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Dystrophin-Glycoprotein Complex and Reactive Oxygen Species

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Abstract

Duchenne's Muscular Dystrophy (DMD) is caused by a deficiency in dystrophin protein. DMD is distinguishable through muscle degeneration and weakness. Dystrophin protein is a necessary structural link between the sarcolemma and the cytoskeleton. Studies show Neuronal Nitric Oxide Synthase (nNOS), a critical enzyme in the sarcolemma, that catalyzes nitric oxide (NO), is a molecular component of the Dystrophin-Glycoprotein Complex (DGC). We will expose the muscle sarcolemma to NO by using gas plasma. Three methods will be tested: 1) treatment with air through a plasma device, as our control, 2) treatment with NO through the plasma device, and 3) treatment with NO via Cold Atmospheric Pressure Source (CAPS) to generate a NO plasma. rt-RT-PCR analysis and confocal microscopy will allow quantification of DGC stability. We propose to answer mechanistic questions such as: 1) does increased NO levels affect the expression of muscle specific genes in the presence and absence of dystrophin, 2) will increased levels of NO stabilize the DGC within the cell, and 3) are other types of muscle cells (skeletal, cardiac, and smooth) affected by increasing NO in cells. Thus, we predict NO treatment will rescue the deficiency in absence of dystrophin.

I. Background

Symptoms and Diagnosis of Duchenne Muscular Dystrophy

Duchenne Muscular Dystrophy (DMD) annually affects 1 in 3,500 males worldwide. DMD is an inherited, fatal disease causing muscle degeneration and weakness of all muscle types. DMD is found in young boys, between the ages of two to three, due to a X-linked recessive pattern. DMD is displayed when a frameshift mutation occurs on the dystrophin protein gene, Xpr21, the largest gene on the human genome, preventing gene expression (refer to figure 1). A frame shift mutation occurs when a deletion or insertion exists in a DNA sequence, which in turn, causes a shift in sequence interpretation. Abnormal behaviors are first noticed in walking, gait, and speech with the development of pseudohypertrophy, or enlarged legs. Symptoms first occur in proximal muscles and progress to the distal muscles. Death occurs due to weakness of thoracic muscles and a decrease function of the respiratory system. Dilated Cardiomyopathy is the most prevalent cause of death.

Figure 1

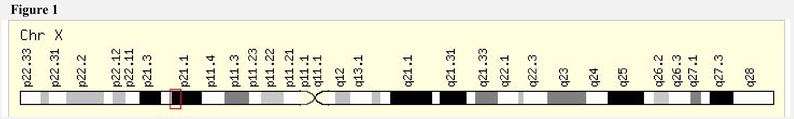

Figure 1: This figure shows where the frame shift mutation occurs on the X chromosome at Xpr21.

Dystrophin-Glycoprotein Complex

The Dystrophin-Glycoprotein Complex (DGC) is a multiprotein complex, functions as a structural link between the sarcolemma-cytoskeleton and the extracellular matrix (refer to Figures 2-3). It aides in blood flow regulation, and in muscle fatigue recovery. A decrease in function of this protein complex causes muscle fibers to become weakened and results in more susceptibility to muscle degeneration and tissue death.

The DGC regulates the recruitment of Neuronal Nitric Oxide Synthases (nNOS), a signaling molecule important in muscle relaxation, catalyzes the production of nitric oxide (NO) (refer to Figure 2). When muscle relaxation occurs, NO diffuses through muscles cells causing the muscle to relax. nNOS has an effect on the DGC, which in turn, affects muscle fatigue, vasodilation, and the structural integrity of the sarcolemma and the cytoskeleton.

Dystrophin, a protein component of the DGC, provides the structural integrity link between the sarcolemma and the cytoskeleton. Dystrophin aids in signaling pathways, such as nitric oxide production, Ca^{2+} entry, and reactive oxygen species production.

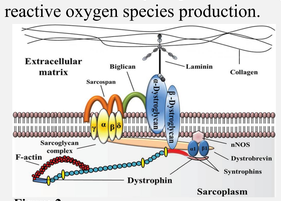

Figure 2

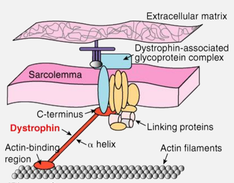

Figure 3

Figure 2-3: These figures show the relationship between dystrophin, the sarcolemma, and the extracellular matrix. Dystrophin-Glycoprotein Complex works to bring stability to muscle by attaching the muscle to different surfaces. nNOS is also shown attached to the glycoproteins of muscle and are important in muscle relaxation.

The Effects of Duchenne Muscular Dystrophy

A lack of dystrophin in patients with DMD disrupts the DGC and nNOS, thereby, causing a decrease in structural integrity. The absence of dystrophin causes the lattice structure of costameres, a structural-functional component of muscle cells, to be disorganized. This leads to a fragile sarcolemma and a decrease in membrane permeability. The fragility of the sarcolemma causes nNOS to detach, preventing the creation of NO. The sarcolemma and associated proteins become damaged and an influx of calcium ions center the cell. This causes necrosis. In turn, muscles are extensively sensitive to lengthening.

II. Methods

Our first step was to establish a time course of how normal C2C12 cells differentiate into myoblasts (Figure 4). We conducted a time course in order to qualitatively determined the normal differentiation time course. We used a serial dilution in a 24 well plate to photograph each dilution (Figure 5A-C). We obtained nine days of photographs for each well (Figure 6).This was done to establish a normal baseline in order to compare it to our future experiments where gene knockdown will be conducted.

Figure 4

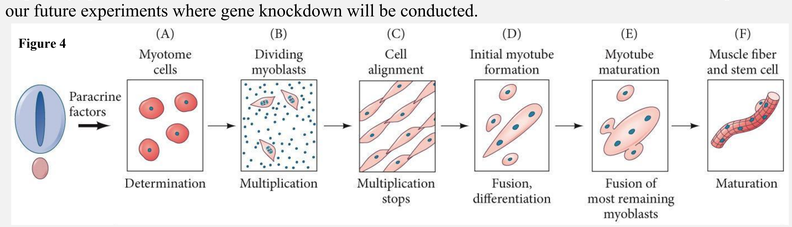

Figure 4: This figure shows the progression of myotome cells to a mature muscle fiber. We used this process to develop a time course for the C2C12 cell line. We have accomplished our time line up through myotube maturation.

Figure 5A

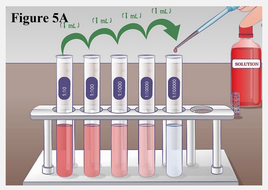

Figure 5B

Column 1-100%- 2.5 X 10^6 cells/mL

Column 2-1:10- 2.5 X 10^5 cells/mL

Column 3-1:100- 2.5 X 10^4 cells/mL

Column 4-1:1,000- 2.5 X 10^3 cells/mL

Column 5-1:10,000- 2.5 X 10^2 cells/mL

Column 6-1:100,000- 2.5 X 10^1 cells/mL

Figure 5C

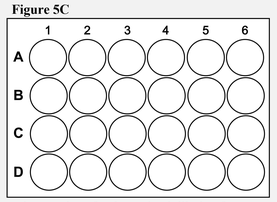

Figure 5A-C: These three figures demonstrate the serial dilution performed in order to obtain various options for the optimal dilution for muscle differentiation. The columns display the dilutions while rows A-D are replications of the dilutions associated with each column.

Figure 6

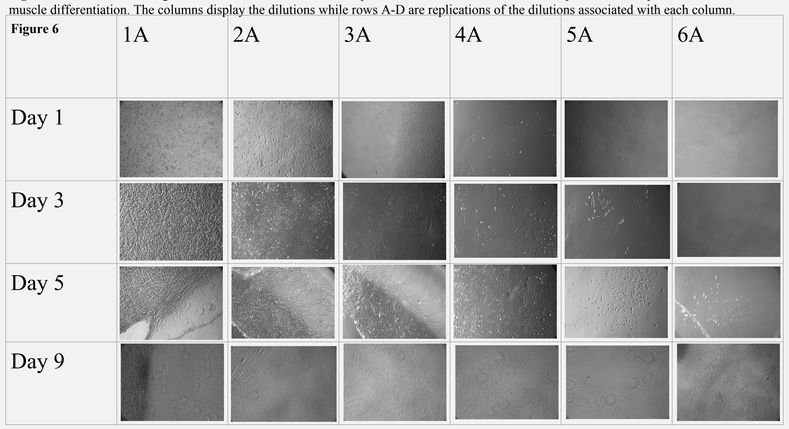

Figure 6: This is an example of our 9 day time course. This figure shows the 6 dilutions from row A on odd days. The columns become more dilute toward column six. As the days progress, the cells multiply and begin to differentiate to become mature myotubes. All photos have been taken using the 10X objective lens.

III. Future Experiments

With the use of Cold Atmospheric Plasma (CAP), we hope to induce physical and chemical changes when applying it to the biological surface, tissue, grown from C2C12 cells. CAP is an ionized gas that can be created using several different gasses such as: helium, argon, oxygen, and nitrogen. CAP has shown to be useful in wound healing, eradication of biofilms, oncology, and tissue regeneration. With the use of a CAP device (Figure 7) and the plasma produced with nitric oxide (NO), we intend to further our studies by applying NO plasma to muscle tissue from C2C12 cells. NO gas is our chosen gas to use due to the circumstantial effects of DMD where NO cannot be created. By exposure of plasma to muscle tissue, we hope to see an increase in muscle stability as NO exposure compensates for the lack of dystrophin and rescues the other components of the Dystrophin-Glycoprotein Complex.

Figure 7

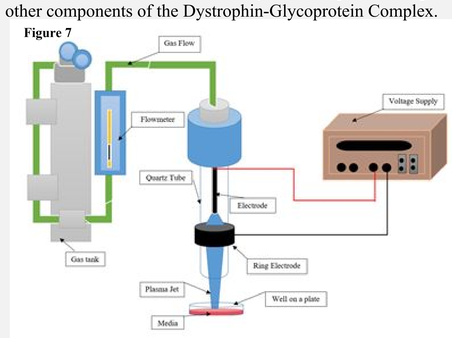

Figure 7: The figure to the left shows a schematic of a CAP device that has been wired to a voltage supply. With the use of gas flow from the gas tank, gas travels through the electrode that is connected to the voltage supply. The gas flows through the electrode to the ring electrode (also connected to the voltage supply) to produce a plasma jet that projects onto a well plate containing media and tissue.

In addition to the use of NO plasma, we will also be using a chemical compound called NONOates. Studies have shown that the use of NONOates for biological purposes have exhibited the release of NO. With this, we hope to see NO levels in our cells rise.

We will be using Polymerase Chain Reaction (PCR) to conclude whether or not the levels of NO has increased in the cells. PCR is a molecular biology technique used to create amplified copies of DNA and RNA segments. PCR will determine if our experiments are successful in the increase of NO levels.

If we are able to increase the levels of NO, we intend on knocking down the dystrophin gene and using the CAP device with NO gas and NONOates to help improve the functionality muscle tissue from the absence of dystrophin. Gene knockdown is a technique used to reduce the expression of one or more gene. We intend on using short hairpin RNA (shRNA) to permanently knockout the function of the dystrophin gene.

IV. Acknowledgement

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