Embryonic stem cells possible
Future application restoring
Damaged and severed
Optic nerves

By

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Abstract
In the first part of this paper we shall describe the principles of stem cells research, the progress that has already been made and its application in medicine. We will then propose how it will be possible to repair the optic nerve by injecting immature or partially differentiated stem cells into it. This would restore vision to those who are suffering from Optic Nerve Hypoplasia and Optic Nerve Atrophy, diseases which currently have no cure. This procedure would not only benefit those with ONH and ONA, but allow future research into the treatment and repair of those suffering from glaucoma and those with completely severed optic nerves, caused by blunt force trauma.

Introduction
Stem cell research is a fast advancing field in the medical world. In 1998, scientists discovered how to harvest stem cells from embryos and grow them in laboratory conditions. Stem cells are a unique type of cell and have three general properties: they can divide and renew themselves for long periods of time, they are unspecialised and they can develop into specialised cell types. Stem cell research seeks to investigate the potential to develop stem cells into various cell types, which can be used to repair parts of the body and can theoretically divide continually, either into specialised cells or to remain as stem cells when they divide.

There are two types of stem cells: Embryonic and Adult. Embryonic stem cells are considered more useful as they are completely undifferentiated. They are found in the inner cell mass of a four or five day old blastocyst. Embryonic stem cells can be differentiated into any type of cell through the process of pluripotency. Stem cells have also been found in the blood of the umbilical cord and the placenta. However they are not as useful as embryonic stem cells as their capacity to differentiate is limited.

Adult or somatic stem cells, are undifferentiated cells found amongst differentiated cells in a tissue or organ. There are fewer adult stem cells; these are not as functional as embryonic stem cells as their primary role is to maintain and repair the tissue in which they are found, therefore differentiation is limited. Adult stem cells are already specialised which limits potential to regenerate damaged tissue. Recent research has discovered that adult stem cells are in more tissues than originally thought. They are found in the brain, bone marrow, the skin, the liver, blood vessels, skeletal muscle and peripheral blood. Unfortunately adult stem cells are not in the vital organs and due to specialisation they cannot become cells, which can repair the organ once damaged.

Embryonic stem cells are of great interest and importance in the medical field. In theory it would be possible to grow cells of medical importance by directing their development in a culture, such as bone marrow, muscle or neural tissue. Treatments may be developed for cell based diseases and to grow tissues needed for transplantation. (4) Potentially many diseases could be treated such as Parkinson’s, diabetes, Alzheimer’s, spinal cord injuries, stroke, burns, leukaemia, other cancers, heart disease and rheumatoid arthritis.

They are grown in a laboratory as a cell culture to form an embryonic stem cell line. The inner cell mass is transferred to a culture dish and is kept isolated. Over 6 months the cells are divided and subcultured until the original 30 cells have replicated into millions of undifferentiated stem cells. Embryonic stem cells can proliferate for more than a year in the laboratory without differentiating but adult stem cells cannot.
There have been some medical breakthroughs in recent years and slowly stem cells are being used to treat various diseases. For example, Koizumi et al investigated “the outcome of cultivated corneal epithelial transplantation for severe stem cell deficiencies using denuded amniotic membrane (AM) as a carrier.” In this study 13 eyes with various diseases and conditions were studied. All had “total stem cell deficiencies”. “Corneal limbal epithelium was cultivated for 4 weeks and showed four to five layers of well differentiated stratification. The cultivated epithelium, including the AM carrier, was transplanted onto the corneal surface up to the limbus, 48 hours after surgery there were no epithelial defects in any eyes, indicating complete survival of the transplanted epithelium. Visual acuity improved in all eyes and vision was restored in 10. However three suffered from epithelial rejection. This study shows how stem cells can be used to restore vision successfully. We want to study stem cell treatment of visual problems further.

**Discussion**

Although a decade ago it was believed that human neurones could not be rebuilt, this doesn’t seem to be true. Research concentrated on therapeutic benefits of limiting further damage. Now research has restarted again as it seems possible that neurones in the brain, under certain conditions do regenerate. They have been found to do this from neural foetal stem cells. This is promising for treatment of many diseases that affect the nervous system, including Amyotrophic Lateral Sclerosis (ALS). The application of the regeneration of neural stem cells could, theoretically, be used to repair partially degenerated or even severed optic nerves, providing relief from conditions such as optic nerve atrophy (ONA) and optic nerve hypoplasia (ONH).

(2) Research into the benefits of neural stem cells to rats with ALS (3) investigated the nerve restoring properties of stem cells, if injected into the spinal fluid. The rats suffered paralysis and weakness in hindquarters and back limbs. Stem cells that showed signs of developing into neural cells were injected into the fluid around the spinal cord in the hope that they would specialize into neurons.

The rats showed significant improvement and had increased mobility in their hind legs, whereas the control group remained the same. Autopsy revealed that the injected cells had begun to migrate throughout the spinal fluid and continued to develop, showing characteristics of neural stem cells.

Researchers advised caution with the results as the effect of the stem cells is not certain and it is too early to tell whether this kind of treatment would translate onto a human scale.
ONA is a permanent visual impairment caused by damage to the optic nerve. The atrophy of the axons in the optic nerve can be partial or profound depending on the number damaged. ONA can occur on one or both eyes and depending on the cause may also get progressively worse over time. There are a number of possible causes of ONA including tumours of the visual pathways, inadequate blood or oxygen supply at birth, rare degenerative and genetic diseases.

ONH is the underdevelopment of the optic nerve due to dying back of the nerve during development of the foetus. ONH usually occurs bilaterally but it has been known to be unilateral. It is the third commonest visual impairment in children.

There are currently no treatments for ONH or ONA as it is not possible at this stage to restore an optic nerve that has degenerated or not fully developed in the first place. Developments in research into the regeneration of nerve cells from embryonic stem cells may change this though:

We propose an injection of undifferentiated or partially differentiated nerve stem cells into the damaged area of the optic nerve, in a procedure similar to that of an optic nerve sheath fenestration, where the eyeball is repositioned so that the optic nerve is exposed and the stem cells are injected into the damaged part.

"In the optic nerve sheath fenestration procedure, the surgeon makes an incision in the optic nerve’s protective covering. He then temporarily removes one of the muscles that rotate the eye inward. The eyeball is rotated to the side, allowing the surgeon to see the optic nerve behind it. Slits are cut into the optic nerve sheath, allowing the excess fluid to escape and harmlessly absorb into surrounding tissues. A special hook removes any adhesions. The eyeball and the muscle are then put back in position."

Completely severed optic nerves can be caused by severe trauma, for example blunt force. Ellis- Behnke and colleagues from Hong Kong University and the institute for Neuroscience in Xi’an have used nanoparticle fibres to create ‘nerve bridging scaffolds’ made from peptide sequences that under the right conditions self assemble into mesh-like sheets. These particles are of similar size and composition to existing salts and sugars found on the surface of axons and they encourage cell growth and migration. In tests using hamsters that had their optic nerves severed and peptide mixtures injected, it was found that some vision had been recovered after six weeks. However caution should be exercised using these findings if applied to reconnecting optic nerves in humans. Injuries caused by blunt force trauma are not as ‘clean cut’ as those deliberately done by researchers and there is the obvious problem of generalising results over different species. Repairing the optic nerve requires axons to extend and bridge gaps and although the research above looks to overcome this problem, it is likely to be more effective if used in conjunction with introducing immature or partially differentiated stem cells into the affected area of the optic nerve.
There are many debates questioning the ethics of stem cell research. As it develops there will be obstacles facing it due to the requirement to use embryos and foetuses as stem cell sources. There are two main issues, the most important one concerning whether it's permissible to destroy human embryos in order to develop stem cell lines. The second is whether using them in human beings is safe. What is the long term effect of transplanting grown tissue? Unfortunately there is opposition as many people consider an embryo to be a potential life and a foetus is a growing life. Many Christians oppose embryonic stem cell research, as they believe in the sanctity of life. To them the use of a foetus is murder. This view can be summed up with the words of Pope John Paul II (10) "Experience is already showing how a tragic coarsening of consciences accompanies the assault on innocent human life in the womb, leading to accommodation and acquiescence in the face of other related evils such as euthanasia, infanticide and, most recently, proposals for the creation for research purposes of human embryos, destined to be destroyed in the process."

Using embryonic stem cells on human beings constitutes a violation of proper ethical considerations regarding experimentation on human subjects, as there is the possibility of the cells becoming cancerous or being rejected (even if they are exactly the same tissue type). There are dangers and complications to all research so stem cell research should not be undertaken on humans until it is shown to be safe. There have been many debates as to whether stem cell research should continue. In order to cool the debate some researchers have come up with imaginative ways to obtain embryonic stem cells without having to destroy an embryo. Four proposals have been floated in recent months. They are (9): The Parthenote Proposal, The Morula Proposal, The Organ Transplant Proposal and the The Alternate Nuclear Transfer (ANT) Proposal. Unfortunately, if these proposals do not work there will be further heated ethical debates.

**Conclusion**

In summary there are major improvements to the treatment ONA and ONH achieved in this proposal. Previously they have been incurable and people have suffered from partial or complete blindness without the hope of restoration of their vision. This research could lead to the eventual treatment of severed optic nerves and glaucoma. This paper has focused on the regeneration of nerve cells in the optic nerve. Therefore further developments to restore damaged or severed nerves could lead to the restoration of nerve cells in the spinal cord. However extensive research will need to be carried out in this field as the treatment of the spinal cord is more complicated than that of the optic nerve as it is a far more complex structure.

Unfortunately, stem cell research is in its infancy, so it is difficult to differentiate stem cells. Research still needs to be carried out into the chemical signals that determine which kind of cells will be produced and the triggers to turn cells 'on'. As with any from of transplant surgery there is the possibility of rejection, which would cause major complications as it would not be possible to remove the cells. There may be greater complications than expected and even the chance of the cells becoming cancerous.

**References**

(1) http://www.ucihealth.com/News/UCl%20Health/Vision-winning-battle.htm
(4) Wisconsin scientists grow two new stem cell lines in animal cell-free culture
Jan 1, 2006 Embryonic Stem cell research at UW-Madison
(5) Stem Cell cultivation Illustration, report by Embryonic Stem cell research at UW-Madison
(6) Noriko Koizumi, MD, PhD, Tsutomu Inatomi, MD, PhD, Tomo Suzuki, MD, PhD, Chie Sotozono, MD, PhD, Shigeru Kinoshita, MD, PhD
http://amalyste.free.fr/documents/1569Shimaz.LSCT.pdf
(7) Glaucoma research foundation, Glaucoma treatment
http://www.glaucoma.org/treating/surgery.html
(8) Paul Marks (2006) Optic nerve regrown with a nanofibre scaffold
(9) “Ethical” Embryonic Stem Cell Research, report by The Center for Bioethics Human Dignity
http://www.americancatholic.org/Newsletters/CU/ac0102.asp
(11) Image for ONA
(12) Image for ONH
(13) Image for ONA from glaucoma
(14) Pediatric Visual Diagnosis Fact Sheet TM, ONA
http://www.blindbabies.org/factsheet_ona.htm
• Borchert, M.S. An Inside Look At Optic Nerve Hypoplasia Research - A Leading Cause of Infant Blindness, USC School of Medicine.
(15): Pediatric Visual Diagnosis Fact Sheet,ONH
http://www.blindbabies.org/factsheet_ona.htm
• Hoyt, C., Good, W. (1 992). Do We Really Understand The Difference Between Optic Nerve Hypoplasia And Atrophy?, Eye, 6,201-204.
• Kjer, P. (1 959). Infantile Optic Atrophy With Dominant Mode Of Inheritance, ACTA OPHTHALMOLOGICA, Sept., 23.