

# *Muscular Dystrophy: Stem Cell Therapy*

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**Abstract:** Genetic disorders affect many people, and muscular dystrophy is a disorder that can greatly decrease the quality of life. Finding treatment to stop or prevent the loss of muscle function by the dystrophin protein is a top goal for scientists in this field. The treatment involves stem cell transplantation by intravenous injection, or local muscle injections. Various stem cells are under consideration, with analysis focusing on the specific stem cells' effectiveness and ability to be used as a treatment.

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## **Introduction: Muscular Dystrophy**

Muscular dystrophy is a genetic disorder, with approximately 30 variations of the inherited disease.<sup>1,3</sup> All of the various forms of this disease can result in both muscle weakness and muscle loss. The variations can differ in their onset, either occurring during infancy, childhood, or middle to late thirties, but all are a genetic disease with similar symptoms.<sup>1,3</sup> Depending on the specific variation of this genetic disease, symptoms can be mild with only physical therapy needed as a treatment, or symptoms can be severe, potentially causing disability and early death. This genetic disease is different from other sicknesses in that there is currently no way to treat it because it is coded into the person's DNA, the instructions for a person's life.

The most common variation of muscular dystrophy, Duchenne muscular dystrophy (DMD), is the result of a mutation of a specific gene in the genetic code. This gene codes for a 427 kD cytoskeletal protein called dystrophin, believed to be the largest gene in the human genome.<sup>2</sup> The mutation of the gene dystrophin is often caused by an accidental insertion or deletion of a nucleotide in the DNA causing a frameshift, or point mutation, which causes an error in the protein formation.<sup>2</sup> The mutation causes dystrophin to be either completely lacking from the muscle cell, or functioning incorrectly. With dystrophin not working correctly, muscles begin to weaken and deteriorate. Dystrophin acts as the shock

absorbers for muscle cells and prevents fibre damage of the muscles; without this protein the muscles wear out.<sup>2</sup> To cure the disease the cells would need to be repaired, so a treatment would need to be found that can correct the dystrophin protein and its complex.

There are several different treatments for muscular dystrophy. The simplest therapies for the disease involve keeping the muscles mobile and trying to promote an upright posture. Braces are used to keep the spine and joints in desired locations with the muscles functioning in the correct manner.<sup>1</sup> Surgeries may also be performed as treatment to release tendons that tighten as the disease progresses.<sup>1</sup> Medications, belonging to a group called corticosteroids, are being used to slow the effect of DMD.<sup>1</sup> Gene replacement therapy is a treatment that uses viruses or plasmids to deliver dystrophin gene sequences to cells with incorrect sequences, but the treatment is in the trial stage.<sup>2</sup> Stem-cell therapy treatment for genetic disorders has large potential as a long term solution. Currently stem cell therapy is being looked into readily because it appears to be a possible cure for muscular dystrophy.

Stem cell therapy is the use of stem cells to treat various diseases with no other known cure. Stem cells can be acquired from various locations in the human body or generated in vitro. Bone marrow has been used to treat cancer for quite some time, but the use of stem cells in genetic diseases is being continuously researched. Stem cells are considered a possible cure-

all because of the potential for stem cells to replace cells with malfunctioning proteins.

Stem cells have been used to treat diseases in the past, but the use of these various cells to treat muscular dystrophy is being heavily researched. There is more than just one type of stem cell, so these various types are being researched to determine which cell has the greatest effect on muscular dystrophy. Stem cells can be administered to muscles in different ways, and this is also being researched to see what methods are most effective. Depending on the type of stem cell and how it is introduced to the muscles, the effectiveness can greatly vary and this is what is being investigated.

### **Stem Cell Therapy in Muscular Dystrophy: Methods of Injection**

Early assessment with stem cells used mice to test the effectiveness of different injection methods. The mice used have a genetic disease similar to Duchenne muscular dystrophy found in humans, and the mice are referred to as mdx mice.<sup>5</sup> Experimentation with mdx mice focusing on stem cell injection was conducted by Gussoni et al. Gussoni et al.'s experiment compared the different types of stem cell injections to see which method is most effective at delivering cells to the desired areas.<sup>5</sup> The method of injection is a very critical portion to the treatment, because even if stem cells are found to be highly effective, they will have no effect in the body if the cells cannot reach the area of interest. Gussoni et al. compared two different stem cell injections based on injection methods used to treat other diseases.

In Gussoni et al.'s experiment, mice were injected with stem cells in two different ways: either directly into specific muscle areas of interest, or intravenously, similar to a bone marrow transplant for leukemia patients.<sup>5</sup> The target areas for stem cells are the muscles where dystrophin is not functioning properly, so the method that more accurately reaches these areas is being tested.

Gussoni et al. found that directly injecting stem cells into the muscles ensures that the cells reach the area of interest.<sup>5</sup> A downfall of this injection process is that it is costly because of the instruments and surgery time needed.<sup>5</sup> Muscular dystrophy affects most muscles in the body, so stem cells have to be injected throughout the body. Once the stem cells are injected, the cells are localized to the area of injection, so

many injections are needed to cover the complete body.<sup>4</sup>

Intravenous treatment is an injection into the vascular system, the blood vessels. This method is how leukemia is treated with bone marrow and is found to be very efficient. Introducing stem cells intravenously involves injecting the stem cells into the blood stream and allowing them to move on their own to each area of the body.<sup>5</sup> If the stem cells are small enough, they can diffuse from the blood into all muscle areas. Such diffusion would require much fewer injections and materials, and it would be less painful for the patient in total. This method of injection would be convenient, be cheaper, and put less stress on the patient.<sup>5</sup> The success of the treatment lies completely on the ability of the stem cells to be able to move from the blood vessel walls into the muscle.

Gussoni et al.'s experiment shows that injection by both methods is very possible, with both methods involving the integration of stem cells into the muscles. Intravenous treatment appears to be the most effective, but only if the stem cells can diffuse through the vessel wall.<sup>5</sup> Gussoni et al. found that if the most effective stem cells cannot diffuse through the vessel walls, direct injection is effective but requires many injections to the muscles.

Gussoni et al.'s experiment showed that stem cell therapy is highly probable as an effective treatment. The experiment they conducted also showed that stem cells do work in mdx mice to help restore function to dystrophin.<sup>5</sup> The possibility that function was partially restored was a huge breakthrough since previous studies of directly injecting the dystrophin protein had not worked.<sup>5</sup> There are many different types of stem cells, and testing was next conducted to see which cells resulted in the greatest increase in dystrophin function.

### **Stem Cell Therapy in Muscular Dystrophy: Types of Stem Cells**

The ability of muscle cells to partially regenerate led to the realization that there were stem cells that directly relate to the muscle, or stem cells that could be adapted to allow for increased muscle function.<sup>6</sup> There are several different cells that can perform these functions, and Price, Kuroda, and Rudnicki reviewed different types of stem cells that are being

tested and have the possibility of being used as a treatment for muscular dystrophy.<sup>6</sup> The experiments that Price et al. reviewed were all conducted on mdx mice, same as in testing the injection methods.

Price et al.'s analysis of previous experiments found that regeneration of muscles is led by muscle satellite cells, a muscle specific stem cell. Muscle satellite cells recruit nonspecific cells to fuse and differentiate into the new muscle fibers, so muscle satellite cells facilitate the creation of new functioning fibres to replace the fibres damaged from malfunctioning dystrophin.<sup>6</sup> In muscular dystrophy, muscle satellite cells cannot replace the cells with the malfunctioning dystrophin protein. Price et al. found that these satellite cells are completely exhausted with their function depleted, so new fibres are not replacing the damaged fibres.<sup>5,6</sup> Gussoni et al.'s experiment also found that stem cells were not maturing to functional muscle cells after replication, like the cells' normal process for maturation, but instead remained stem cells.<sup>5,6</sup> Satellite cells were transplanted directly in order to replenish the depleted satellite cells and to function for the stem cells that were not differentiating to muscle cells. Price et al. saw there were positive results of restoring dystrophin function three weeks after injection, a promising start to a possible long term treatment.<sup>6</sup>

In order for satellite cells to be used as treatment, the cells must be isolated from the body. Isolation of these satellite cells is problematic because they are mainly found in core muscles like the diaphragm.<sup>6</sup> Isolating these cells from the diaphragm is difficult and would require surgery, and because cells are not able to be seen without a microscope, it would be difficult to extract the cells. The possible use of muscle satellite cells is diminished by the difficulty in acquiring the cells for treatment.

Previous success with muscle satellite cells had led to experimentation that involved generating a similar cell, but one that could be more easily acquired. Building on this research, Price et al. found that satellite cells could be generated in vitro, meaning the cells were grown in a lab, most likely in an isolated petri dish or test tube.<sup>6</sup> Generating these cells in vitro is a much easier way of isolating this specific type of cell than extraction, and these generated cells are given the name primary myoblasts. Primary myoblasts were given their own name because these cells may

behave differently when generated in vitro compared to the muscle satellite cells isolated from the body.<sup>6</sup> Price et al. determined that myoblasts could be injected right into the muscle of concern, were easily cultivated, and showed success in increasing the dystrophin protein's function.<sup>6</sup>

With high promise from these results in mice, Price et al. observed the effect of myoblasts on non-human primates to see if the myoblasts would be accepted by the body and what the immune response would be.<sup>6,7</sup> The myoblast were successfully integrated into the primates, but it was unclear whether the cells would provide any phenotypic change to dystrophin.<sup>6</sup> The primate trial showed that it was very possible that myoblasts could be accepted by the human body as well, because of the similarity between the two. Human clinical trials with myoblast injections began in the 1990s, but data was originally inconclusive as it proved difficult with limited technology to determine if the dystrophin had been corrected.<sup>6</sup> Studies are still being conducted to determine the total effects of myoblast transplantation on the human body and dystrophin. Even with the success of myoblasts, other stem cells are being researched to see if they have even better results.

Price et al. discussed another set of stem cells in muscles, in addition to the satellite stem cells, called side population cells. These cells can be found in both muscle and bone marrow, and they still possess myogenic potential when transplanted into the muscle.<sup>6</sup> Dyes are used to isolate the side populations. These cells are not as difficult to isolate as are the satellite cells since side populations are found in more accessible areas. The muscle side population cells regenerate muscle, but the regeneration is through a different path than the other stem cells in the muscle.<sup>6</sup> One of the more promising contributions of the muscle side population cells is their ability to move from the blood to the muscle, meaning there is a possibility for the cells to be injected into the vascular system and reach many muscles throughout the body.<sup>6</sup> This is the ideal method of injection that was discussed previously, so if the side populations function as predicted, they will be the ideal treatment. The muscle side population cells' regeneration abilities are not nearly as long term, as are the abilities muscle satellite cells. Despite side populations' ability to be injected intravenously, the cells do not last as long as

desired, so other stem cells were looked in to.<sup>6</sup>

Another type of stem cell that Price et al. observed to be used as a treatment is bone marrow. Bone marrow was tested due to the bone marrow's success in decreasing the effects of certain cancers. When bone marrow is injected into the blood, it differentiates into new blood cells, which is why it is used to treat leukemia.<sup>6</sup> Price et al. found through experimentation with mdx mice that the stem cells in the bone marrow can differentiate into myogenic cells, meaning that bone marrow can be used in muscle repair.<sup>6</sup> In the mdx mice, dystrophin was repaired, and it was found that the bone marrow cells stayed in the musculature longer than did any of the previous treatments while cells still maintained dystrophin expression.<sup>5,6</sup> The process of the bone marrow converting from bone cells to muscle cell is still unknown. Despite the mystery of the process, bone marrow still has many positive aspects to be used as a treatment. One of the major positive aspects is that bone marrow is injected intravenously, the ideal injection method. This type of stem cell changes from bone to muscle, and it is similar to embryonic stem cells in their ability to differentiate into a variety of cell types, and embryonic stem cells have been studied greatly in the past.

Price et al. found that embryonic stem cells were originally thought to have the solution to genetic diseases. The lack of breakthroughs in recent years in the use of embryonic stem cells has decreased research in this area. These stem cells hold a lot of promise because they are the cells that all organs originate from, so there is no question of whether they can differentiate into the desired cell or not.<sup>6</sup> The use of embryonic stem cells to grow new muscles was first conducted approximately 20 years ago.<sup>5</sup> The efficiency of muscle growth by these cells has not been improved in recent years, so it is not often used as a viable transplantation option. Embryonic stem cells do show promise in gene analysis, where specific genes can be targeted. Genes of interest can more easily be isolated to see how their function relates to muscle activity.<sup>5</sup> By studying embryonic cells in vitro, there is an increase in knowledge regarding the molecular process of muscle growth and formation.<sup>5</sup> The information found from the in vitro gene analysis is currently not being used clinically, and more studies are needed to be conducted before proved

useful. Currently embryonic stem cells are not used as a treatment, but there is still a great deal of potential that may arise with future technology and research.

### **Stem Cell Therapy: Health Concerns**

In stem cell therapy, some of the cells that may hold the most information are embryonic stem cells, cells that are the subject of great controversy. The embryonic stem cells used in research are those that have been grown in vitro, meaning they were cultivated in a petri dish and never created inside a human being with the intent for life. Others still believe that use of in vitro stem cells is unethical, and experiments can have difficulty being funded due to the controversy surrounding the subject.

Besides ethical concerns with stem cell treatments, there are also health concerns. The body's immune response to stem cells is something that must also be considered during treatments.<sup>7</sup> Anytime a foreign substance is injected into the body, the body's immune response has to be closely monitored. When stem cells were injected into mdx mice and humans, immunosuppression had to occur to allow the foreign stem cells to be accepted by the body and not destroyed.<sup>7</sup> This means the immune system, in a sense, must be turned off to allow for treatment to take place. The suppression of the immune system is why many people undergoing cancer treatments can become sick very easily, because other illnesses have a higher chance of infecting individuals when the individuals are being treated. When a person's immune system is suppressed, the microbes that are commonly destroyed by the immune system are allowed to thrive and can lead to severe illness. The immunosuppression is required, because studies have shown that within an hour of injection, 90% of the injected myoblasts were removed by the immune response, meaning they would not reach the muscle to have a chance to affect dystrophin.<sup>7</sup>

Scientists have also considered the possibility that the immune response could be attacking the newly functioning dystrophin. The new dystrophin from the injected stem cells is not a naturally functioning protein in the person's body, so its function may be seen as a foreign threat.<sup>7</sup> With clinical trials of several stem cells being tested, the immune response is a present concern for doctors.

The immune system is the major concern, but the

differences in the subjects of the stem cell treatment have to be considered. There are differences between muscular dystrophy in mice and humans. The muscle in mice is more resilient, so the fibers can reform and the disease is less severe.<sup>5</sup> Mice muscles and human muscles have different characteristics, and these differences must be known before testing can be done with human stem cells.<sup>5</sup> The differences also mean that there is no definitive proof that these treatments will have any effect in humans. Studying mice allowed for a great deal to be learned about stem cells and how they function in muscles, but clinical test on humans must proceed with caution because of possible unexpected side effects that may have not been seen in the mdx mice.

### **Conclusion**

Muscular dystrophy is a genetic disease that greatly decreases the quality of life for many individuals by decreasing muscle function. There are many studies being conducted on the possible application of various stem cells. Dystrophin is isolated as the protein responsible for the muscle malfunction in Duchene muscular dystrophy, the most common form of muscular dystrophy. At this time, myoblasts are the type of cells that have had the most clinical trials, and show a great deal of promise. Other cell types are currently being tested, with various clinical trials underway. The clinical trials focus on the ability of the stem cell to increase dystrophin, as well as how easy it is to acquire the cells and how easy it is for them to be injected into the body. With any type of medical treatment, there are several controversial aspects that present themselves as well. These include ethical concerns that arise with generating cells in vitro and how the immune system will respond to new stem cells in the body. Despite concerns, research still holds promise with many stem cells being clinically tested, myoblasts being the most favored stem cells currently. All of the different stem cells have certain characteristics that work as a possible treatment, and only after clinical trials will the most effective cell be found.

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