth for a new species.

After many years, both fishermen catch the new fish. It turns out that this new fish prefers the new lure designed by fisherman 1, and resides at the depth identified by fisherman 2. Both strategies led to new discoveries, both based on past knowledge. However while both fishermen tend to swear by their specific method, a combined approach of both tactics would have yielded the fastest results. The engineer knew how to create a novel tool (the new lure), yet not where to direct that tool in the most efficient manner (the proper

spawning spot). The biologist knew where the new fish would be, but only after a methodical series of experiments testing where similar species were, all the while fishing with an existing lure preferred by existing fish.

Both fishermen tend to swear by their method, as is often the case with engineers and biologists more broadly. The reality is both have advantages and both have limitations. Engineers might complain that heavy reliance on existing knowledge to form hypotheses is both slow and limits the ability to make fundamentally new discoveries (that prior knowledge could have never predicted). On the other hand, biologists state the need for hypotheses to organize our thinking to know we are moving towards a new discovery, and that this is needed to avoid years of fishing with cool new tools but in the totally wrong spot, therefore catching nothing. As a trained engineer in my undergraduate years, I had the opportunity to learn hypothesis-driven biology research for a year at the NIH in Washington D.C. before starting medical school. And I was bad at it. Learning how to form and evaluate hypotheses really is a skill that takes years to develop. Given a desire to learn both approaches, I chose to be co-advised by both an engineer, Dr. Dave Beebe, and a biologist, Dr. Anna Huttenlocher for my Ph.D. This enabled exposure to the tactics of both fishermen, which I then yielded to answer questions about how cells work over the next four years of Ph. D training.

DEFINING THE QUESTION: HOW DO IMMUNE CELLS MOVE THROUGHOUT THE BODY

The first step to uncovering new discoveries is defining a broad question to investigate. In my case, I was interested in how immune cells move throughout the body to fight disease.

The journey of immune cells throughout the body

Immune cells must travel all throughout our body to fight infections and help us heal from tissue damage. This could be minor damage like a stubbed toe, or major damage like a heart attack. In either case, the first immune cell to arrive at the scene is the neutrophil, the most abundant cell in the immune system, which circulates throughout our blood vessels surveying the body for infection or injury. Let's consider the journey of neutrophils with an example we're all too familiar with: a paper cut. In this scenario, the damaged skin cells produce molecules that indicate that tissue damage has occurred. Neutrophils in nearby blood vessels immediately sense these molecules, and start to exit the vasculature, squeezing through tiny gaps in our blood vessels to get into the skin. Once out of the vessel, neutrophils then start their journey through the skin, pushing and pulling their way through skin cells to reach the papercut and begin the healing process.

Diseases that impair the ability of neutrophils to migrate throughout our tissues result in poor wound healing and recurrent infections, often resulting in an average life expectancy of 8-10 years old. On the other hand, too much recruitment, that is excessive

accumulation of neutrophils in places they shouldn't be, is implicated in autoimmune diseases like Lupus, Crohn's disease, or Multiple Sclerosis. Therefore, scientists have spent decades trying to develop drugs that control immune cell migration. These endeavors have proven useful in the context of treating human diseases. By understanding the molecules used to attach to and exit the vasculature, scientists have developed FDA approved drugs that keep neutrophils in the blood vessels to treat autoimmune disease. That said, exiting the vasculature is only the first part of an immune cell's journey. They then must push and squeeze through surrounding cells in a process termed "interstitial migration" (**Figure 1**). How they do this is not well understood, setting up the broad question of my research. How do immune cells move through cell-dense tissues during interstitial migration?

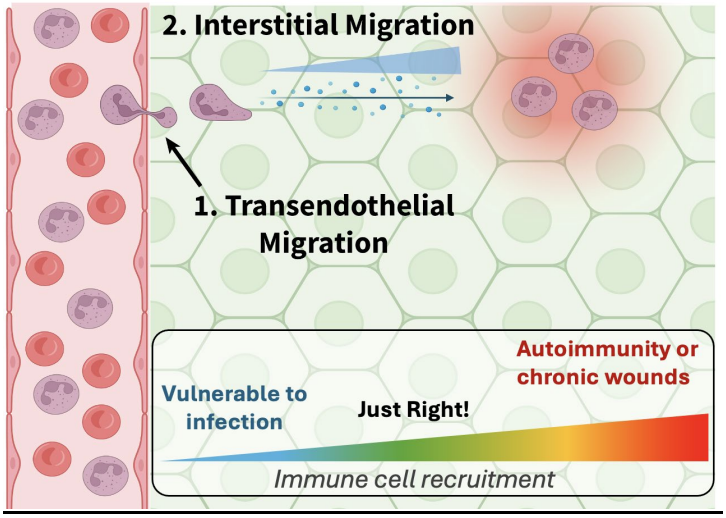

Figure 1: Schematic of a neutrophil's journey to sites of inflammation. In the context of tissue infection or injury, neutrophils exit the vasculature in a process termed "transendothelial migration" before migrating through surrounding cells termed "interstitial

migration.” In the case of a paper cut, these surrounding cells would be skin cells. In the case of a heart attack, they would be heart cells. Too much recruitment is implicated in autoimmune conditions; however too little recruitment leads to poor wound healing or infection clearance.

Current tools to study interstitial migration

The ability to answer this question depends on the quality of tools available to study interstitial migration. While many scientists often use mice as an animal model to study disease, the skin of mice is opaque, limiting the ability to image the movement of immune cells using microscopy. Our lab has instead spent decades inserting fluorescent molecules into the genome of immune cells within the larval zebrafish, which has clear skin, allowing us to image the movement of immune cells throughout the organism. Because I was interested in interstitial migration, I used a fish that had fluorescent skin cells and fluorescent neutrophils to visualize immune cell migration through the “maze” created by surrounding cells (**Figure 2a-b**).

In addition to using animal models, engineers have developed systems to study interstitial migration in the laboratory outside of an animal’s body. Most commonly, they build tiny microchannels that immune cells must crawl through, which mimic their migration between surrounding tissues (**Figure 2c**). An advantage of this approach is that scientists can use human cells isolated from blood donors rather than fish neutrophils, which might make findings more relevant to human disease. However, a disadvantage is that these microchannels are made of very rigid materials, which do not replicate the soft

cellular environment within living organisms. This change in stiffness matters, as immune cells can sense the mechanical forces in their environment, which impacts migration strategies. Thus, replicating the physical forces present during interstitial migration in living organisms is important. For that reason, I sought to construct microscale channels bound not by rigid materials, but by a soft liquid-walled interface that replicates physical properties of surrounding cells during interstitial migration (**Figure 2d**).

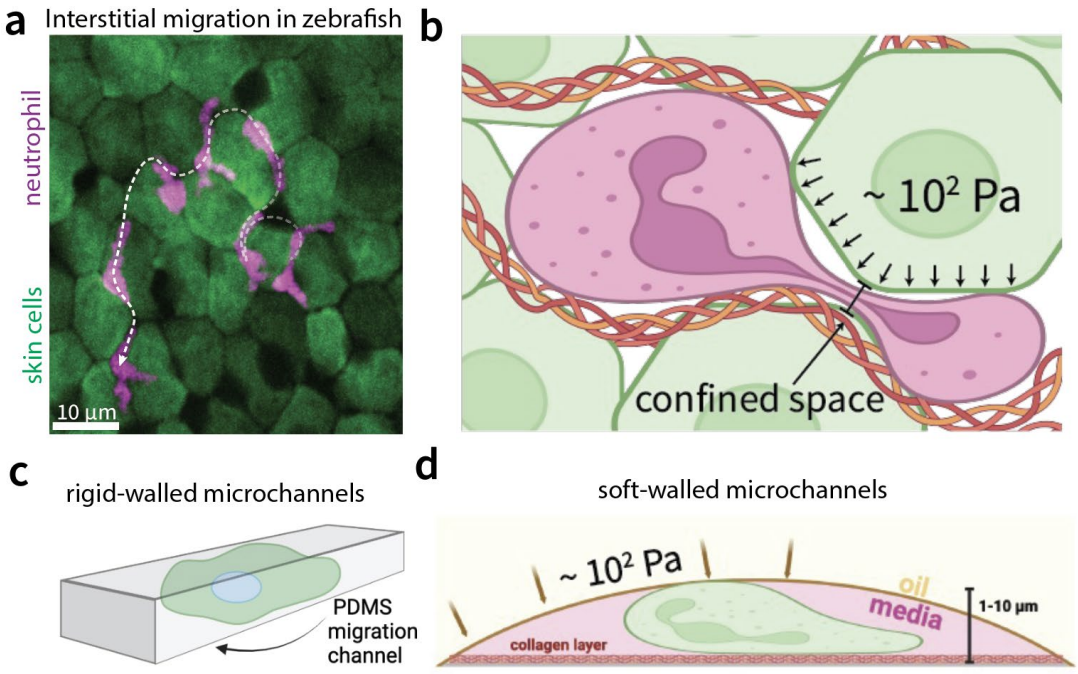

Figure 2: Tools to study interstitial migration. a) Path of a neutrophil migrating between skin cells in the zebrafish. **b)** Schematic depicting neutrophil (magenta) migration through skin cells (green) that are soft (~100 Pa stiffness) and deformable. **c)** Existing rigid-walled microchannels to model interstitial migration made from a material called PDMS. **d)** Schematic of liquid-walled microchannels, made of an oil-media interface, which exerts similar forces to that of surrounding cells (~100 Pa).

Cells as tiny machines

While off to a solid start, with initial tools in place, the question of “how do immune cells move through cell-dense tissues” is too broad. That is, what do I mean by “how do cells move”?

In order to narrow the question to become experimentally testable, we must understand existing knowledge of cell migration. In the 1970s, scientists began to study how cells move on flat petri dishes, learning that they are tiny machines made of mechanical components like the pistons of a car, which migrate towards chemical cues in the environment like humans do to the smell of cookies. I specifically like the analogy between how cells and humans move. The first step to moving towards freshly baked cookies is extending our leg forward, thereby generating “expansive force.” We then link that foot with the environment, the friction between our foot and the floor resulting in “tractional force,” that then will enable forward motion when we contract the muscles in our trailing leg to push ourselves forward, termed “contractile force.” Immune cells move in a similar way, coordinating expansive, tractional, and contractile forces together to generate forward motility towards chemical cues produced by injured or infected cells. Instead of using arms, legs, and muscles, they use tiny molecules called actin and integrins to generate these forces, described in the following section.

How cells generate migration forces: Actin and Integrins

To push the cell membrane forward along a flat surface, cells rely on chemical reactions to control the growth of rigid fibers made of a molecule called actin. These fibers can be thought of like tall Lego towers, with each actin molecule representing an individual Lego block. Chemical reactions within the cell drive the addition of new blocks to heighten the tower, or removal of individual blocks to shorten the tower. In the case of a cell, these fibers point outward against the cell membrane, such that addition of actin molecules results in growth of the fiber, resulting in outward, expansive forces pushing the cell membrane forward.

Just as our front leg is then attached to the floor through friction forces, these actin fibers are linked to the substrate (the floor that cells walk along) via molecules called integrins. Integrins act as linkers between actin fibers within the cell to molecules on the substrate, forming adhesions with the outside environment. These adhesions, like the friction between our foot and the floor, generate tractional forces that enable forward motion when the whole cell contracts. All that said, these studies have largely been performed on flat surfaces in the laboratory, a very different environment from three-dimensional, densely packed cellular environments that make up tissues within our body. I sought to build upon this established knowledge by narrowing my research question to ask: “How do neutrophils generate expansive and tractional forces to migrate through three-dimensional cellular tissues?”.

There are now two approaches to answer this question. Fisherman 1, the engineer, might design a totally new tool to study how cells migrate in the face of mechanical resistance. Fisherman 2 might use existing tools, in combination with prior knowledge from how other immune cell types move, to test hypotheses of how cells generate these forces during interstitial migration. During my Ph.D. I took both approaches, which I describe below.

RESULTS

FISHERMAN 1: DEVELOPING NEW TOOL TO STUDY IMMUNE CELL MIGRATION

How the physical environment regulates migration forces

In the body, immune cells do not move along flat petri dishes. Instead, they are constantly surrounded by other cells, which act as mechanical barriers that push against immune cells in three dimensions (**Figure 3**). These forces squish, or “confine” cells. In the 2000s, engineers leveraged fluid mechanics principles to construct tiny devices that squish, or confine cells. This laid the groundwork for discoveries into how cells sense confining forces to modulate intracellular processes, resulting in the Nobel Prize awarded in 2021. These systems have since been applied extensively to understand how confining forces regulate cell migration strategies. That is, how cell mechanical confinement alters the balance of contractile, expansive, and traction machinery to enable cell migration. In general, when squished by the environment, cells rely less on tractional forces and greatly on contractility to squeeze through tiny gaps.

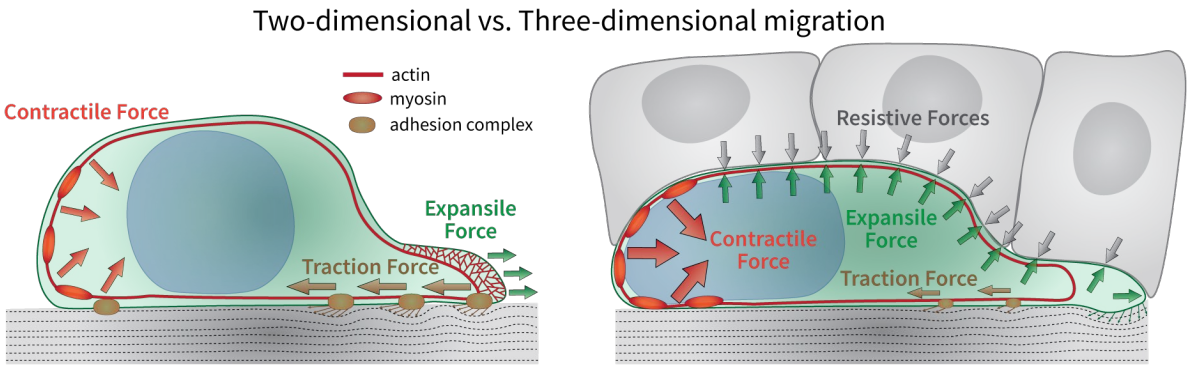

Figure 3: Schematic depicting forces generated during cell migration on a flat surface in the laboratory (left) versus three-dimensional migration in living tissues (right). In general, actin exerts expansive force at the cell front, which is linked to the substrate (floor) via integrins that make up adhesion complexes. Contraction of the cell rear results in forward cell movement, release of existing adhesions and initiation of another cycle.

Current methods to study confined cell migration

Among the many methods available to confine migrating cells, the most common is constructing tiny channels that cells must migrate through. These channels are made of a material similar to rubber, called Polydimethylsiloxane (PDMS), which is soft enough to mold into shapes, yet firm enough to hold shape and confine migrating cells. That said, while this rubbery material feels soft and deformable to us humans, it is extremely stiff from a cell's perspective. It therefore does not accurately model interactions between immune cells and surrounding cells in the body. Given the ability of immune cells to sense very small physical forces, this drives a need for new platforms that confine cells with a

soft interface that mimics the forces applied by surrounding cells. Cells themselves can be thought of as sacs of fluid. So what better material to use than fluid itself?

Akin to the tactic of fisherman 1, who developed a new lure in hopes of catching a new species of fish, I sought to develop a platform of tiny channels bound by soft fluid walls, which I hypothesized might better replicate the forces applied by surrounding cells during interstitial migration. I designed a system to visualize neutrophils migrating under confinement by soft deformable walls. I compared interactions between neutrophils and the soft interface in our device with those between neutrophils and surrounding cells during interstitial migration in the zebrafish, finding them to be very similar. After showing that this system replicates the mechanical resistance provided by soft surrounding cells, I studied how cells exerted expansive and contractile forces to enable migration. By imaging actin dynamics in cells, I found that instead of actin being at the leading edge to push against the membrane, under soft confinement by liquid walls more representative of living tissues, actin is located at the center of the cell, pushing up against the soft interface. This work adds to our understanding of how immune cells generate forces to migrate through soft tissues. Future work might enable targeting of these mechanisms to alter immune cell migration to treat autoimmune diseases.

The new liquid-walled microchannels used here better replicate the soft environment apparent during immune cell migration in the body, and enable precise control over experimental parameters important to studying human immune cell migration. However, this engineered system cannot replicate the environmental complexity exhibited in living tissues. Thus, after engineering this new system, I sought

to use established animal models to further investigate mechanisms by which immune cells migrate through cell dense tissue in living organisms.

FISHERMAN 2: DISCOVERING HOW IMMUNE CELLS GENERATE FORCES TO MIGRATE THROUGH LIVING TISSUES

In contrast to the tactics of fisherman 1 (the engineer), who designs new lures in hopes of catching new fish, I next sought to learn the tactics of fisherman 2 (biologist), who forms hypotheses based on existing knowledge and designs experiments to methodically test these hypotheses. I studied emerging research findings in other immune cell types, and learned that scientists in Canada and Austria have recently discovered that some immune cells use actin at the center of the cell to push out against the rigid microchannel walls previously described.

With this in mind, I used the zebrafish as a model organism to investigate how neutrophils use actin as they migrate between surrounding cells within living tissues. As predicted by experiments in liquid-walled microchannels, I found that neutrophils migrating in between surrounding cells also positioned actin at the center of the cell. Considering recent work, I figured that formation of this central actin occurred in response to the physical forces applied by surrounding cells. I therefore needed to find a way to alter the forces applied by skin cells. I leveraged recent work out of the University of Washington in Seattle that shows that wounding the zebrafish alters the spacing between their skin cells. I figured that this might serve as a good method to limit the physical forces

applied to migrating neutrophils. I learned how to wound zebrafish with a powerful laser and showed that when the space between skin cells increases, neutrophils lose actin at the center of the cell, instead repositioning it back to the front.

I then sought to investigate the hypothesis that the central actin was *required* to exert pushing forces on surrounding cells to make space for the neutrophil to migrate. To test this, I had to uncover and then inhibit the chemical reactions that regulate formation of the central actin. Using previous knowledge from around the world, I identified a couple molecules that might regulate the central actin. As hypothesized, inhibiting these pathways via drug treatments and genetic perturbations resulted in loss of central actin, inability to generate expansive forces to create space between surrounding cells, and inability to migrate. Through this work, I identified how neutrophils exert expansive forces to push their way through cell-dense tissues.

After uncovering the molecular pathways that regulate expansive forces, I turned my attention to traction forces. Recall that this is analogous to generating friction forces between our foot and the ground. While it is known that immune cells use integrins to adhere to non-cellular components within the extracellular environment (i.e. substrate), I hypothesized that neutrophils might adhere directly to surrounding skin cells themselves. While it has been suggested that immune cells might be able to adhere to surrounding cells to generate traction forces, it has not yet been proven in existing literature. To investigate this, I proposed a hypothesis whereby neutrophils use a specific integrin (αEβ7) to recognize specific molecules on surrounding skin cells. I used CRISPR-based gene editing to delete the integrin αEβ7 gene, finding that loss of this molecule impairs motility through cell-dense tissues. Therefore, this work provides the first evidence that

immune cells can adhere to surrounding cells to generate traction forces to enable migration in a living organism. Furthermore, it identifies the specific mechanism by which neutrophils do this, by identifying a role of integrin αEβ7 in regulating cell migration that had not previously been known. In the future, development of drugs to target this integrin might enable new treatments to modulate the immune response in the context of infection, tissue injury, or autoimmune disease.

CONCLUSION

The challenge of a Ph.D. is learning how to develop and refine methods in how to think about complex questions. After beginning with the broad question of: "*How do immune cells move through our tissues*," I spent time learning and evaluating the existing tools – mouse models and engineered microscale systems. By noticing a gap in how poorly these microscale systems modeled interstitial migration in the body, I first took an engineering-minded approach, akin to fisherman 1, constructing a new "lure" in liquid-walled microchannels which mimics interactions between neutrophils and surrounding cells in living tissues. I used this tool to show that neutrophils migrating under conditions similar to living tissues transition from using actin at the cell front to a distinct migration strategy with actin at the cell center to migrate under soft confinement. This work enhances our understanding of the molecular mechanisms utilized by neutrophils to deform surrounding cells and generate space for migration.

Following this work, I transitioned to a more "biologist-minded" approach, like that of fisherman 2, using recent findings in similar immune cell types to form a hypothesis regarding the mechanisms by which neutrophils generate expansive and tractional forces to migrate through cell-dense living tissues. I tested hypotheses regarding how neutrophils generate expansile and tractional forces using drug treatments and genetic engineering in a zebrafish model. Through these experiments, I was able to uncover two new mechanisms by which neutrophils exert expansive and tractional forces to migrate through cell-dense tissues.

In all, this work exemplifies the utility of combining different approaches to answering complex questions. I used an engineering approach to develop a system to study cell migration in a totally new way and then transitioned thought processes to delve deeply into the mechanisms that regulate cell migration in a biological context. Altogether, this work opens the door for development of drugs to target these mechanisms to therapeutically control the immune response in the context of human disease.

Throughout this process, I cannot help but harken back to the question raised at the start of this chapter:

"Why get a Ph.D.?"

While words cannot explain the complexity of what is learned throughout the Ph.D. process, it can most simply be stated through the words of Dr. Sachs from my undergraduate training:

"*A Ph.D. teaches you how to think.*"

<u>It really is a degree in philosophy</u>. While at a practical level a Ph.D. is measured by the ability to learn and apply different methods of thinking to discover new truths about nature, the insights that one learns throughout this process extend much deeper. In life, we are

faced with challenges on a daily basis, whether they be tangible (i.e. what choice should I make in this current moment?), or more emotional (i.e. how can I efficiently regulate my thinking to best approach this problem?). While these moments are often clouded by an infinite number of potential routes, a Ph.D. teaches you how to cut through noise to identify the fundamental question underlying any problem, and from there how to organize one's thinking in the most efficient way to arrive at a solution. With that, I am eternally grateful for my experience in graduate school which has undoubtedly equipped me with tools to become a better scientist, physician, and person.