

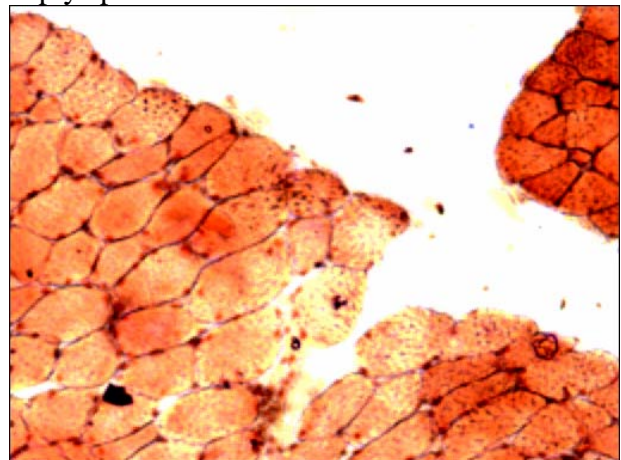
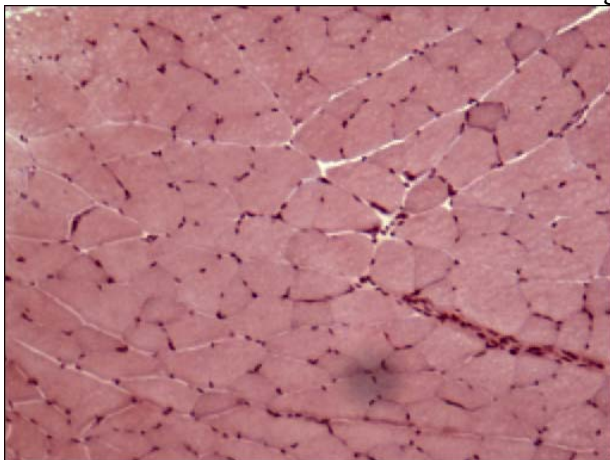
Update #7 from the Lab Team
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Hello Polar Scientists!

This is a very exciting time for the Lab Team! Now that we have been working very hard at perfecting our procedures on mouse muscle tissue, we are beginning to perform experiments on Weddell seal muscle tissue. In this update I will describe: the differences I have noticed between mouse and Weddell seal tissue; the recent obstacles I have had to overcome in my immunohistochemistry experiments and; results from immunohistochemistry on both mouse and Weddell seal muscle tissue.

This week I began sectioning Weddell seal muscle tissue using the cryostat. One characteristic that was immediately noticeable was the small size of the Weddell seal tissue samples compared to the mouse samples (figure 1). Of course, this is not because mice have a larger muscle mass than seals. This phenomenon is due to the difference in fat content of the two animals and differences in muscle harvesting environments. When we harvest mouse muscle in the lab, the conditions are easy to control and the mice have relatively little fat that may otherwise interfere with the harvesting technique. In contrast, when the Ice Team harvests a sample of muscle from a Weddell seal they are working in freezing cold conditions with large animals. Furthermore, when they obtain a sample of tissue a large portion of it will likely be fat tissue. This difference in size of the sample may prove to be the next challenge that faces the Lab Team. Since we had so many fibres to examine in the mouse tissue sections, the probability of getting good results in at least one area of a section was fairly high. Now that we are working with Weddell seal tissue samples that are much smaller, we may find that if the experiment did not provide good results in one area of the section, there will be no other area to look for good results. This means that we will have to be extremely careful in our procedures to make sure that we get good results and do not waste any of the precious Weddell seal muscle tissue.

Figure 1: Cross-sections of muscle tissue samples from a mouse and a Weddell seal stained with hematoxylin. In the mouse muscle tissue (left) notice the high number of fibres in the sample. In the Weddell seal muscle tissue (right) notice the lower number of fibres and large empty spaces.



As I mentioned in my previous updates, there are two proteins that I am attempting to visualize by performing immunohistochemistry: m-cadherin to visualize muscle satellite cells and dystrophin to visualize the muscle cell membrane, also known as the sarcolemma. Remember that I am looking for cells that are m-cadherin positive and are located outside the sarcolemma, which is outlined by dystrophin. The dystrophin antibody is working very well, as can be seen in figure 2. Unfortunately, I have been experiencing a few problems with the m-cadherin antibody. It appears as though some areas that are not muscle satellite cells are positive for m-cadherin (figure 3). The areas that are marked off with white arrows do not exist where a muscle satellite cell should be (outside the sarcolemma). I consulted Dr. Hawke for some guidance and we deduced two possible explanations for these findings: (1) The primary antibody may clump together in random places leading to a high amount of fluorescent secondary antibody binding to the area or; (2) Other structures embedded in the muscle tissue such as nerves and vasculature have proteins similar to m-cadherin that may bind to the primary antibody. To resolve these problems I will have to ensure that the primary antibodies do not clump together. This can be accomplished by using some very basic science. It is well known that things that have a higher density tend to sink in a solution. Therefore, in the vial that holds the solution of m-cadherin antibodies, the antibodies that stick to each other will be more dense and sink to the bottom of the tube. If I only take antibodies from the top of the vial, I should avoid using the antibodies that are clumped together. Solving a problem in the lab often requires thinking of the most basic scientific principles!

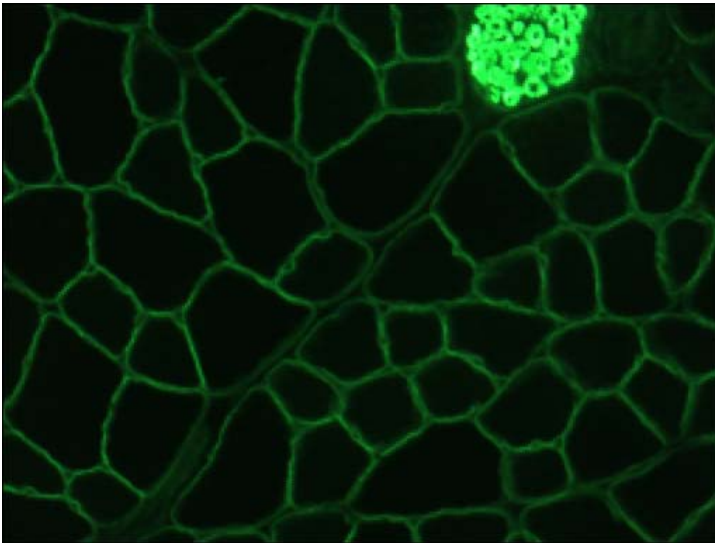


Figure 2:

Immunohistochemistry results from mouse muscle tissue. The dystrophin antibody has bound to the dystrophin protein which is in abundance at the sarcolemma of each muscle fibre. The structure in the top right corner with a high amount of dystrophin is likely a nerve.

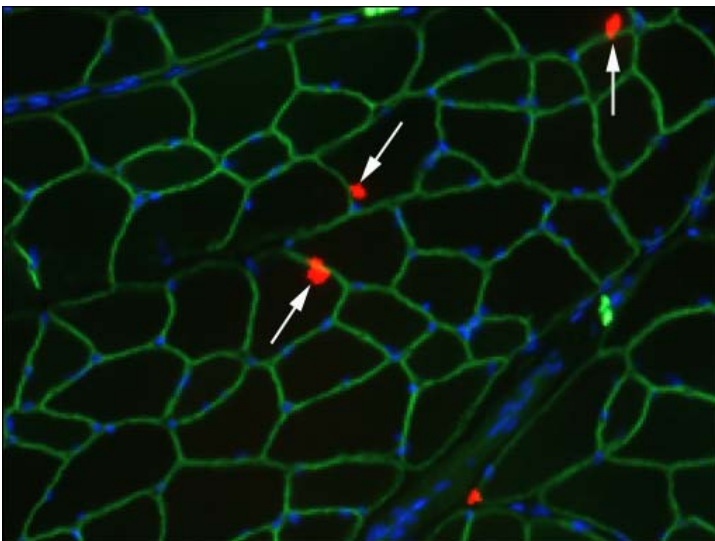
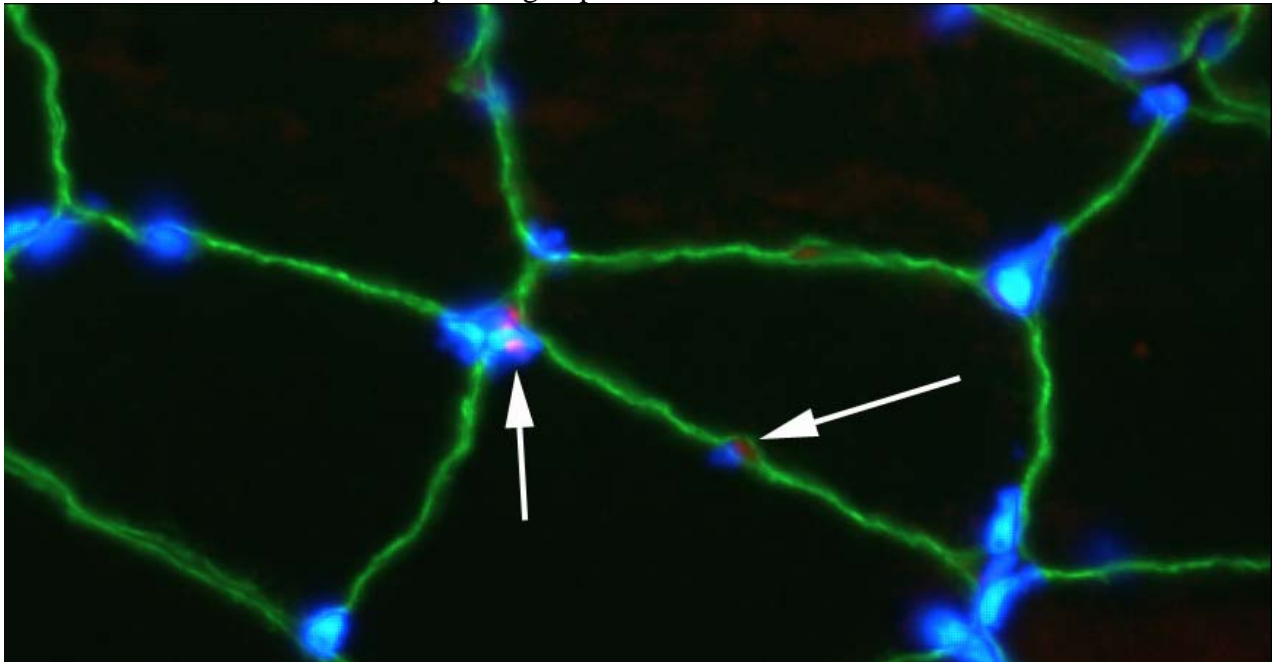


Figure 3:

Immunohistochemistry results for mouse muscle tissue. This is an overlaid image of dystrophin (green) and m-cadherin (red). The blue areas are nuclei. The white arrows are pointing to m-cadherin positive areas that are not muscle satellite cells.

I had some success in finding muscle satellite cells in the mouse muscle tissue. As you can see in figure 4, the white arrows are pointing to what I believe to be muscle satellite cells. I came to this conclusion because the areas that were positive for m-cadherin (red) were outside the sarcolemma, outlined by dystrophin (green). Furthermore, the areas that were positive for m-cadherin were positive for DAPI, which stains for the nucleus of a cell. The nucleus of a muscle satellite cell is usually large compared to the small size of the satellite cell and m-cadherin is a protein that is a well known identifier of muscle satellite cells.

Figure 4: Immunohistochemistry results for mouse muscle tissue. This is an overlaid image of dystrophin (green) and m-cadherin (red). The blue areas are nuclei. The white arrows are pointing to possible muscle satellite cells.



My results for Weddell seal muscle tissue were not as promising as my results for the mouse tissue. I found a number of areas that were positive for m-cadherin, but not positive for DAPI. These results from the seal tissue provide an excellent opportunity to practice one of the most fun aspects of science: problem solving! Examine figure 5 below and before you read the next paragraph see if you can think of some problems that may be occurring that explain why I am not finding muscle satellite cells in the Weddell seal muscle tissue.

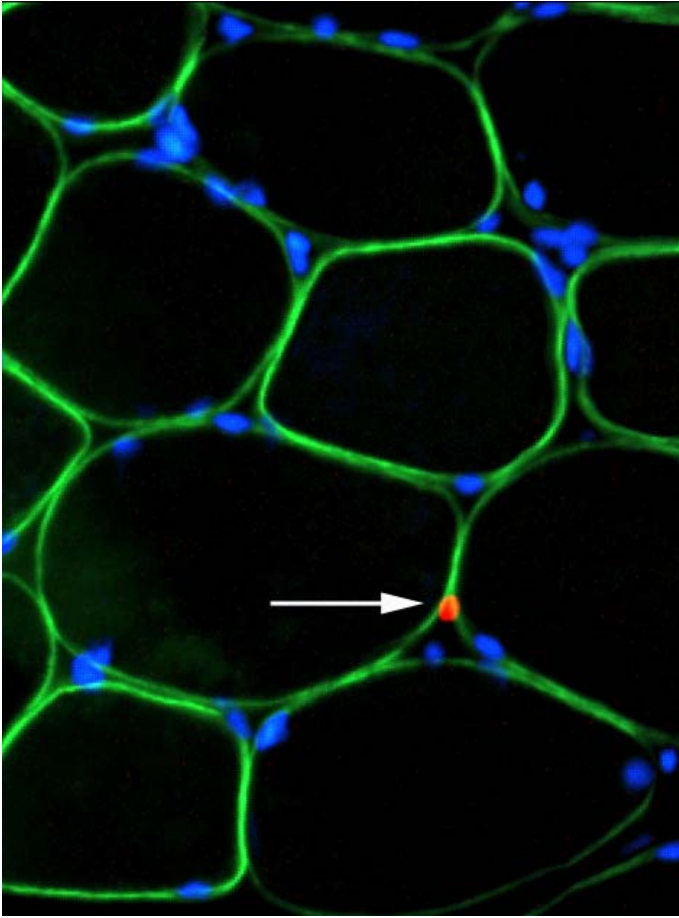


Figure 5:

Immunohistochemistry results from Weddell seal muscle tissue. This is an overlaid image of dystrophin (green) and m-cadherin (red). The blue areas are nuclei. The white arrow is pointing to an area that is positive for m-cadherin, but is not a muscle satellite cell.

As previously mentioned, the red area may merely be antibodies that have clumped together. This collection of antibodies likely bound to the secondary antibody and fluoresced red. It is probably just a coincidence that the red clump of antibodies landed between the muscle fibres. This coincidence makes it very tempting to jump to the conclusion that this is a muscle satellite cell, but since there is no nucleus in this area I cannot make the assumption that this is a muscle satellite cell. I will be able to avoid this problem by employing the technique of avoiding dense clumps of antibodies, as mentioned earlier. Another source of the problem may be with the structure of the primary antibody that I am using. Antibodies are made to bind to specific proteins. The problem is that a protein such as m-cadherin in mice may be slightly different in Weddell seals. Antibodies bind to a specific area on a protein called an *epitope*. It may be that the area where the m-cadherin antibody binds to the m-cadherin protein in mouse muscle is different in Weddell seal muscle. The process can be compared to making a puzzle. The m-cadherin protein and the m-cadherin antibody are like puzzle pieces and need to fit together just right. I may have to consider trying a primary antibody that is made to bind to a slightly different epitope.

Next on my agenda will be attempting to get good results using the m-cadherin antibody on Weddell seal muscle tissue. Keep checking my blog for details on what I am doing to overcome my obstacles in the lab. If you happen to be wondering what our laboratory looks like, below are two pictures of me working on immunohistochemistry in the lab.

Figure 6: Peter working on immunohistochemistry in Dr. Hawke's lab at York University, Toronto, Canada.



Challenge Questions:

1. What three characteristics am I looking for in my immunohistochemistry results that will allow me to verify the presence of a muscle satellite cell?
2. Put the following three substances in order from most dense to least dense (Instead of looking for the answer on the internet, try designing your own experiment to test your hypothesis. Tell me how you designed your experiment when you answer the question.)
 - a. water
 - b. lead
 - c. ice