The Application of Stem Cells in Muscle Culture Relating to Duchenne Muscular Dystrophy

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Abstract

In this research paper we will be discussing the advantages and possible disadvantages of the future uses of stem cells in medicine, as well as their potential applications. In particular we will focus on four main areas of stem cell research; how stem cells are stimulated to differentiate into a specialised cell, as this is fundamental in realising stem cell research’s potential to provide new and previously unimaginable cures; how one may culture them for tissue transplant; how stem cells may be used to treat muscular dystrophy and finally the potential hurdles and issues surrounding this area of research. By looking closely at these few areas we hope to deduce whether there is a viable future for stem cells in medicine, what this future may be, and whether these benefits outweigh the drawbacks.

Introduction

After the initial discovery of stem cells in the early 1900s, when scientists discovered that certain cells could generate blood cells, the world of medical research began to experiment and discover the origin and nature of these totipotent and pluripotent cells, which would be the focus of study in years to come. In the 1900’s there was a sizeable increases in bone marrow stem cell (mainly mesenchymal and haematopoietic stem cells) research, until in 1973 a team of physicians carried out seven successful bone marrow transplants which then confirmed their potential and highlighted their usefulness in treating diseases. Although these bone marrow stem cells are particularly useful in treating conditions such as leukaemia and lymphoma, there are obvious disadvantages. Firstly, there is the fact that adult stem cells are rather difficult to culture for a variety of reasons. Initially, they will not multiply or proliferate, limiting their potential as a cancer treatment. Moreover, in order to avoid the rejection of these bone marrow stem cells they have to be taken from the patients themselves (autologous transplant) or in 25%-30% of situations from a person with identical tissue genetics (allogeneic transplant.) Hence, because of these issues, adult stem cells are particularly difficult to culture and to collect in enough amounts to have a significant therapeutic effect.

Therefore it was a considerable breakthrough in 1998 when James Thompson (University of Wisconsin - Madison) isolated a new type of stem cell from an embryo in the blastocyst stage of development and then went on to develop and culture the first embryonic stem cells. These new stem cells where quickly seen to be superior to the earlier adult stem cells, as not only were they totipotent, meaning that they could differentiate into over 220 cell types within the human body, but were also far easier to culture as they had the ability to proliferate, and hence had far greater therapeutic potential. There are, inevitably, disadvantages with these ESCs as they raise many ethical issues, consequently leading to a reduced social acceptance, while also provoking a strong immune response from the patient’s body which must be suppressed via strong immunosuppressive drugs, leaving the patient vulnerable to previously harmless microorganisms. Throughout this research paper we will look at other possible uses of ESCs and the ethical issues involved.

Muscular Dystrophy

Duchenne Muscular Dystrophy is a genetic condition which stems from a deficiency of the protein dystrophin, essential for muscle formation. Essentially, this causes muscle to waste, as the myofibres present in skeletal muscle become irreversibly shortened and damaged, leading to severe physical degeneration. This lack arises from a mutation on the DMD, or dystrophin, gene, causing it to form a missshapen type of the protein, unable to fulfil its normal function. Dystrophin acts as an anchor for skeletal muscle tissue, forming a vital part of the protein complex (the costamere) that attaches the muscle fibre to the extracellular matrix, in this case the basement membrane of the muscle. Even though it is typically present in miniscule quantities, averaging 0.002% of muscle proteins, it is crucial to muscle formation, as without it muscle fibres cannot attach to the cell membrane, resulting in progressive muscle weakness and degeneration. The mutation that causes this is not always the same; over one thousand different mutations have been observed. Typically the condition
is carried on the X-chromosome, making it a recessive X-linked condition. Duchenne Muscular Dystrophy is far more common in males than females; women can be carriers of the gene, but are generally unaffected by it, unless they are one of the few ‘manifesting carriers’. The progression of the disease is one of worsening muscular weakness, sometimes coupled with learning difficulties, until death at the age of 25-35, mainly from respiratory difficulties. Duchenne’s, and its milder form Becker’s, is thought to affect approximately 1 in 3,500-5,000 boys worldwide.

Presently there are no truly effective treatments for MD. The most available treatment currently is drug therapy for the recently diagnosed, such as prednisone, a drug that delays the onset of muscle degeneration. However treatments such as these merely prolong the inevitable as well as causing side effects such as bone fragility and weight loss which can amplify the mental vulnerability experienced. Our hope is that, with a drive in research and a boost in finances, embryonic stem cells could not only be used to treat muscular dystrophy but to cure it permanently. Additionally, for muscular dystrophy to be treated successfully, a high quantity of stem cells will be required, and since embryonic stem cells are able to quickly proliferate this would be suitable for therapeutically treating the disease. As embryonic stem cells are totipotent, meaning that they can differentiate into almost all types of cells, scientists may be able to induce the differentiation of ESCs into myosatellite cells with a functional dystrophin gene, stimulate it to proliferate (which may be unnecessary with ESCs) and then finally inject it into the patient’s skeletal muscles with the hope that it will decelerate or even stop the effects of MD. However for this complex process to go ahead there would need to be experimental trials carried out on animals, like those done by Dr Marita Pohlschmidt. Here, highly beneficial effects were seen, yet there is still much work to be done in improving and making safe stem cell treatment. To quote Dr Pohlschmidt: "This area of research is still in its infancy and much more work must be done before stem cell technology can be regarded as a viable route for treatment of muscle disease." During these trials on mice there was evidence, from the coloured dyes used, that these implanted cells had penetrated deeply into targeted muscle, indicating that there was successful growth and development in the muscle tissue. Consequently after a single month the muscle ability of the mice had significantly increased with no tumour growth, which had previously been a drawback. Similarly, scientists have discovered a gene in ESCs called Pax3 which causes muscle cell growth; hence if this gene were to be injected into the muscle it would induce new muscle growth. However there is a strong possibility of tumour growth as a result of this treatment due to the Pax3+ cell line. While there are many and varied approaches to treating Duchenne’s muscular dystrophy, this discussion will contemplate whether it may be possible to replace these wasted muscle with muscle tissue grown in vitro, with stem cells taken from the sufferer’s own body.

**Muscle Culture**

Muscle fibres are generated by myosatellite cells, progenitor cells found embedded between the basement membrane and sarcolemma layers. In this case, progenitor cells are equated to somatic stem cells, since they do not possess a precursor found throughout the body and can differentiate to form a more specialised muscle cell, in this case forming a myotube. Normally quiescent, these cells are activated by microtraumas to the muscle fibres, microscopic tears that disrupt muscle organelles, and triggering the myosatellite response. The myosatellites migrate and proliferate to the site of the trauma. Some myosatellites remain as organelles upon the muscle fibre, ready to activate in the event of another microtrauma, but the majority differentiate, fusing with each other or the damaged muscle fibre to form myofibrils (muscle fibres). Therefore, by this process the number of myofibrils will become greater, leading to an overall increase in size of the muscle tissue. Usually, dystrophin is
present to attach the muscle fibre to the cell membrane, but for sufferers of MD, this process is lacking, leading to poorly-developed muscle tissue and degeneration.

Ideally, since one of the highest causes of death with MD is breathing impairment, it would be possible to generate new muscle fibres in the laboratory, which could then be implanted into the body to replace previously-wasted groups, to restore a degree of normal function. While more complicated muscle groups will require a higher degree of technical prowess, simpler groups could more easily be emulated *in vitro*. With respiratory problems in mind, growing replacements for the intercostal muscles is of paramount importance, to re-establish a level of normal breathing capacity. Once it has been established that this is achievable, more complicated muscle groups could be developed, to restore mobility and body strength. This would not only be applicable to sufferers of muscular dystrophy; muscles could without doubt be implanted into those with major muscle damage, for example burns victims. The application of this tissue culture would be limitless for the repair of the human body.

In normal tissue culture, the process is a relatively simple one; in June 2011 a trachea was grown for an unnamed patient from their own somatic stem cells. Stem cells were extracted from the patient’s body and seeded onto a synthetic scaffold before being grown in a bioreactor for two days. Upon completion, the scaffold and resultant trachea was implanted into the patient, continuing to grow and eventually fulfilling the normal role of a trachea. Previous examples of this had the stem cells seeded onto a donor scaffold, taken from a cadaver; this synthetic method has the benefit of not being dependant on a regular supply of donors, but can instead be pre-prepared and stockpiled until required. The process for *in vitro* muscle culture would be similar: stem cells would first be extracted from the body of the donor, ideally being myosatellite cells, as these are the principal cell in the process of myogenesis. They would then be seeded onto a synthetic framework, and then developed in a specialised bioreactor in order to achieve the correct structure and form. Upon completion, the resultant skeletal muscle tissue would be implanted into the recipient’s body, replacing previously wasted or damaged muscle groups. While this process may be sufficient for injury victims, for those with Duchenne’s muscular dystrophy the process would be a little more complicated, which will be discussed later.

The process by which the muscle tissue could be grown from stem cells is a more elaborate one than for less complex tissues such as the trachea. As previously discussed, the muscle must undergo shear forces and movements in order to form properly, by causing microtraumas and so generating new myofibrils, so any bioreactor used must be able to simulate these tensile forces throughout the development of the muscle tissue. Secondly, the developing tissue must have an adequate blood supply able to permeate it; it is all very well to grow the tissue in the first place, but in order to sustain it in the body a sufficient quantity of blood must be able to reach it for oxygen and nutrient deliverance. Currently, only a thin layer of muscle can be grown, since this issue has not been resolved; further research is required in this area to be able to develop a realistic network of blood vessels throughout the muscle, and hence thicker muscle may be grown. Another area which necessitates further study is that of its stimulation by nervous tissue. All muscle requires a nervous impulse in order to contract; this is delivered by neurons embedded throughout the muscle tissue. For the muscles to be usable in the body they must have this network present, otherwise they will be unable to contract. Currently a method has not yet been found to stimulate development of neurones in the muscle tissue, so further research must be done to aid the development of *in vitro* muscle growth.

Muscle Culture and Muscular Dystrophy

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In any tissue culture, the ideal would be to use somatic cells from the body of the intended recipient (autologous donation), thus avoiding the problem of outright rejection or having to take immunosuppressive drugs to lessen the chance of this, which would be difficulties encountered if the donor tissue came from another. Therapeutic cloning is expensive and carries with it the ethical problems previously discussed, while few people have their cord blood cells saved at birth. Therefore, autologous tissue culture would clearly be the ideal option. However, in the case of Duchenne’s Muscular Dystrophy, it would be far more difficult to achieve this, as the sufferer’s stem cells would still possess the mutated form of DMD. Therefore, the myosatellite cells would express this mutated form, and so form misshapen muscle tissue, which would be unusable for implantation. Therefore, in order to achieve normal dystrophin transcription, this faulty gene must be silenced to prevent its effects. Gene silencing can be achieved by several different ways; a particularly promising area is that of antisense therapy. However, in order to fully understand the mechanism by which this works, the exact structure of the DMD gene and its mutations must first be discussed. The DMD (dystrophin) gene is found in the Xp21.1 cytogenetic location, and thus is expressed only in males. In females, two X-chromosomes are possessed, so the ‘true’ version of the gene is expressed in preference to the mutated version. In approximately 72% of cases, exons (sections of coding DNA or RNA) are deleted, and in 7% exons are duplicated. Therefore, when the DNA is transcribed into RNA and then translated, amino acids are added or left out instead of adhering to the proper pattern, leading to a misshapen dystrophin protein unfit for purpose, as it is no longer the correct shape for attachment.

There is a similar type of muscular dystrophy to Duchenne’s, albeit in a much milder form, known as Becker’s. In Becker’s MD, different, less severe, mutations upon the DMD gene lead to a malformed, but usable, version of dystrophin which is still partially capable of fulfilling its role. This is typically found when base pairs are deleted rather than duplicated, in effect showing that it is more beneficial to lack bases than to have surplus to requirements. If the duplicated bases were silenced (characteristic of Duchenne’s), this would in effect be converting the condition into Becker’s MD, since this tends to have more deletions than duplications. If it were possible to convert Duchenne’s into Becker’s, the muscle tissue cultured would not be perfect, but would still be eminently usable, without any possibility of rejection or an immune response, and so could easily be implanted into the body to replace wasted tissue. As well, this less effective form of muscle would be more sustainable for the receiver, as it would not require the same level of myogenesis and exercise to maintain, being smaller and less powerful than usual. The receiver would then be able to utilise the implanted muscle to be able to breathe properly, regaining a degree of normal function. However this would necessitate gene manipulation, which will now be explained.

Antisense Therapy

Antisense therapy works by relying on the complementary nature of base pairs in DNA and RNA. When the mutation causing muscular dystrophy in the sufferer is identified, a strand of RNA (known as an oligonucleotide) can be synthesised which is complementary to the mRNA that the mutation codes for. Therefore, when the mutation is transcribed, the synthesised oligonucleotide can bind to it, preventing it from being translated, thereby silencing its expression. As well as RNA oligonucleotides, DNA oligonucleotides and synthetic compounds can also be used. Antisense therapy has been shown to have some success; in 2007 van Deutekom et al injected 20-mer (twenty base) antisense nucleotides into the tibialis anterior muscle, in order to induce exon-skipping of the mutated sections of DNA. This was successful; normal dystrophin expression was temporarily restored up to levels of 35% of the norm, proving that it would be possible to silence the mutated sections of the DMD gene during muscle culture. This means that the muscle stem cells used would be able to form dystrophin that would be either normal in shape or similar to Becker’s, depending

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on how targeted the antisense therapy is to the individual's own mutations, and thus would now be capable of forming usable muscle tissue when grown *in vitro*.

**Delivery of Antisense Therapy**

While antisense oligonucleotides only last a short duration, with the muscle being cultured in the laboratory it would be relatively simple to repeat treatments through the growth period of the tissue. Another major problem with antisense oligonucleotides is that of delivery. They are not capable of penetrating the cell by themselves, requiring a delivery mechanism to reach the nucleus and thus the DNA to be blocked. The most common approach to this delivery issue is that of cell penetrating proteins, or CPPs. This technique involves attaching the oligonucleotide to a polypeptide chain capable of crossing the cell surface membrane, and delivering the oligomer to the specified part of the cell, in this case the nucleus. Oligonucleotides are generally incapable of crossing the cell surface membrane; they are far too large and lipophobic to simply diffuse across, while attachment to a peptide chain also has the bonus of giving a degree of protection from enzymes found within the cell. While other delivery mechanisms exist, such as lentiviruses, this area holds greater promise, as it does not bring with it the elevated risk of cancer that other methods pose, an important consideration to be sure. Therefore, by these methods, antisense oligonucleotides could be delivered by cell-penetrating proteins, to manipulate the gene expression of myosatellite cells, thus causing them to produce partially-functional dystrophin, which in the muscle tissue cultured could then be used to restore respiratory capacity to sufferers of Duchenne’s Muscular Dystrophy.

**Ethical issues**

Although the possibilities of stem cells and their capacity to differentiate into all manner of specialised cells is seemingly endless, there are however numerous ethical issues which shroud them, being some of the most controversial and poignant that humankind is likely to encounter. The central argument is whether the destruction of a human embryo (in the blastocyst stage of development) is worth the alleviation of pain and suffering felt by millions of people. This argument is not black and white and must be scrutinised and observed from all perspectives in order to build a detailed and ethical conclusion. We will start by elaborating on the basic ethical issues involved.

Firstly there is the fact that embryonic stem cells originate from “spare embryos” left over from IVF treatment, as not all eggs are needed for implantation back into the woman’s womb, hence whether the embryos are used for research purposes or not is immaterial as the embryo will inevitably expire if not used. Therefore would it not be more beneficial to alter the unavoidable expiration of these embryos into a process in which their potential value can realised, not neglected? Similarly the actual spare embryos are directly caused from IVF hence it is *this* that should be looked at, not the stem cell research which only serves to benefit others with these spare embryos. However, many philosophers believe that there is a subtle moral difference between actively destroying an embryo and passively allowing the embryo to expire through an act of omission, irrespective of the outcome.

Perhaps the underlying disagreement which needs to be solved before a conclusion is reached is when life really begins and the overall status of the embryo. This concept is heavily disputed as many believe (Church Of England) that life begins when the first spasmodic brain impulses occur, typically 9-16 weeks, the reasoning behind this is that it is our brain and our ability to reason which seems to set us apart from more primitive life forms such as household pets. Similarly personhood is a developing capacity hence there is no real weight to a blastocyst (5-6 days after fertilisation) being a person as it is too early in its development. In these cases above there is no real problem when

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considering spare embryos as a blastocyst is too early in its development to coincide with any significant points in growth, additionally in the UK licensed research can only take place on embryos up to 14 days.

Conversely, Roman Catholics believe that life begins at the moment of conception which is both logical and non-arbitrary meaning it can’t be subjected to an individual’s perception, "Life must be protected with the utmost care from the moment of conception" Second Vatican Council. This highlights the fact that many believe that it is the responsibility of those in a position of moral stability and accountability to help preserve the fundamental rights of the “unborn baby” as it has the potential to become a human with a soul and thoughts. This argument is strengthened by the fact that the advantages of stem cells are, at this moment in time, just an expectation and may not actually be realised hence it may just be an unnecessary waste of resources and time. In addition to this one particular viewpoint would be that it is up to the mother to decide the fate of her spare embryos as to force a woman to make a particular decision would be immoral and would neglect the principle of autonomy. The principle of autonomy encompasses the idea that everyone is able to comprehend notations of what is right and conversely what is wrong; therefore an individual should act on these views accordingly. Therefore in this context, as the spare embryo can still be viewed as belonging to the mother, she should have control over it. However opposed to this idea is the view that an embryo has a separate genetic constitution to the mother hence has to be viewed as a separate entity.

Lastly, in many cases embryonic stem cell research is being funded and run by private companies such as Geron, a Californian biotech company which has poured millions into its stem cell research. This is strongly opposed by many pro-life organisations such as the Christian Defence coalition who recently protested at Geron itself. Many believe that embryonic stem cell research should not be funded at all let alone with the sole purpose of creating profit as the commercialisation of ESCs completely undermines the value of potential life. Conversely funds should be given to support advances in adult stem cells hence circumventing the moral issues involved in embryonic stem cells as these types of stem cells are just as viable in the world of medicine.

Conclusion

To conclude, the major advances in stem cell research may prove to be invaluable in treating previously incurable diseases and conditions, such as the previously-discussed Duchenne Muscular Dystrophy, through such methods as tissue culture. However despite these desirable properties, there are inevitably various problems involved such as the lack of social acceptance towards ESCs, and the need for more extensive research into areas such as successfully growing muscle tissue, with a sufficient blood vessel network and all issues of mutation addressed. Having said this, in spite of the potential flaws in stem cells therapy there is no doubt that these new advances in this area of research has opened the world’s eyes to the seemingly endless possibilities of stem cells and their application in the world.

Bibliography

http://stemcells.nih.gov/info/ethics.asp

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