

Update #4 from the Lab Team

Peter Jaksa, York University, Toronto

Hello again fellow Polar Scientists!

I am very excited to tell you about the progress I have made in the last few weeks. As Jesse and Sophia described, we have had our fair share of obstacles, but we have learned to become very skilled at cryosectioning. In my last update I briefly described the process of immunohistochemistry that I will be using to study muscle stem cell number and distribution in Weddell seals. It may be helpful to take a look back at my first update to refresh yourself on the procedure.

Immunohistochemistry is very exciting for me because it is something I have never done before. The first time I went through the procedure it took me approximately five hours, but the procedure may take even longer in the future. Scientists often need to adjust their experiments in order to get the best results. In the following paragraphs I will describe in more detail how I performed the experiment.

As you know, I have made many thin slices of mouse muscle using the cryostat machine. These thin slices are stored in a freezer on a microscope slide at -80°C . The first step in the procedure was to warm the samples to room temperature. It is important to not let the sample dry, or else it may crack, curl or fall off the microscope slide. To avoid this, I could not bring the tissue to room temperature simply by removing it from the freezer and placing it on the table. Instead, I left the sample in a *humidifier* until it became the same temperature as the air in the room. The humidifier is a lot simpler than it sounds. It is a large dish with wet paper towel and a lid. The wet paper towel keeps moisture in the air inside the dish. This moisture ensures that the sample will not dry out (Figure 1).

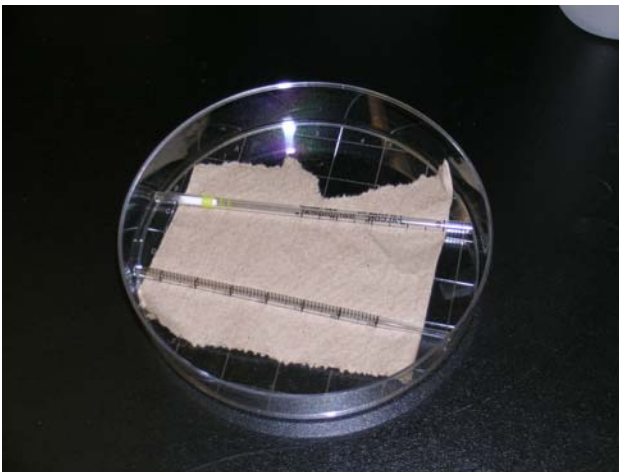


Figure 1:
Humidifier without a lid. The slide is placed on the two clear bars to make sure it does not touch the moist paper towels.

Once the sample was warmed to room temperature, I incubated the slide and sample in a solution which will bind to “sticky” molecules on the sample that may otherwise bind to the secondary antibody. This solution is called *blocking solution*. If I do not block these “sticky” molecules, the secondary antibody may bind to them. When I later shine fluorescent light on the sample, I would not be able to tell the difference between the protein I am interested in and the “sticky” molecules. The next steps in the procedure were described in my first update. I placed the primary antibody, which will bind to the protein I am interested in, on the muscle sample. Following this, I placed the secondary antibody on the muscle sample. If you recall from my first update, the secondary antibody will bind to the primary antibody and also has a fluorescent molecule attached to it. Thus, when I shine a fluorescent light on the sample under a microscope, I will see a bright colour in the places where the antibody exists. Antibodies are very small and not visible to the human eye. They are dissolved in a liquid and the solution is placed on the sample. After placing the primary antibody solution on the sample, I have to let it incubate in the humidifier for at least one hour. This ensures that the primary antibodies will bind to the protein I am interested in visualizing. The same must be done when I place the secondary antibody solution on the slide to make sure it binds to the primary antibody. It is also important to wash off any extra primary or secondary antibody after their one hour incubation period, or else I may be visualizing left-over antibodies instead of my protein of interest. This washing step is done in a special apparatus that allows the entire slide to be immersed in a liquid (Figure 2).



Figure 2: The microscope slides are placed in this special glass container and then it is filled with a liquid like such as a washing solution.

Since this was a practice experiment, I did not use dystrophin and m-cadherin antibodies as I described in my first update. Furthermore, I used mouse muscle tissue because we will have very little seal tissue to work with and I want to perfect my procedure before I use the seal muscle. It is as true in science as it is in life to say, “Practice makes perfect”. I practiced performing the immunohistochemistry procedure with an antibody that binds to a protein called desmin. Below are the results of my experiment (Figure 3). The areas on the cells that my primary and secondary antibodies have attached to are a red colour, indicating that the muscle has desmin. The blue coloured areas are the nuclei of the cells, which I labeled with a dye called DAPI.

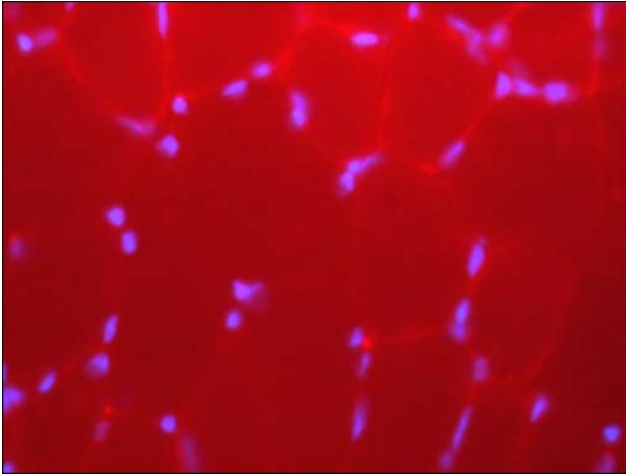


Figure 3:
Immunohistochemistry
results for desmin.

Red ⇨ desmin

Blue ⇨ nuclei
(Stained with DAPI)

This picture is similar to the one in my first update. It is also a cross-section of muscle tissue, but it appears all red because desmin is found in abundance in muscle cells. You may notice that some areas have a more fluorescent red colour than other areas. This is due to a higher concentration of desmin in the area, leading to more binding by the primary and secondary antibodies. The more secondary antibodies that bind, the brighter the colour will be because of a greater number of fluorescent molecules. You may also notice that there are many blue nuclei. This is because *skeletal muscle*, which is responsible for all voluntary movement in the body, is *multinucleated*. This means that each muscle cell has more than one nucleus.

The next steps of my research will include testing the m-cadherin and dystrophin primary antibodies on muscle tissue. Once this procedure is perfected, I will begin taking serial sections of Weddell seal muscle tissue and performing immunohistochemistry on it to discover the number and distribution of muscle stem cells.

I am very excited for the weeks to come as I will begin to investigate the hypotheses I described in my first update. In the mean time, keep checking my blog for updates on my progress in the lab.

Challenge Question:

As described in my update, skeletal muscle cells have many nuclei within a single cell. What is the job of the nucleus within a cell? Why do you think skeletal muscle cells have more than one nucleus?