BUILDING ON SCIENCE: MY CAREER (SO FAR) IN CELL RESEARCH

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THE BACHELOR'S DEGREE – ENTERING THE SCIENCES

Growing up, I always wanted to become a medical doctor. Like many young students pursuing medicine, I entered my undergraduate degree in the biological sciences. The science courses were a struggle for me to get through and I found myself even more interested in my option courses such as psychology or even economics. Nevertheless, I finished my biological sciences degree. I did apply to medicine but did not get in, so I decided to get a master's degree in order to improve my curriculum vitae in hopes of eventually getting into medicine.

THE MASTER'S DEGREE – ENTERING THE SCIENCES

When deciding which research area to pursue for my master's degree, I gravitated toward bone and joint research based on my involvement in playing sports growing up. I met with several different scientists at the Bone and Joint Institute (formerly called the Joint Injury and Arthritis Research Group) at the University of Calgary, and chose to work in the laboratory of Dr. David A. Hart. The first task I was given by the principle investigator was to choose a particular project to work on. I was given several different scientific papers to read, which turned out to be a daunting task. Despite having a strong science background, I struggled with those papers, getting lost in the scientific jargon. Nevertheless, based on what I could glean from those papers, the area of bone cell mechanotransduction seemed interesting to me. Mechanotransduction is aimed at understanding how cells sense, transduce, and respond to mechanical loading. It is the process that can explain why weightlifters have increased bone mass whereas astronauts have decreased bone mass.

My mechanotransduction research focused specifically on how the bone forming cells, osteoblasts, respond to different types of mechanical loading. I would apply different loading modalities onto cells, such as stretching or compressing cells using special instrumentation. From my studies we found that osteoblasts not only responded to mechanical loading by regulating the expression of genes, but they also responded by physically modifying their environment. In other words, osteoblasts, which are in a collagen matrix, are able to reorganize collagen by pulling on it. This pulling force was established by the cytoskeleton, which is the structural framework of the cell. Two major components of the cytoskeleton are actin and tubulin. Actin and tubulin are similar to the molecular cables and struts of a bridge – they provide cellular architecture. Without proper organization of actin, the pulling force exerted by the cells on the collagen matrix is diminished, reducing the potential for matrix remodelling.[1, 2] Insufficient remodelling forms the basis of several bone pathologies such as non-union healing of bone and osteoporosis.

My master's degree allowed me to perform real research through which I was actually able to

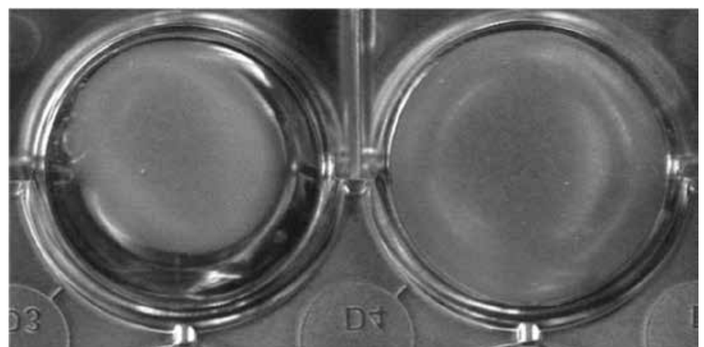

__Figure 1:__ Cell mediated contraction of collagen gels. Top view of gels within a culture dish. Cells are placed within a collagen solution, which gels when placed in a 37°C incubator. After 48 hours, the gels are detached from the sides of the culture dish. The cells that are within the gels contract the collagen gels as shown in Panel A. However, this contraction is dependent on actin as treatment of cells in collagen with actin inhibitor (latrunculin B) prior to detachment prevents collagen gel contraction as shown in Panel B. Dashed lines represent perimeter of gels.

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contribute to the world's wealth of scientific knowledge. I was even given the opportunity to present at scientific conferences and publish my findings scientific journals. These experiences left me thirsty for knowledge and hungry for more research.

THE PH.D. – NO LOOKING BACK

After completing my master's degree, I wanted to further advance my research expertise by embarking on a Ph.D. I discussed this interest with my master's degree supervisor. He urged me to pursue research away from the University of Calgary as this was where I did my master's degree and a change in the research environment would be an asset in terms of my career. Through my exploration of other areas of research, I became intrigued by bioengineering, which involves developing replacement body parts using a patient's own cells. This interest inspired me to move to Toronto to work with Dr. Rita Kandel, a pioneer in articular cartilage bioengineering research.

Articular cartilage is the white glistening tissue that resides on the top ends of bone joints and provides a nearly frictionless surface for joint movement. Unlike other tissues, such as bone or skin, articular cartilage is incapable of self-repair. Damage to articular cartilage results in progressive degradation and, as a consequence, joint replacement is often necessary. Replacements consist of replacing cartilage with metal/plastic/ceramics, which have a limited lifespan and eventually require revision. Thus, these replacements are not optimal and patients would certainly benefit from bioengineered articular cartilage.

Articular cartilage has a unique composition. Two essential molecules present in cartilage matrix are proteoglycans and collagens. The cartilage cells, which are called chondrocytes, mainly produce the proteoglycan aggrecan and type II collagen; these molecules are essential for cartilage to withstand mechanical loads. A major aim in bioengineering cartilage is to produce tissue that approximates articular cartilage with high expression of these molecules. In 1995, a breakthrough study showed that culturing chondrocytes at high density in three-dimensional culture could result in the generation of articular cartilage tissue.[3] Importantly, this study demonstrated that bioengineering of articular cartilage was possible.

At this point, a lingering issue in the field of bioengineering was how to scale up to replace an entire human joint. To accomplish this, a large number of chondrocytes capable of depositing matrix rich in type II collagen and aggrecan are needed. Unfortunately, chondrocytes are limited in number. Chondrocytes can be placed in culture dishes to proliferate and increase the number of cells. However, expanded cells undergo a process of dedifferentiation, which results in the failure of cells to express cartilage matrix and they express high levels of type I collagen instead. This is indicative of fibrocartilage, which is inferior to articular cartilage and is incapable of meeting the mechanical demands of the joint.

For my research, I was tasked with finding out what regulates this dedifferentiation process in chondrocytes. The first thing I noticed when I looked in the microscope was the stark contrast in the sizes and shapes of the cells. Initially chondrocytes were small and round, but after a few

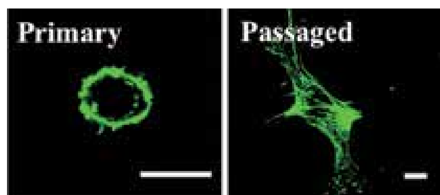

Figure 2: *Confocal microscopy images of freshly isolated primary chondrocyte and culture expanded passaged cells stained for F-actin using FITC-phalloidin. Primary chondrocytes are smaller and have a cortical distribution of actin whereas passaged chondrocytes have actin organized into stress fibers throughout the cytoplasm. Scale bar = 10um.*

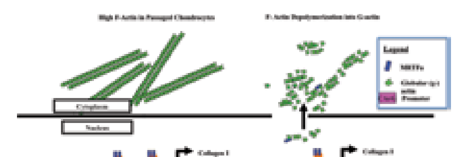

Figure 3: *Control of type I collagen gene expression through the actin polymerization in passaged chondrocytes. Passaged chondrocytes contain a high proportion of F-actin. Actin depolymerization through latrunculin B treatment increases G-actin monomer concentration. G-actin attracts MRTF and pulls MRTF from the promoter CArG promoter regions of type I collagen and brings it into the cytoplasm of the cells. This supresses gene expression of type I collagen.*

days chondrocytes became larger and began to spread out. From my previous studies, I knew that the cellular cytoskeleton could regulate this change in cell size and shape. Upon further investigation of the literature, I discovered that it was initially demonstrated over several decades ago that the actin cytoskeleton could regulate the production of cartilage matrix. But the molecular mechanisms by which the cell did this were still was unclear. So it became my objective to find the connection between the actin cytoskeleton and matrix gene expression.

Work being performed in other types of cells had shown that a transcription factor called myocardin-related transcription factor-a (MRTF) could regulate the expression of type I collagen. 4 MRTF, when inside the nucleus of cells, binds to the promoter regions of genes and drives the expression of certain genes such as type I collagen. MRTF can also bind actin. Actin could exist as monomeric, globular (G-) actin, which can polymerize to form filamentous (F-) actin and MRTFa has a high affinity to G-actin. When F-actin is depolymerized into G-actin, MRTF binds to the G-actin, is exported from the nucleus to the cytoplasm, and gene expression could be decreased.

Based on these findings in other cell types, the hypothesis for my project formed: *Actin polymerization regulates type I collagen gene expression in passaged chondrocytes through MRTF*. To test this hypothesis I first measured G-/ F-actin and MRTF localization in primary and passaged chondrocytes. In support of my hypothesis, I found that there was more G-actin (less F-actin) in primary chondrocytes as compared to passaged chondrocytes. 5 Next I examined the effect of actin depolymerization on passaged cells in increasing the proportion of G-actin. I treated passaged chondrocytes with an actin depolymerization drug and found that not only did this increase the proportion of G-actin but it also resulted in MRTF export to the cytoplasm and reduced type I collagen expression. The last piece of support for my hypothesis was the finding that inhibiting MRTF decreased type I collagen gene expression. Thus, actin regulated type I collagen through MRTF. This research indicated that MRTF regulation should be considered for improving bioengineering of articular cartilage.

I completed my project, defended my thesis, published my work 5-7 and graduated with my Ph.D. However, I still have that hunger for more research. The next step for me is to complete a post-doctoral research fellowship to gain more scientific expertise, which is often a required step before starting up as an independent researcher. I have decided to gain further expertise in actin research. For this pursuit, I will be heading to The Scripps Research Institute in San Diego, California to a renowned actin research laboratory. Although I will not be working in musculoskeletal research there (I will be conducting eye research), I would like to one day bridge the expertise I gain and apply it in musculoskeletal research. I hope to eventually return to Canada to start my own research lab.

MY ADVICE FOR STUDENTS

Pursue what interests you

There is a world of opportunity, so do the things that interest you. For me it was bone and joint research. Look at what research is currently being done at institutions in your area. Contact researchers at the labs that interest you and speak with them about potential projects. When you go to speak with them, be prepared; for example, demonstrate your interest in their lab by reading some of their published research papers. Also, make sure you jive with your potential supervisor. You can get a sense of their personality by speaking with them and other people in their lab.

Start early

I entered the world of research relatively late. Many labs accept undergraduate students for small research projects during the summer or for course credit during the school year. Some even accept high school students. So look for these opportunities. If you find a laboratory you are interested in, it does not hurt to contact them to see if there is opportunity for you to do work there.

Read, read, read

Although it is very daunting at first to read a scientific paper, mainly because of the jargon, you will

eventually understand and even begin to use this jargon. It will be a new language to you and will take time to learn, so keep at it. Know what research has been done in your field, and find studies that are comparable to your own research, so that you do not get stuck trying to reinvent the wheel. In addition, read papers from outside of your field. Other fields of expertise can give inspiration for your own work. For me, the research being performed in vascular research gave me insight to my cartilage project.

Consult with others

Others have tackled the same or similar problems that you face, so speak with them. When you start in the lab you will have to learn methodology and how to perform certain experiments. Often other lab members can provide some guidance, so don't be afraid to ask them for help. When they do teach, make sure to listen and take good notes. Along with asking specific questions about your research, consult with others about the 'big' decisions, like the decision to do another degree or post-doc. Make a point to develop good relationships with your supervisors. I was lucky to develop great relationships with both my master's and doctoral supervisors. For instance, my master's supervisor guided me in choosing my doctoral supervisor. Subsequently both my master's and doctoral supervisor gave some advice on which lab to select for my post-doctoral research. Remember that these people can only provide you with advice, but ultimately these decisions come down to you. Nonetheless, it can certainly be helpful to hear their opinions. For me, my previous supervisors continue to act as my mentors and have been fundamental to my development and success.

Don't be afraid to try something new

During your research you will invariably ask questions to which no one has the answers. To me, the exciting part of research is answering those types of questions. Along the way you may encounter naysayers that want to discourage you from attempting something new in your research, but try to ignore them. Discovering and observing something no one has observed before has to be one of the most exhilarating feelings. Enjoy it and never stop asking questions.

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