Lab Summary- Fall Semester, 2015
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Using Stem Cells to Study the Presence of Dystrophin in Patients with Duchenne Muscular Dystrophy

Duchenne Muscular Dystrophy is a genetic disease that involves progressive muscle degeneration and weakness. DMD is caused by a mutation in dystrophin, a protein that keeps muscle cells intact. Dystrophin provides muscle cells with strength by connecting the internal cytoskeleton to the surface membrane. Without its structural support, the cell wall becomes less stable, and eventually the cell bursts and dies. Eventually, in DMD patients, the rate of cell death becomes increasingly larger than the rate of cell regeneration, leading to the deterioration of the muscle.

Stem cells have an incredible capacity to replicate and thus produce large amounts of cells. Because of this function, with normal wear and tear on the muscle, stem cells from within the muscle aid in the repairing of the damage. However, this leads to the generation of more and more cells with mutated dystrophin in DMD patients. The exact mutation of dystrophin is unknown. Studying the stem cells of DMD patients would allow for further research of what is happening with dystrophin in people with this disease.

Over the past semester, our lab has been practicing maintaining induced pluripotent stem cells (iPSCs) in hopes to eventually differentiate DMD patient stem cells into cardiac muscle for further research on dystrophin. The stem cells were received from a lab in Israel, who made the iPSCs by taking a skin biopsy from the patient and then using fibroblasts and reverse-differentiate them back into stem cells, or hiPSCs. The lab sent us four different lines of patient stem cells: lines 28.1, 28.12, 29.3, and 30.6; however, half way through the semester, only lines 29.3 and 30.6 had the dystrophin mutation confirmed. Additionally, we have been maintaining BJiPSC cells we had in our liquid Nitrogen tank. These stem cells do not have a dystrophin mutation, so they will be our control. Unlike the hiPSCs, they are embryonic stem cells, and therefore have never been differentiated before.

We followed the protocol and used the materials suggested in [insert paper name/author here]. In September, we began growing the cultures on plates coated with 100μg matrigel. Following the protocol, we plated the thawed cells in one well with 2mL mTeSR1 media with a rock inhibitor suggested by the paper. Daily, we would change the media on the cells, only using rock inhibitor within the first 24 hours after passaging the wells. While monitoring the plates in-between passages, we would mark any suspicious looking cells with a marker on the microscope in the culture room. We would then aspirate off the media from the well and use a pipette tip at the end of the aspirator and touch it to the plate in the areas that we had marked on the plate, removing the unwanted cells. Before passaging, we would allow the cells to grow until they were 80-90% confluent, or until they reached around day 12 in culture. To split the plate, we would freeze down 5 wells each in their own cryovial to store in the liquid Nitrogen tank. We would then split the one remaining well into all six wells of a new matrigel-coated plate.

Before we began growing our own stem cells, we were growing some from the Herron lab, using geltrex instead of matrigel to coat the plates, as geltrex is cheaper. Using geltrex, we saw lines covering the plate that were either weird congregations of cells or scratches at the bottom of the plate. We switched back to matrigel when we began plating our own cells and never had issues with these lines again. We did not figure out exactly what caused this problem, but we decided that it is best to use matrigel, as it is suggested in the paper as well.

Once we were growing our own cells, we began to make adjustments to the paper's protocol based on what we observed to work better on our cells. Primarily, we decided to use iPS Brew as a media instead of mTeSR1. We began using iPS Brew primarily because we ran out of mTeSR1, but realized the cells seemed to recover after freezing and grow faster with iPS Brew (Figures 1 and 2).

Next, we started splitting the plates into 3 wells instead of 6 while passaging because we wanted the cells to be more confluent. This was the case for the BJiPSCs because we wanted to prepare them for differentiation soon, which requires ~90% confluence. As for the hiPSCs, we were unable to get any colonies to form and had very few cells on any of our plates. Figure 3 shows our BJiPSC colonies on the left and a possible hiPSC colony on the right. There is a clear difference between the successes of the two plates.

We are currently in the process of beginning the differentiating process of our BJiPSC line. As for the hiPSC line, we are continuing to monitor them, but we have changed our recovery process. We plan on leaving them in culture after thawing for 48 hours with rock inhibitor to see if colonies establish better. We will watch for colony formation after the first media change and two days after thawing without pick cells of the plate to keep from aspirating off unnecessary cells.

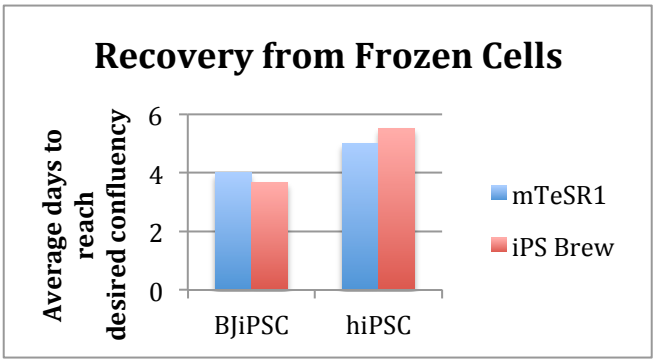

Figure 1. Average days to reach desired confluency when recovering frozen cells.

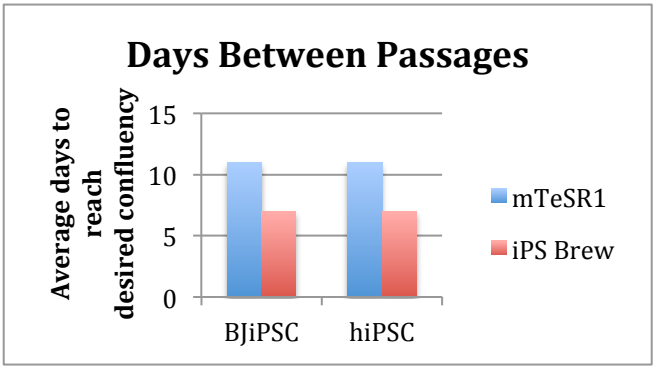

Figure 2. Average days to reach desired confluency between passages

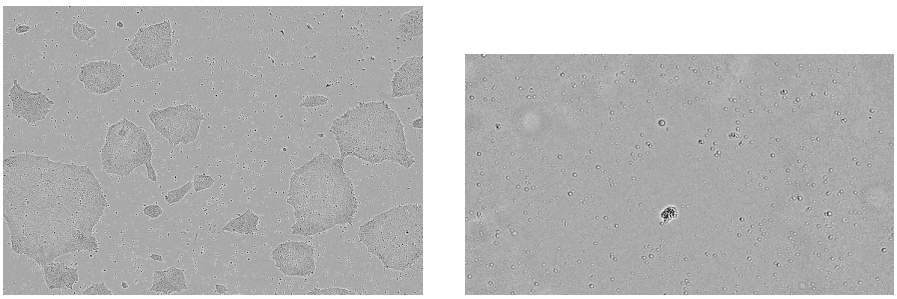

Figure 3. Picture of stem cells. Left: BJiPSC colonies. Right: Possible hiPSC colony