

## Update #1 from the Lab team

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Hi everyone!

Welcome from Dr. Hawke's Lab Team at York University. I want to tell you all about what I will be studying and why it is so important.



The Weddell Seal is an amazing animal. When a Weddell Seal is born it is referred to as a *pup*, as seen in the picture on the left. At this stage of life it mostly feeds from its mother and does not enter the water. Later, as a *juvenile*, it lives on the ice edge. It is a fast swimmer, but not strong enough to travel long distances under the ice. Additionally, if it did travel far under the ice it would encounter larger adults guarding breathing holes which could lead to a fight, very much in favour of the larger adult. As the Weddell Seal matures into an

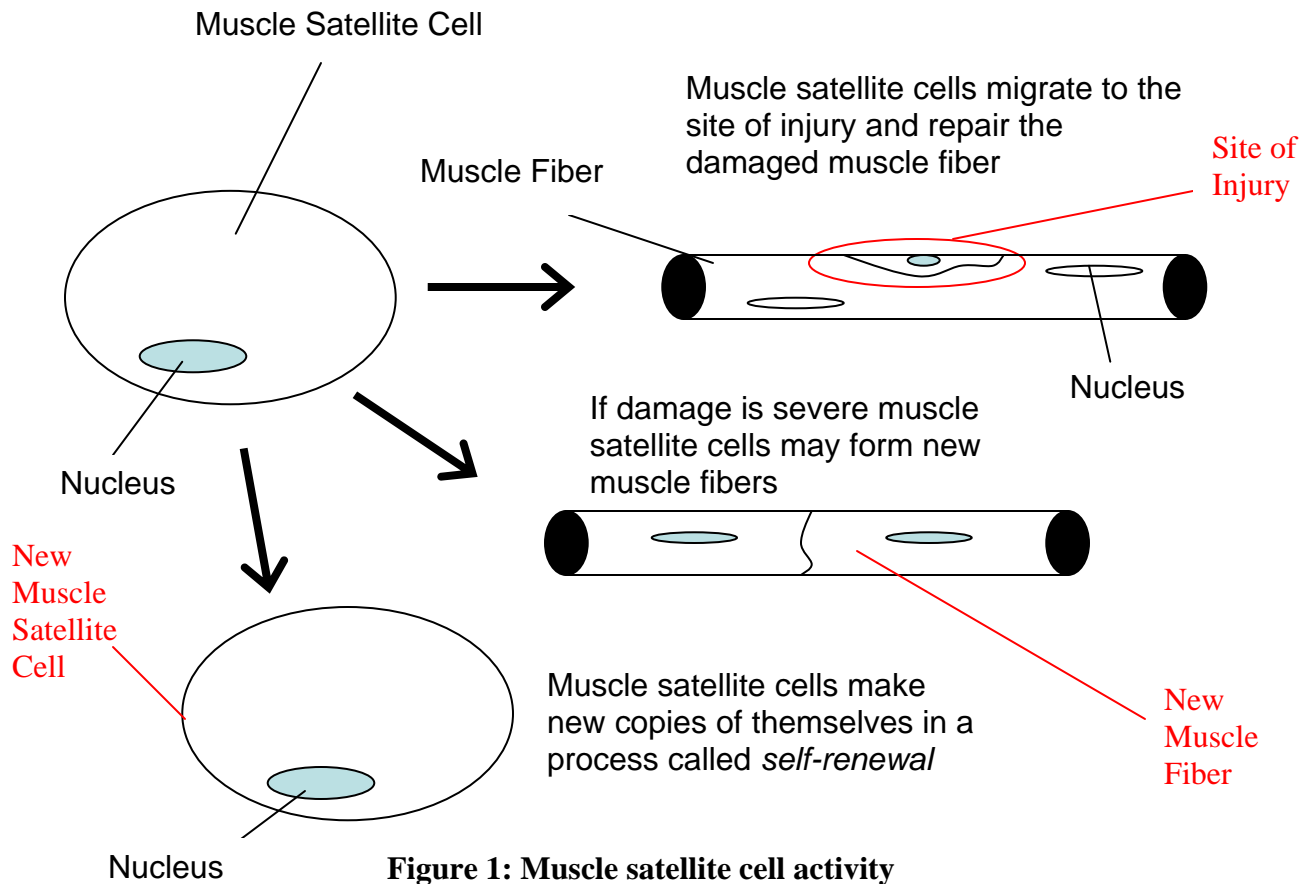
*adult* it gains the ability to dive deeper and for longer periods of time. With its new strength it can venture farther under the ice and claim its own breathing holes. The Weddell Seal has been known to dive to depths of 700 meters and hold its breath for 80 minutes!! On these long adventures under the ocean they accomplish most of their activities. Everything from mating to feeding is done under water.

So why are we so interested in the Weddell Seal? The ability of the Weddell Seal to exercise so deep under water and for so long without taking a breath must have some application to us! Think of the last time you jumped into a pool and swam under water for a few seconds. Like me, you probably came to the surface gasping for air. Why did this happen? The reason is that your cells were starved for oxygen. They need the oxygen to efficiently make energy or your cells could die. This is especially true when exercising. Think of the Weddell Seal again. When it dives under water, it is moving quickly as it attacks prey and avoids predators. All this work in the muscles means that the muscles need more energy and therefore more oxygen. It's amazing! How can the Weddell Seals exercise for over an hour underwater while we can barely last 30 seconds?

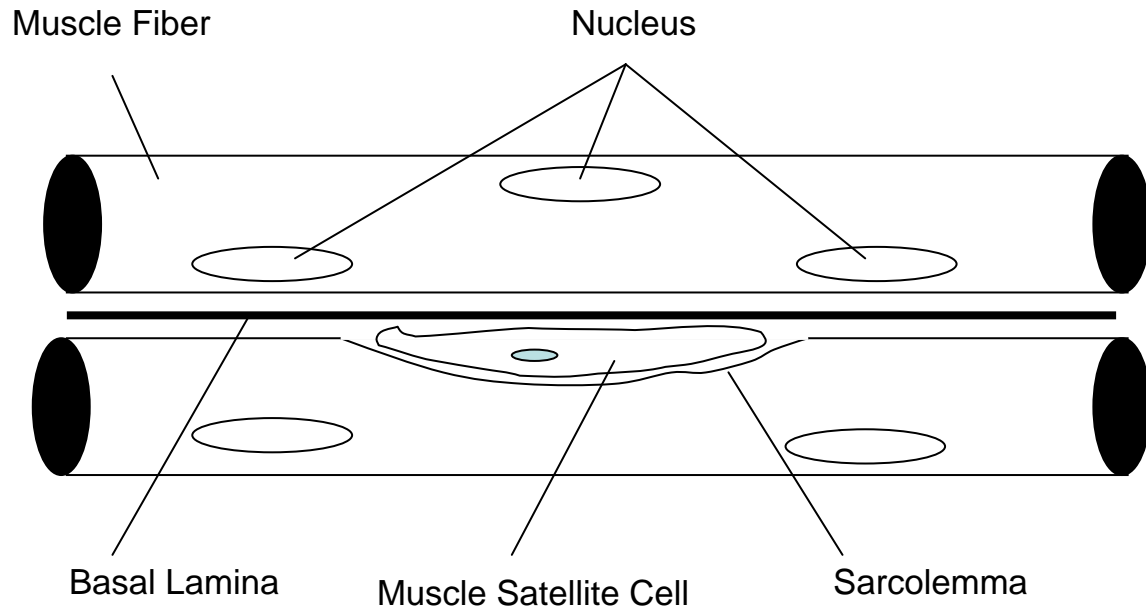
Are you getting an idea of why we are so fascinated by this animal? We want to know how the Weddell Seal can outperform the greatest human athletes underwater. Maybe an example from the human health perspective will help clarify our interests in the Weddell Seal...

We breathe in order to get oxygen into our blood. The blood then moves through *arteries* carrying the oxygen to all of our cells so that the cells can use the oxygen to make energy. Also, the blood carries away waste from the cells to be expelled from the body. What happens when the path of the blood to the cells is blocked? This is what happens in a heart attack or stroke. A blockage in an artery is often caused by a build up of cholesterol called *atherosclerosis*. When the blood is blocked, the cells that are normally supplied blood by the artery become starved for oxygen. This is known as *ischemia*. If enough time goes by without oxygen reaching the cells, the cells may die. Now, think of the problems this could cause if the cells that die are part of a very important organ such as the brain or heart. Having a heart attack or stroke is similar to the Weddell Seal's adventure into the sea. The amazing thing is that even though there is no new oxygen getting to their cells, their cells do not die. This condition of a lack of oxygen is known as *hypoxia*. So there you have it...if we can understand how the Weddell Seal can survive the conditions of a heart attack, maybe we can prevent human beings from being seriously injured by a heart attack or stroke.

Now that we understand why it is so important to study the Weddell Seal, I am going to explain more specifically what my research will be investigating. Muscle, like all other tissue in your body, is composed of cells. What is unique about muscle is that the cells elongate and form fibers. When you contract your muscles, the fibers shorten and this is what allows us to produce the forces needed to walk or participate in any physical activity. When we exercise or cause damage to our muscles the muscle tissue is able to repair itself. One of the cells that are crucial to the repair of muscle is the *muscle satellite cell*. These cells, also known as *muscle stem cells*, will migrate to a site of injury and either repair the damaged fiber or replace it with new fibers if the damage is more severe. Additionally, when the muscle satellite cells divide to make new muscle, it also produces another satellite cell to make sure that there are enough satellite cells for future repair of damage (figure 1). Since the Weddell Seal is able to exercise for so long under hypoxic conditions, we hypothesize that it must have an amazing ability for muscle repair which may be linked to their muscle satellite cell characteristics.



I will be investigating the number of muscle satellite cells and their distribution in the Weddell Seal as it matures from pup to juvenile and to adult. Perhaps there will be differences in the number of muscle satellite cells as the Weddell Seal ages. This will give us information about how the Weddell Seal can accomplish its amazing daily tasks. Also, I am interested in knowing where the muscle satellite cells are located. These cells are normally dormant until they receive some sort of signal that activates them to migrate and divide. The phenomenon in which the satellite cell sits quietly waiting for signals is called *quiescence*. Perhaps knowing where the satellite cells are located will tell us what kinds of messages signal the Weddell Seal's muscle satellite cells to divide. To study where these cells are we have to know a little bit about the characteristics of muscle satellite cells. The muscle satellite cells sit in a groove between two structures in the muscle. It is located outside the cell membrane of a muscle cell called the *sarcolemma*, and inside a membrane that covers the muscle fibers called the *basal lamina* (figure 2).



**Figure 2: Location of quiescent muscle satellite cells**

To conduct my experiments I will be using samples of Weddell Seal muscle tissue sent to us from the Ice Team in Antarctica. To obtain the tissue samples a process called *muscle biopsy* is performed. The animal is unharmed in this process. A small incision is made in an area with high muscle content. A device is then used that removes a small piece of muscle. To give you an idea of the amount of muscle taken, only 10 to 15 milligrams of muscle is taken from a Weddell Seal that weighs as much as 800 kilograms! That's less than 0.00000002% of its body weight! The same procedure is done to humans for research purposes. The sample must then be properly frozen for transport from Antarctica to the lab here in Toronto, Canada. To do this, the sample is frozen in what is called OCT compound and placed in liquid nitrogen to quickly freeze the muscle and OCT compound. At the end of the procedure the sample looks like a frozen chunk of toothpaste (figure 3). We then place the sample in a machine that we use to take thin slices of the muscle (figure 4). A very sharp blade cuts through the muscle to obtain a *cross-section*. This allows us to see the arrangement of all the muscle fibers by using a microscope (figure 5). Each red circle in the cross-section represents a different muscle fiber that we have cut through. If you are having difficulty picturing the procedure, it is similar to how a deli worker would slice thin slices of lunch meat (just much more precise). We take repeated cross-sections of the muscle and place them on microscope slides and put them directly into a freezer for storage at  $-80^{\circ}\text{C}$ . Each slice of the muscle that we place on a microscope slide is only  $8\mu\text{m}$  (micrometers) thick (That's about 10 times thinner than a piece of paper!!). This entire procedure is called *cryosectioning*.



Figure 3: Muscle and OCT compound  
(The muscle is the pink circle in the middle)



Figure 4a: Cryosectioning machine (outside)

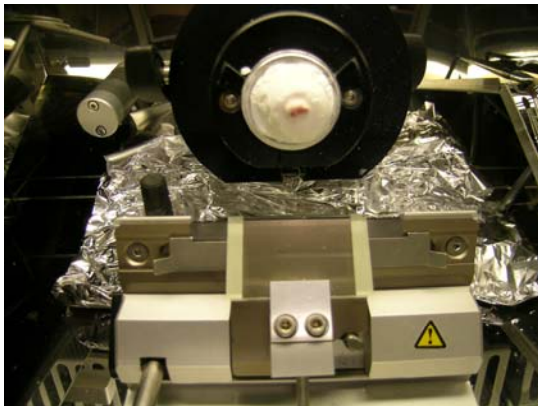
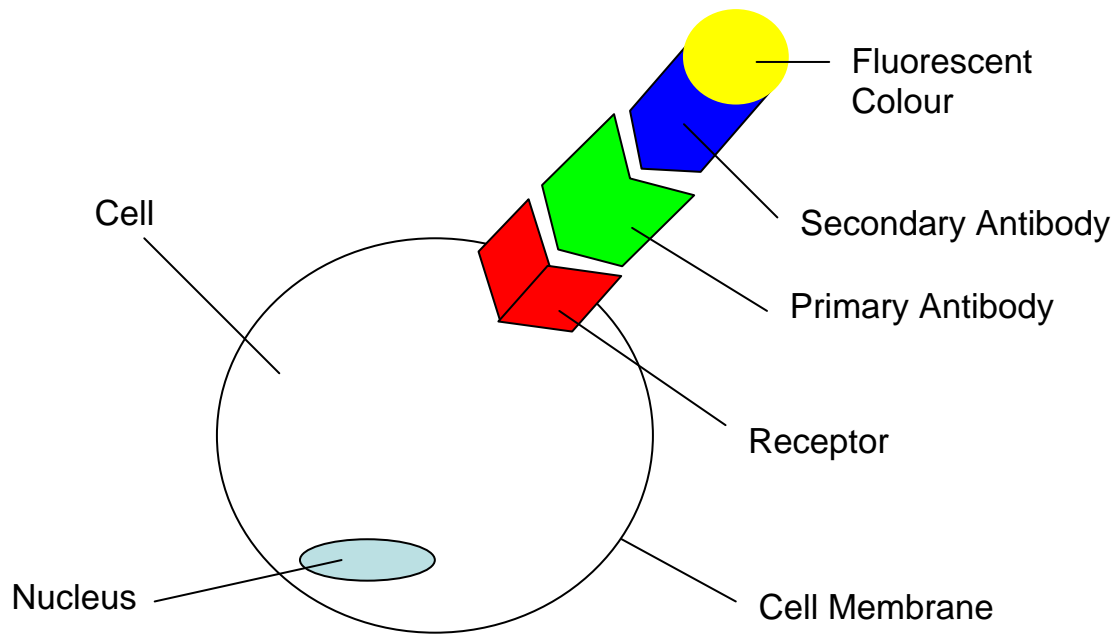


Figure 4b: Cryosectioning machine (inside)



Figure 5: Cross-section of mouse muscle  
stained with a red dye called  
*hematoxylin*

Finally, to examine the location and number of muscle satellite cells, I utilize the cell's ability to adhere to certain molecules. On the surface of all of our cells there are receptors. *Receptors* are proteins that are a specific shape so that they only bind to a specific molecule called an *antibody*. There are many different antibodies that bind to cells and some cells only bind to certain antibodies and not to others. We place the slides from the muscle cryosectioning in a liquid that contains an antibody, known as the *primary antibody*. We then place the same slide in another bath of liquid that has an antibody that binds to the first antibody. This is called the *secondary antibody*. The secondary antibody has a molecule that shines in a specific colour when placed under a microscope that uses fluorescent light. This allows us to tell what kinds of cells we are looking at. This process is called *Immunohistochemistry* (figure 6).



**Figure 6: Immunohistochemistry**

For my experiments I will be using two different primary antibodies. The first is called *m-cadherin* and it binds to muscle satellite cells. The other primary antibody I will be using is called *dystrophin*. Dystrophin is a protein that is very abundant directly beneath the cell membrane (also known as the *sarcolemma*). Do you remember how I mentioned earlier that the muscle satellite cells are just outside the sarcolemma? If I can see where the sarcolemma is using dystrophin antibody and where the satellite cells are using *m-cadherin*, then I will be able to confirm which cells are indeed muscle satellite cells.

I have started to practice the above procedure on some mouse muscle tissue obtained from Dr. Hawke. In the upcoming weeks I will begin my experiments using the actual Weddell Seal muscle tissue from the Ice Team. I hope to have some interesting results to share with you!

**Challenge Questions:**

1. What does the prefix “cryo-” mean in “cryosectioning”?
2. Based on what I have told you about the job of the muscle satellite cell, can you think of things that make them active?
3. Now that you know what cell receptors are, why do you think it is important for cells to have receptors?