

# Finding a Method of Curing Cleft Lip and Palate Using Stem Cells

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**PASS WITH DISTINCTION**

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## Abstract

In the following paper we will discuss stem cells in depth, exploring their nature and their discovery. We will include the research that has been conducted thus far and its implications on modern medicine. We will then discuss the ethics surrounding stem cell research and go on to suggest the use of alternative sources for stem cells that are more morally viable. We will then go on to look at cleft palate and lip as a case study, outlining the problems with their current treatment and the discussing possibilities for two alternative therapies using stem cells. Finally we will conclude the viability of this application of stem cells and discuss the future of stem cell research.

## Introduction

Stem cell therapy is a rapidly expanding medical field which promises to eradicate many of the “incurable” diseases of today. The stem cell is considered by scientific researchers across the globe to be the most promising medical tool that this age has to offer. Its huge potential to single-handedly save the lives of millions of people is rooted in its undifferentiated state; a rare quality that gives it the potential to become, given the correct conditions, any cell, tissue or organ in the body of its organism. For this reason there is hope that it can excel where many other medical treatments have previously failed.

This exciting prospect became a possibility in November 1998, when James Thomson of the University of Wisconsin-Madison successfully conducted the “experiment that shook the world”, creating the first sustainable line of human embryonic stem cells (ESC’s). Thomson obtained the donated embryos from a local IVF clinic, which consisted of eight cells surrounded by a thin membrane. He placed the embryos in culture dishes with carefully prepared nutrients and grew them into blastocysts, which are hollow balls of approximately 100 cells, within which lie an inner cell mass of about 30 pluripotent embryonic stem cells. Using a hollow glass needle he extracted the inner cell mass under a microscope and placed them in a Petri dish. The next stage in his experiment was revolutionary to the field. He worked out the conditions in which to culture these cells in vitro (latin for in glass) and cause them to carry on dividing indefinitely by mitosis without differentiating, a quality that no other specialised cell has. This makes stem cells incredibly useful as it allows a huge number of genetically identical cells to be created from just one parent cell. This process is known today as clonal expansion. There were many hurdles to overcome in order to perfect this process. The growing environment must be sterile to prevent air-borne contaminants from altering the levels of nutrients and gases in the Petri dish. Other difficulties included perfecting the nutrient concentrations and maintaining them at a constant level to mimic the normal external environment of the cell and maintaining the correct temperature, carbon dioxide and oxygen. By optimising the conditions to cause embryonic stem cells to multiply without specialising, Thomson formed the base knowledge needed for all experiments on stem cells that were to follow. Researchers of today still try to replicate the conditions laid out by Thomson, but now commonly culture the stem cells with bacteria genetically engineered to maintain these optimum conditions.

The medical potential of stem cells is rooted in their DNA. This is a protein that comprises a sequence of bases. Three sequential bases are known as a gene, which codes for the synthesis of one particular amino acid. As amino acids polymerise to form long protein chains, many genes within a section of DNA together code for one particular protein to be synthesised by the cell. In-between genes there are also inactive parts of the DNA that do not code for a particular amino acid but are thought to be associated with turning the genes “on” or “off”. If the genes are turned off, they do not synthesise that particular amino acid. As more and more genes are “turned off” further into their development, the possible application of that cell within the body decreases further and further. This process is known as specialisation. Of course, there are varying degrees of specialisation. A cell that is completely unspecialised, for example an embryonic stem cell, is known as a pluripotent cell and has the potential to differentiate into almost any cell within that particular organism. An example of a multipotent cell is an adult (somatic) stem cell that has partly specialised and so has the potential to specialise into cells of multiple, but a limited number of cell lineages. Both types of stem cell are widely used in stem cell therapy. Following the first stem cells being discovered in bone marrow and used successfully in the treatment of leukaemia, many more somatic stem cells have been discovered such as stem cells of the brain, heart, skin and even cancerous tumours.

Since Thomson’s breakthrough many researchers have been enticed by the potential of stem cell therapy (clinical use of stem cells) resulting in a rapid growth in the field, leading us today to being on the brink of the successful use of stem cells in preventing and curing a huge array of ailments. One use of ESC’s in stem cell therapy is genetically engineering them in vitro into specific specialised cells for transplantation to replace damaged tissue and organs. If used successfully in humans this could mean the end of the global organ shortage for transplantation as theoretically as many organs as were needed could be created on demand. These organs would also be of an identical genetic match to the patient so this would eradicate the possibility of rejection, unlike when a foreign tissue is transplanted into the body. In December 2001 two independent research teams using different techniques engineered neural progenitor cells – the multipotent stem cells that go on to differentiate into brain and nerve cells, from ESC’s. This led scientists closer to curing a wide range of neurological and brain diseases such as cerebral palsy and Parkinson’s (a disease caused by the inability of neurons to produce enough dopamine). In July 2003, a type of human neural cell called oligodendrocytes, created from ESC’s, were injected into paralysed mice. These cells were found to have formed new myelin sheaths around nerve cells and had secreted growth factors that stimulated the birth of new neurons. A technique has been perfected to cause ESC’s to specialise into dopamine and serotonin-producing stem cells for use in the future treatment of Parkinson’s. These stem cells begin their lives in a Petri dish as ESC’s grown in a medium of insulin, transferrin, fibronectin and selenium. This induces the formation of nestin-positive stem cells, which are selected as it is these that will go on to differentiate into the neurons. This process is known as clonal selection. The second clonal expansion phase involves the addition of laminin (a protein) and a basic fibrous growth factor, bFGF, which causes the cells to undergo mitosis whilst remaining in an undifferentiated state. This allows them to divide into nestin-positive neuronal precursor

cells before they differentiate. The third phase involves the removal of the bFGF to prevent further mitosis, allowing the cells to differentiate into dopamine-producing neurons. These neurons could then be injected into a person with Parkinson's. However, when a similar technique was used to grow dopamine-producing neurons from monkey ESC's to treat a primate version of Parkinson's the new cells integrated well with the brain tissue and reduced the symptoms of the disease in the short term, but in the long term the cells did not survive. This example demonstrates the practical problems that need to be overcome in stem cell therapy despite an already vast and fast-growing bank of knowledge in the field.

Another possible use of ESC's is to simply inject them into the site of disease, where they will differentiate due to their surrounding conditions, however this technique has less credibility. Nevertheless, in January 2002 embryonic stem cells were injected directly into mice with Parkinson's disease. Scientists were amazed to find that the completely unspecialised cells "homed in" on the sites of brain damage and specialised into functioning, dopamine-producing neurons. This treatment would never have been allowed in humans as scientists cannot be certain that these completely unspecialised cells would affect their targeted site rather than elsewhere in the body. However, it seems from this experiment that they could detect their needed site and the chemicals surrounding them within the body caused them to specialise into the correct cells. This has huge implications on the use of pluripotent stem cells in medicine, but much more testing needs to be done before this can become a standard procedure in humans.

The questions that grab the public's attention are those concerning the prevention and cure of disease, but the possibilities for the use of stem cells also extend into research in other areas of medicine. For example, it has been recently discovered that cancerous tumours have stem cells that fuel their consistent return, and further knowledge into the workings of these stem cells may help scientists develop cures for cancers.

## Ethics

There are a number of ethical issues surrounding embryonic stem cells that have slowed their use in research. In Thomson's experiment the stem cell that was used came from an embryo that was destroyed after he removed the inner cell mass from the blastocyst. Certain religious or ethical groups feel that by destroying an embryo you are destroying a potential life and therefore they compare stem cell research to murder. George W. Bush holds this opinion and has hindered stem cell research in the world's leading research nation. Since the first embryonic stem cells were isolated in 1998 America has received only a minimal amount of Government funding and strict laws have been passed against stem cell research. In 1987 foetal tissue research was banned, bowing to the pressure due to concerns that legalising the research could lead to more elective abortions amongst women. However, with the presidential election of Barack Obama all bans on stem cell research have been lifted.

There are still huge ethical concerns surrounding the use of stem cells in medicine. The question is, at what point is the foetus considered to have a soul? Some groups feel that

life begins at conception and is sacrosanct. Others object, as embryos used in these experiments are the excess embryos that would not otherwise be transplanted into the mother during IVF. Although there is an option of donating embryos to women that cannot produce their own ova, still 60% of embryos created during IVF never fulfil their potential for life.

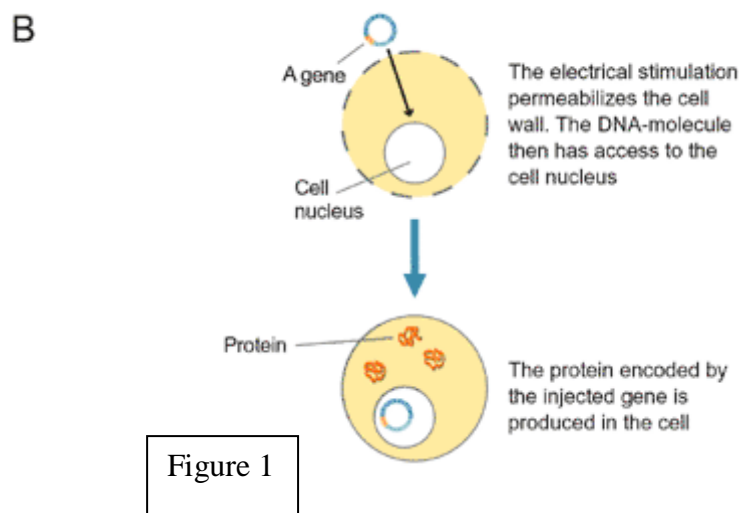
### Alternative sources of Stem Cells

In response to the fuelled debate over the ethics of embryonic stem cells, researchers have sought after alternative sources of undifferentiated cells.

In the human body many somatic stem cells are being discovered that have similar properties to ESC's. For example, scientists have found extremely potent somatic stem cells in the nose. If they responded in the same way as ESC's they would be a good non-controversial source of stem cells to repair any failing body part. A study published in science magazine also described the developmental potential of somatic stem cells when placed in different environments. They proved that neural stem cells from the brain of an adult mouse could form chimeric chick and mouse embryos, giving rise to cells of all germ layers. The Minnesota group also derived a rare multipotent stem cell found in the brains, bone marrow and muscles of humans, dogs, rats and mice. They are named multipotent adult progenitor cells (MAPC's). In the lab, MAPS's can undergo mitosis without differentiation indefinitely, unlike many other somatic stem cells. They also express telomerase, an enzyme associated with immortality. Unlike many other multipotent stem cells, they appear to be able to differentiate into any cells of the three germ layers, displaying properties of pluripotent stem cells. When placed in a mouse embryo with ESC's they will integrate with the embryonic cells, eventually contributing to all the parts of a baby mouse. The fundamental problem with the use of these stem cells is that they are rare and difficult to grow. The criticism of the research group that published these results is that nobody has yet managed to reproduce the results. This brings to light another issue associated with stem cell research. Because of the rate at which the research is moving forward many groups are feeling pressure to keep up, and there have been many cases where results have been proven to be unreliable.

Although in somatic stem cells some of the genes have "switched off" there is evidence to suggest that this process is reversible, and in certain cases scientists have reverted somatic stem cells back into stem cells that are as pluripotent as ESC's, called induced pluripotent stem cells (iPSC's). Dopamine producing neurons have also been produced from iPSC's and have been successfully implanted into a rat with Parkinson's reducing its symptoms. Shinya Yamanaka, a stem cell scientist at Kyoto University, thought of creating these iPSC's whilst studying the process of cloning. He knew that during cloning the reprogramming of the adult nucleus (switching the genes back "on") must occur due to "factors" present in the egg cell. He thought that by trying to recreate these factors in vitro he would be able to reprogram stem cells without cloning. The breakthrough came when Yamanka successfully reprogrammed mouse skin cells into pluripotent stem cells by transfecting extra DNA into the cells and creating iPSC's. He originally did this using viral

vectors, such as retroviruses, to transport the four extra genes needed to unspecialise the somatic cell. These genes include Oct-3/4 and Sox2. The genes were inserted into the DNA of the retroviruses, which were multiplied and cultured in a Petri dish with the skin cells. They attacked the cells in the same way as normal viruses, merging the new genes with the DNA of the skin cells, which reprogrammed the skin cells back into pluripotent cells. The problem with this method was that they had the potential to activate oncogenes, causing cancer in the cell. Therefore, scientists could never use this technique in vivo. A new technique has recently been developed to produce iPSC's without the use of viruses, with a technique called electroporation. This technique involves the use of electricity to cause the pores of the cell membrane to open temporarily so that the genes can be inserted directly into the cell (see figure 1).



Although iPSC's seem to hold much potential, there is a problem. As the parent cells are somatic, they have undergone mitosis many times before extraction. During mitosis copies of DNA are made by the cell and there are often mutations formed in the new strand of DNA. The mutations may prove harmful when transplanted to other areas of the body.

We will use the experiments previously discussed as evidence to help us to explore the possible application of stem cells to treat cleft palate and lip. We will devise new techniques for their treatment that have not yet been fully explored by scientists, whilst building on concrete science.

## Discussion

### Cleft Palate and Lip

One in 700 babies is born with a condition called cleft palate and in the majority of cases cleft lip is also present. The combined affect of these two conditions can cause: serious facial disfigurement; speech problems; problems in the development of the jaw and teeth; problems of proper air flow in breathing and there is even the possibility of hearing problems.

This condition is caused when plates of skull that form the hard and soft palate do not completely join, leaving a gap that runs down the roof of the mouth. This can occur as a complete cleft palate, which results in an open connection between the oral and nasal cavity, often resulting in cleft lip. An incomplete cleft can form instead, which results in a hole in the soft palate. Cleft lip is an open connection above the upper lip that can either be unilateral (distress of one side of the lip) or bilateral (distress of both sides of the lip). The most commonly used procedure to repair a cleft lip is the Millard procedure, first carried out in Korea by Ralph Millard. This is a simple surgical technique that involves incising and then sewing the flaps of skin together in such a way that the scarring will occur along the edges of the philtrum and as far up the nose as possible (see figure 2).

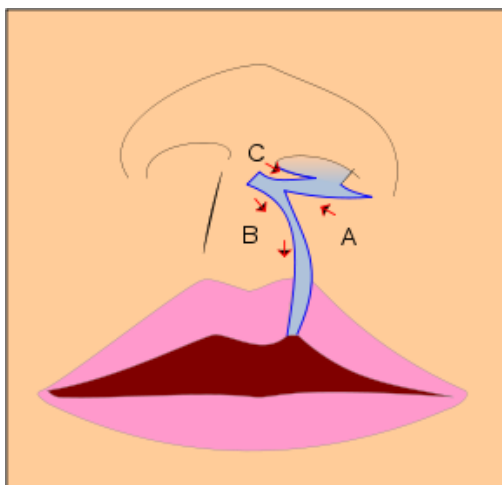


Figure 2 (<http://upload.wikimedia.org/wikipedia/en/e/e1/Millardrepair2.svg>)

Cleft palate is normally treated by surgery. The key thing in cleft palate surgery is the reconstruction of the soft palate and hard palate layers. The fistula (hole) in the roof of the mouth and the cleft in the jaw are often treated by alveolar bone grafting. This involves transplanting bone from the patient's hip or rib. The surgeon will then bring excess tissue and muscle together to close the cleft, often using transplanted tissue from the inner cheek or other areas of the body. The soft palate does not contain bone, so in correcting the soft palate a surgeon will also use transplanted tissue and connect it to the palate, creating a functional muscle sling. This process is called an intravelar veloplasty.

The above treatments are good ways of tackling cleft lip and palate but they all come with drawbacks, and it is these drawbacks that bring us to the proposal of using stem cells in the treatment of cleft palate and lip.

### Proposed Cleft Lip Treatment

Although the surgery used to treat cleft lip is simple and cost-effective, there is often a residue of scar tissue formed on the face. This tissue can be visible if the colour does not

blend in with the skin surrounding it. Scar tissue has many other drawbacks. It is less elastic than other tissues of the face so if extensive, can restrict natural movement. Scar tissue also has less protection from carcinogenic UV radiation. This is a particular problem as the scar will be situated on the face, where there is increased exposure to sunlight. It is also a problem in tropical third world countries where UV radiation is high. To eradicate this danger we propose the use of somatic stem cells or ESC's to inject into the healed site which will drastically reduce scar tissue as mentioned in the introduction. Somatic stem cells of the skin are found in the basal layer of the epidermis and a small skin biopsy would be enough to extract and clonally expand these multipotent stem cells. When the stem cells were injected into the site of the scar tissue they could promote the growth of normal skin tissue and reduce the scar tissue. We also propose the possible use of iESC's or ESC's in the treatment, as initial results are encouraging. However, further tests will be needed as side effects have been recognized when injecting these pluripotent stem cells into the body. In certain cases, the ESC's have displayed uncontrolled tumour-like growth and there is always a danger that the stem cells will not differentiate into the correct tissue.

### Proposed Cleft Palate Treatment

An alveolar bone graft for the fistula or cleft jaw requires a bone biopsy. This involves removing a bone fragment from the hip or rib and then transplanting it into the fistula and cleft jaw. The bone biopsy is painful and can cause problems after the transplant if the bone is not taken with great care. Stem cells are already being used as an alternative to bone grafting in the healing of severe fractures. In treating severe fractures orthopaedic surgeons' extract bone marrow from the patient, and then process the bone marrow stem cells before transplanting them into the fracture. Some 40,000 people have already undergone the procedure but the bone marrow has to be extracted, which has a risk and can also be painful depending on where you take it from. Therefore, scientists have looked at alveolar bone marrow stem cells, which can be extracted from the jaw bone with minimal pain. It is these alveolar stem cells that would be used to treat the fistula and cleft jaw. The alveolar bone marrow stem cells extracted from the jaw would be grown in vitro. They would be processed in the three stages previously mentioned. The alveolar bone marrow stem cells would then need to be injected into the fistula or cleft jaw. The transplanted cells have to try and survive in a very harsh environment once implanted and many of them could die or become dislodged. Therefore a modified comb scaffold would be used to try to increase the growth and survival of the stem cells transplanted. The comb would consist of a Plexiglas backbone with molecular tethers that would hold epidermal growth factors at their tips. The epidermal growth factor is a protein that plays a huge role in the differentiation and growth of many cells. Trials have already been done, showing that stem cells grown on these modified comb scaffolds were more likely to survive and there is even evidence to show that the scaffold boosts cell division and growth. Therefore, it is hoped that with the help of a modified comb scaffold the alveolar bone marrow stem cells injected in the fistula and cleft jaw would divide and grow thereby filling in the hole or gap. ESC's or iPSC's could be converted to skin cells

using methods described in the introduction, or somatic stem cells of the muscle or skin could be used to produce skin of muscle cells to cover the bone using a similar technique.

### Conclusion

There is no doubt that the experiments conducted thus far imply that stem cell research will hold enormous benefits for all of mankind. Our research paper has led us to ponder two questions. Depending on the answers, we believe that stem cell research will either excel in the future and reach its anticipated potential or will end up suffering a huge anti-climax. Firstly, should we as a community place a higher priority in protecting the possible lives of the unborn over those that are already seriously ill? If this is not considered viable, stem cell research will have to be abolished, despite its potential. Secondly, will cells ignore the conventional wisdom of developmental biology and unnaturally transform from their finally specialised state to other cells with long-term success? Natural cell differentiation is overwhelmingly one-directional and therefore perhaps stem cell therapy is man's naïve and arrogant belief that biological fate can be overruled.

A problem with the research that we have explored is that it has mostly been undertaken on much less complex organisms than ourselves. When the same biology has been tested on more complex organisms it has often failed, for example the long-term problem of using genetically engineered neurons to treat monkeys with Parkinson's which was unforeseen in a similar study on mice. This proves the huge leaps that stem cell research still has left to make. The use of iPSC's in stem cell therapy may involve problems due to the age of the cells used, but this can only be verified or nullified with further experimentation. Although our proposed technique for the treatment of cleft palate is based on successful scientific experiments involving other areas of the body, we cannot be sure that all tissues will react in the same way to similar treatment. Only time will tell if the hype of stem cell research is justified.

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