

EMBRYONIC STEM CELLS AND THEIR POTENTIAL IN THE
TREATMENT OF PARAPLEGIA

By

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PASS WITH DISTINCTION

RESEARCH PAPER
BASED ON
PATHOLOGY LECTURES
AT MEDLINK 2008 AND VET-MEDLINK 2008

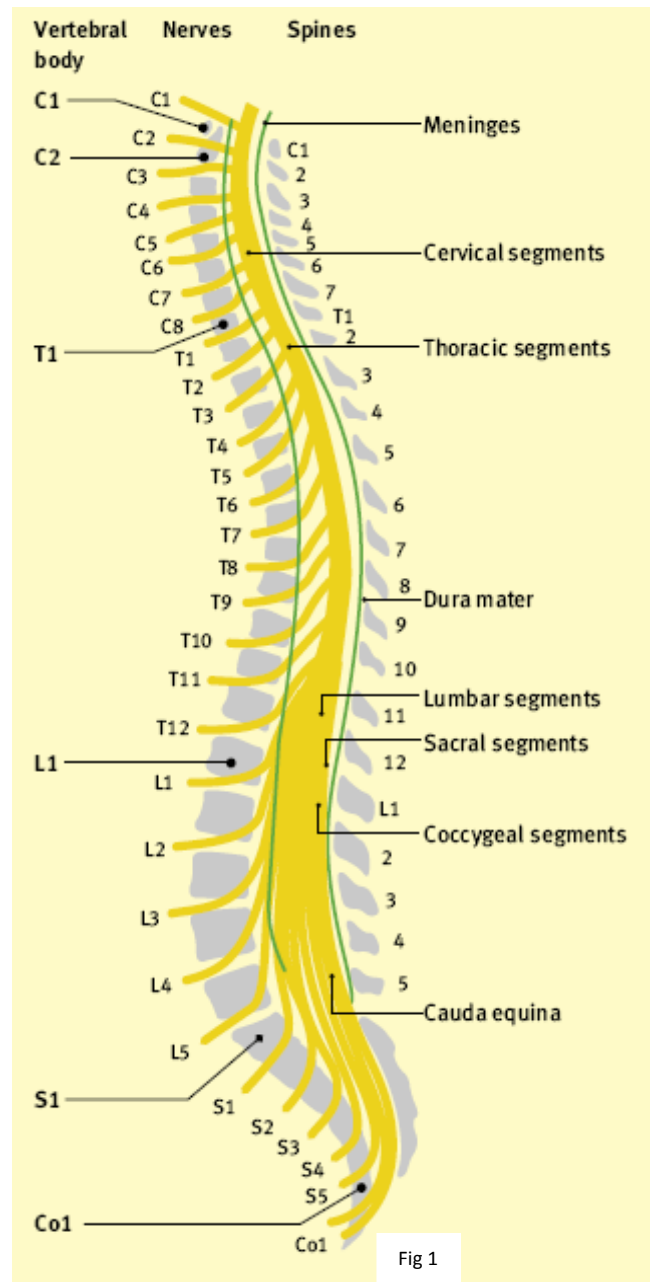
Abstract

The potential for the usage of stem cells in medicine is vast and widely seen as one of the greatest possible advancements in the field for this century. Within this paper, we will discuss the possibilities of using embryonic stem cells to help cure paraplegia, more specifically paraplegia due to injury rather than congenital conditions such as spina bifida. As we are talking about Paraplegia and not tetraplegia, we will focus on damage to the nerves T1 and below (see fig 1). We propose the use of embryonic stem cells to produce sensory and motor neurons genetically identical to the undamaged neurons within the patients spinal cord to allow mobility of the lower body without attack as a result of rejection by the body's immune system. We will introduce the method of using therapeutic cloning to create embryonic stem cells in order to avoid rejection.

Introduction

Stem cells are cells found in most multi-cellular organisms. Their two main properties are that they are able to divide numerous times via mitosis without differentiating into any other type of cell and the ability to differentiate into any type of specialised cell (potency). Embryonic stem cells are stem cells taken from the inner ball of cells found in young embryos known as the 'blastocyst'. A blastocyst may contain between 50-150 embryonic stem cells 4-5 days after fertilisation.

All stem cells are pluripotent meaning they can differentiate into any of the three main germ layers. In our case we will focus on the ectoderm germ layer, responsible for the formation of the cells in the nervous system and epidermis in order to create neurons that can be transplanted to the patient. Gail R. Martin is credited with the term 'embryonic stem cell' after he successfully isolated them from mouse embryos in 1981. Embryonic stem cells from humans are a fairly new development in medicine as they were first isolated from blastocysts in 1998 by a group lead by James Thomson. Embryonic Stem cells can be used to fight cancer in the creation of cancer killing cells as shown by the works of Kaufman in 2005.



In the future it is thought that embryonic stem cells will be used to create organs specific to the patient; this massive advancement may save millions of lives due to prevention of coronary heart disease, diabetes and pulmonary diseases such as COPD, collapsed lung and many more. As embryonic stem cells are pluripotent, many problems arise when trying to get them to differentiate into a specific type of cell. The cells require certain signals for accurate differentiation; they cannot be injected directly into the body as this will result in the formation of a neoplasm with areas that resemble all 3 germ layers (teratoma). 'Cell Therapy' has unimaginable potential within the field of medicine as it could save up to millions of lives. Stroke results in cell death within the brain as neurons are destroyed, there is potential to create and transplant neural stem cells into the brain to counteract this cell death in order to return function to the damaged areas of the brain. In February 2005, Yehoash Raphael and his US-Japanese team successfully restored hearing to a guinea pig by re-growing hair cells within the cochlea of the animal. It is thought the same technique could be applied using embryonic stem cells to grow the hairs inside a deaf patient's cochlea.

On the 1st of February 2003, Robert Aramant and his team successfully restored vision to a woman with the hereditary disease retinitis pigmentosa. The team transplanted new retinal cells to the eye created using embryonic stem cells. The success is put down to the transplant of a 2mm square sheet of the upper retinal layer which has helped to preserve the circuitry of the light-sensing cells as well as the 'nourishing' cells.

Currently a lot of research is going into the use of stem cells. The main aim for stem cells is to cure many diseases including some debilitating ones such as diabetes and Parkinson's disease. Parkinson's Disease is a degenerative disease of the brain which is caused by a destruction of cells in the brain producing a neurotransmitter called dopamine. This can lead to many symptoms such as "tremors" which is caused by inability to control limbs, impaired balance, fatigue, insomnia and many more. Embryonic stem cells were transplanted into the brain of a PD sufferer. The Stem cells differentiated into fully working brain cells which produced dopamine, which then relieved the symptoms of PD. Type 1 Diabetes is another illness that is suffered by 1 in 250 people in the UK, with that figure set to rise due to our Americanized eating/living habits. Type 1 Diabetes develops when the islets of Langerhans (cells that produce insulin) are removed by the body's own immune system (auto immunology). Research is progressing to try and harvest stem cells to replace the islets of Langerhans which would remove the need for frequent insulin injections. The list of diseases which could be cured could go on and on, however we are going to focus on one particular disease which is paraplegia. We will discuss just how the use of stem cells can allow the paralyzed full use of their legs once more.

The following sources contain further information on all topics discussed:

http://www.scienceblog.com/cms/human_embryonic_stem_cells_used_to_kill_cancer_cells_9064 - Information on the creation of cancer killing cells by Dan Kaufman

<http://www.newscientist.com/article/dn7003> - 'Gene therapy is first deafness cure'

<http://www.medicalnewstoday.com/articles/15535.php> - Information on retinal transplant

Discussion

Before we delve into paraplegia, we must first understand nerve cells and the central nervous system (CNS). The Spinal Cord contains bundles of nerves which run down from the brain to the spinal cord and connect to motor neurons which allow you to move. There are 31 segments in total and each controls a specific function. The one that is most commonly injured is the C 5-6 section which controls breathing. The spinal cord is especially important as it controls all the voluntary functions in the body, such as speech, movement of limbs and even urination. Inside the spinal cord, are highly specialized cells, called nerve cells. There are approximately 28 billion of these cells in the body, which span out to the depths of the human body. They vary greatly in length, from a few micrometers, to over a metre.

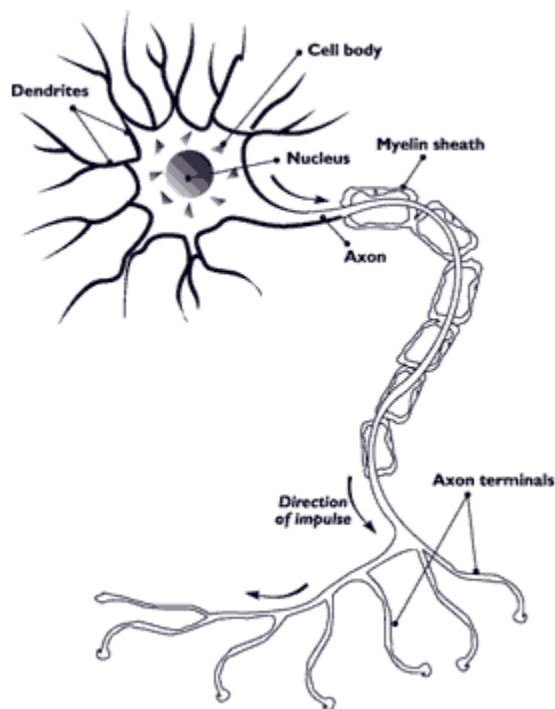


Fig 2

The brain sends messages through these brain cells. The dendrites connect to other cells to form a chain to pass the message, in the form of an electrical impulse, along. Electrical impulses are received at the dendrites, and transmitted through the axon which connects to other nerve cells. The electrical impulses travel across synapses which are junctions between two neuron cells. The myelin sheath layer that wraps around the axon is a form of insulation which ensures that the electricity keeps flowing. Normally, there will be what is called a motor pathway. This involves a chain of nerve cells which starts at the brain, moves through the spinal cord and ends at muscle tissue.

Paraplegia is paralysis of the legs due to damage in the central nervous system. A patient with paraplegia has a break in their motor pathway, which could have been caused by a variety of reasons such as: stroke, multiple sclerosis or a trauma (maybe a fall or a blow). However paraplegia is usually caused from a trauma to the mid/lower back. An accident such as a car crash or a gunshot wound could cause the dendrites to be torn apart and literally cut off the connection between nerves. It can be thought of as a simple circuit, if one of the wires is cut then the circuit breaks and it no longer functions. This is essentially what happens for trauma induced paraplegia. The problem with nerve cells is that they cannot divide by mitosis like other cells thus making it impossible for them to heal after being damaged. As well as the loss of function of the legs and trunk of the body, there are also many secondary illnesses caused by paraplegia. For example, Osteoporosis, where the bones become brittle and likely to break can occur as a sufferer of paraplegia is unlikely to get a lot of exercise and muscle/bone activity. Paraplegia can also lead to fatal illnesses such as chronic heart disease because, as mentioned before, paraplegia sufferers are unlikely to partake in much physical activity thus increasing their chances of heart disease. As well as physical problems caused by paraplegia, there are also mental ones. The realisation of being paralyzed can take a toll on a patient mentally and cause panic and lead to depression.

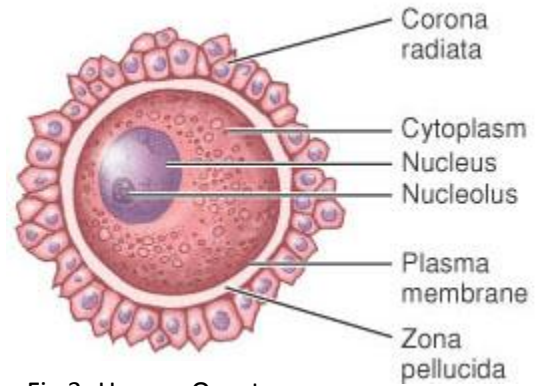


Fig 3- Human Oocyte

Unfortunately Due to the intricacy and complexness of the CNS, it is virtually impossible for doctors to try and operate on the area. There is still no definitive cure for paraplegia; however research into stem cells is sure to change that.

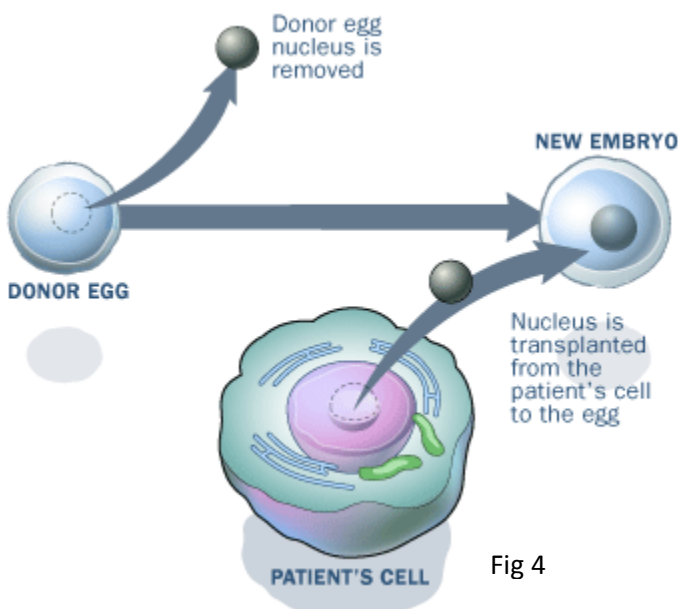


Fig 4

Our aim is to come up with a successful method of creating fully functional motor, sensory and relay neurons. To do this we must first find a way of creating neural stem cells from the embryonic stem cells taken from the blastocyst. This can be achieved using a technique known commonly as Therapeutic Cloning or 'Somatic Cell Nuclear Transfer'.

Therapeutic Cloning

In essence, therapeutic cloning involves removing the nucleus from a donated unfertilised

ovum and inserting a nucleus from a patient's own cell to create an embryo within which contains stem cells matching the DNA of the inserted nucleus and therefore the patient.

The process begins with donated unfertilised egg cells. The egg cell (known as an oocyte) now has its DNA removed. This process is done using a holding pipette and a micropipette. The holding pipette as its name suggests, is used to hold the oocyte in place whilst the micropipette extracts the nucleus via the use of vacuum. The micropipette punctures a hole in the zona of the oocyte. Once the nucleus has been removed from the cell, a nucleus from a somatic cell is inserted into the enucleated cell. The nucleus is removed from the somatic cell via the same process as the removal of the nucleus from the oocyte. In our nuclear transfer, we need to insert the nucleus of a neuron. This can be done by extracting the nucleus from the soma in any fully functional sensory, motor or relay neuron. Once the nucleus has been extracted we can insert it using the reverse of the previous removal technique. A hole is drilled in the membrane using the micropipette and the nucleus is forced along it into the cytoplasm of the cell. The chemical nocodazole is used to aid the process by keeping the oocyte in metaphase of mitosis to make the job of removing the nucleus easier as the nuclear membrane is dissolved at this stage. The nucleated cell is given a burst of electrical energy that is used as a substitute for fertilisation to stimulate cell division:

Development of the Oocyte

Embryogenesis is the name given to the growth and development of an embryo. There are 3 main stages known as blastula, gastrula and organogenesis. As we are aiming to isolate stem cells from the blastocyst, we need only focus on the first stage of embryogenesis which is blastula. The oocyte develops into a sphere containing around 130 cells

surrounding a cavity called the blastocoel. The blastocoel develops to grow an inner cell mass known as an embryoplast. If left to grow, the embryoplast would develop into a foetus. The embryoplast is the source of embryonic stem cells and is surrounded by a wall of cells known as the trophoblast which if left to develop would grow into the placenta. After 4-6 days, the

embryoplast will consist of between 50-150 cells all of which are pluripotent.

We must now develop a method to isolate the inner cell mass from the blastocyst. There are various tried methods for doing this including:

Natural Hatching of the Blastocyst:

In this procedure, an outgrowth of inner cell mass is removed from the blastocyst mechanically.

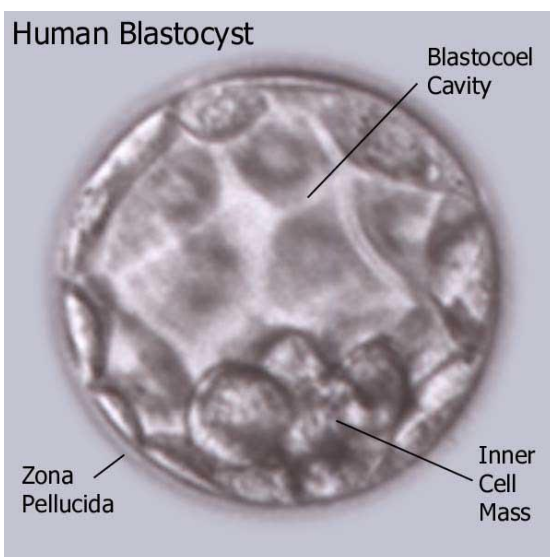
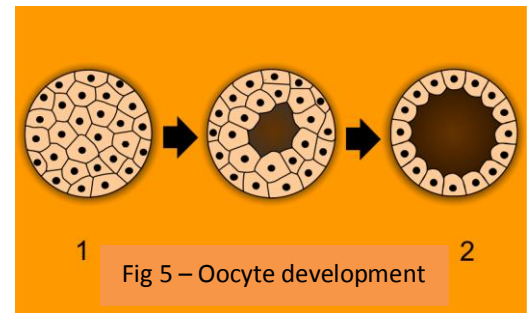


Fig 6

Microsurgery:

This method is similar to that of nuclear transfer. The blastocyst is held using a holding pipette and is rotated to a point where the embryoplast is at '9 O Clock'. A beveled shape biopsy needle is then used to penetrate into the blastocoels cavity. The disadvantage of this procedure is that great precision is required to remove the embryoplast without damaging the embryo.

Immunosurgery:

The 3rd method and the one we would most likely make usage of is immunosurgery. This method involves the removal of the surrounding wall known as the Zona Pellucida. This can be achieved by adding 0.5% Pronase to the blastocyst. The blastocyst is then incubated at body temperature for 30minutes with an oxygen concentration of 5%. The blastocyst is then placed in diluted rabbit anti mouse serum for 30minutes. If the blastocyst was only placed into this serum, all cells within the blastocyst would be killed. However the blastocyst is then added to a guinea pig complement which results in only trophoblastic cells being killed, leaving the inner cell mass which can then be separated from the remaining dead trophoblastic cells. Trypsin is added to the inner cell mass and then incubated to allow for the cells to disaggregate.

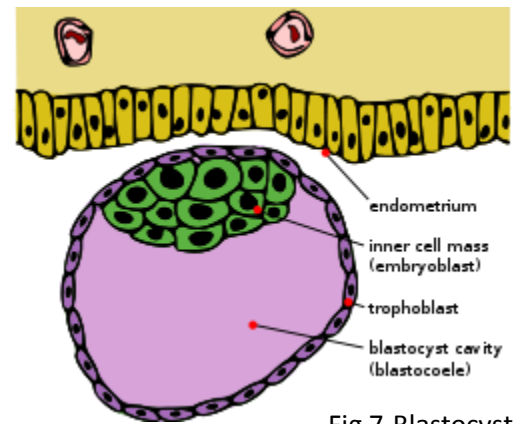


Fig 7-Blastocyst

Now that we have isolated the inner cell mass of neural stem cells, we are able to begin the differentiation of the stem cells into neurons that can be transplanted into the spinal cord. To do this the stem cells need to be surrounded by 'supporter cell's which will give them signals to trigger the development of the stem cells into neurons. This may be achieved using astrocytes.

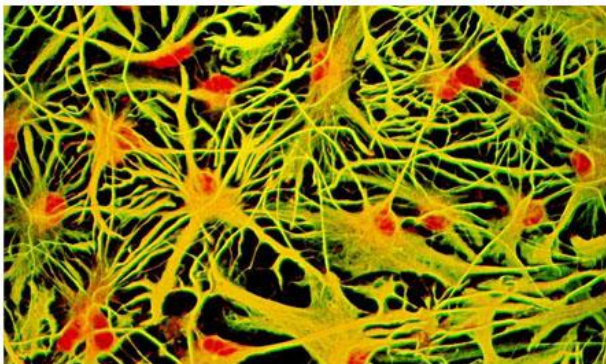


Fig 8-Astrocytes

Astrocytes are glial cells found in the spinal cord and brain and play a vital role in repairing the brain and spinal cord following injury. They are known to give out chemical signals that trigger neuron growth. Astrocytes are crucial in the structural support of neurons and maintenance for the environment of the neurons; they are also known to regulate the formation and activity of synapses connecting

neurons. It was shown in the work by Charles Stevens at the Salk institute in California that when they grew neuronal stem cells from adult rat brains together with astrocytes, the stem cells produced more neurons. It is thought we could use the astrocytes to stimulate the production

of neurons within the lab under similar conditions to that of the brain and spinal cord. The neural stem cells would be placed in a petri dish containing the astrocytes at body temperature with a good supply of oxygen and nutrients needed by the cells to develop into the desired neurons.

Now that neurons can be successfully grown in the lab, the hope is to transplant them into the spinal cord of a patient. The main objective is to successfully transplant the grown neurons into the spine of a patient. Normally for a transplant procedure, immunosuppressive drugs would have to be taken by the patient to prevent rejection of the tissue by the body's immune system. However, the neuron cells have come from the patient's own cells, so the cell surface antigens are identical and there will be no rejection by his/her immune system. Neurons are transplanted via surgery.

The transplantation is very risky and complex and has only been attempted by a few doctors. Patients are first anesthetised and magnetic resonance imaging (mri) is used to locate the coordinates of insertion. The spine is a very vulnerable area so mri is used to make sure that no more further damage will be done to the patients central nervous system. Two needle tracks are then created each side of the spine and the surgeon carefully bores a hole in the spine. The neurons are then placed into the spinal cord. The hope is that the grown neuron cells will interact with the damaged ones and replace them. Neuron surgery has proved successful on tests done on rats. The test proved to be positive as it was found that the cells that were injected into the spinal cords of the rats were interacting with the damaged neurons. The neurons matured and repaired the motor pathways of some of the rats fully repairing their spinal cords although this wasn't the case for all the rats. The results have varied in humans though. In Chennai, four paraplegic patients all had stem cell therapy. After the procedure 2 of them were able to walk and the other two reported to have feeling in their legs. The operation they underwent was slightly different to the one mentioned earlier. A small amount of fluid was extracted from each of the four patients' bone marrow which would provide a source of adult stem cells. The stem cells were then processed with the appropriate growth factors at the Centre for Regenerative Medicine Laboratory. The patients then had to undergo another surgery, where the stem cells were re-injected into the spine at the point where the injury occurred. The stem cells differentiated to neurons and started to grow. This is certainly great news in favour of the use of stem cells, but stem cells aren't all good news.

The use of stem cell transplants gives side effects similar to that of chemotherapy. Platelet levels fall which means that blood doesn't clot effectively; this increases that chance of internal bleeding. Anaemia is another side effect which is caused by the drop in red blood cell count; this can leave patients lacking in energy, breathless and fatigued. Patients may have trouble eating and drinking, general tiredness and worst of all they may become infertile. As well as

physical side effects, there is a huge ethical debate behind the use of stem cells. Obviously embryonic stem cells are extracted from embryos which soon die as a result. The destruction of embryos for research has caused much controversy and concern, especially amongst the religious. The argument amongst Christians is that every life is sacred and that scientists have no right to grow and culture embryos as each one could be a potential life. Stem cell research can also be seen as sinful as the researchers are “murdering” potential lives. The embryo is totally unconscious during research and is said to be just cells undergoing mitosis, however some people believe that the cells should be respected as they are the beginnings of life. The other side of the story is that stem cells can potentially cure many diseases and improve the standard of life for so many people. Surely it’s better to remove cells from the unborn to help those already living? The argument of destroying potential life is arguable because a “potential life” is destroyed every month in a woman if her egg isn’t fertilised. Potential life isn’t life and the two shouldn’t be confused; the research is conducted before there is any consciousness unlike other procedures such as abortions. We mustn’t forget that stem cell research is in their infancy and we’re sure that there will be more effective ways of acquiring stem cells in the future.

Conclusion

In summary, we propose to use therapeutic cloning to create neural stem cells which can develop and grow in the lab into neurons that can then be successfully transplanted into the spinal cord of the patient. The main aims for our ideas are:

- To successfully create neural stem cells capable of developing into neurons
- Making sure these neurons are specific to the patient from which the nucleus was created in order to avoid rejection by the body
- To use the ideas discussed to branch out into other fields of medicine and uses of embryonic stem cells i.e. organ regeneration

There are many limitations to our ideas that need to be resolved. The therapeutic cloning technique requires immense precision in order to successfully transfer the nuclei of the neuron into the oocyte. Keeping the oocyte within metaphase of mitosis to remove the nucleus may prove difficult as the chemical nocodazole may be ineffective. The immunosurgery technique used to remove the inner cell mass of the blastocyst requires a lot of chemicals of the correct concentrations and specific oxygen concentrations; any alteration in the surroundings of the oocyte may result in damage to the stem cells that are extracted. The full potential of astrocytes to give out chemical signals to the neural stem cells is not known. Links have been

made between astrocyte usage and the rate at which neurons grow from stem cells but the individual chemical signals that allow this process to happen are still being investigated and so this may be a setback in our proposition. The transplant of the neurons into the spinal cord is very problematic due to the complexity and vulnerability of the Central Nervous System. Re-establishing connections between the created neurons and previous undamaged ones could be a problem when the neurons have been transplanted but has been proved possible in a lab as grown neurons have re-established connection with rat motor neurons.

The ethics involving the use of embryonic stem cells may also prove a large obstacle as the process will require donations of oocyte's which it could be argued are potential lives. However as the eggs are not fertilized, it may be argued that the eggs are put too much better use with the extraction of the inner cell mass of stem cells. It has also been suggested that the growing embryo may be left to grow by some scientists so as to see what would happen if left to develop; this may give rise to so called 'human cloning'.

In conclusion, stem cells have the potential to be extremely beneficial and useful within nearly every branch of medicine. Here we have outlined the possibility of utilizing the stem cells to help those with paraplegia as opposed to more life threatening urgent cases such as organ transplants. Therapeutic cloning is a very effective way of creating stem cells specific to the patient yet there are many further improvements that need to be made. With the USA recently lifting the ban on embryonic stem cell research, the next few years and decades are sure to show us just how much of an influence these remarkable cells will have within medicine and on the world.

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Methods of Nuclear Transfer

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Information on the uses of immunosurgery to extract the inner cell mass of stem cells

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Astrocytes instruct stem cells to differentiate into neurons

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