EUROPEAN JOURNAL OF PHARMACOLOGY & TOXICOLOGY

EJPT

Journal homepage: www.mcmed.us/journal/ejpt

STEM CELLS - A REVIEW

Shankar M*[1], Roopa Kumar D[1], Ramesh B[1], Niranjan Babu M[2]

*[1]Department of Pharmaceutical Chemistry, Seven Hills College of Pharmacy, Venkatramapuram, Tirupati-517 561, Andhra Pradesh, India.
[2]Department of Pharmacognosy, Seven Hills College of Pharmacy, Venkatramapuram, Tirupati-517561, Andhra Pradesh, India.

Article Info

Received 25/11/2013
Revised 15/12/2013
Accepted 18/12/2013

Key words Embryoic stem cells, Transplant, Multicellular Organism.

ABSTRACT

There are three accessible sources of autologous adult stem cells in humans are, Bone marrow, which requires extraction by harvesting, that is drilling into bone, Adipose tissue, which requires extraction by liposuction and Blood, which requires extraction through apheresis, where in blood is drawn from the donor, passed through a machine that extracts the stem cells and returns other portions of the blood to the donor. Tissue-specific stem cells, which are sometimes referred to as adult or somatic stem cells, are already somewhat specialized and can produce some or all of the mature cell types found within the particular tissue or organ. Recent developments in human stem cell research have raised hopes that new therapies will become available that will serve to relieve human suffering. These developments also have served to remind society of the deep moral concerns that are related to research involving human embryos and cadaveric fetal tissue. Serious ethical discussion will continue on these issues. We were persuaded that carrying out human stem cell research under federal sponsorship is important, but only if it is conducted in an ethically responsible manner. And after extensive deliberation, the Commission believes that acceptable public policy can be forged, in part, on widely shared views.

INTRODUCTION

Stem cells have the remarkable potential to develop into many different cell types in the body. Serving as a sort of repair system for the body, they can theoretically divide without limit to replenish other cells as long as the person or animal is still alive. When a stem cell divides, each new cell has the potential to either remain a stem cell or become another type of cell with a more specialized function, such as a muscle cell, a red blood cell, or a brain cell. This document covers basic information about stem cells [1]. Stem cells are undifferentiated biological cells, that can differentiate into specialized cells and can divide to multicellular organisms. In mammals, there are two broad types of stem cells: embryonic stem cells, which are isolated from the inner cell mass of blastocysts, and adult stem cells, which are found in various tissues. In adult organisms, stem cells and progenitor cells act as a repair system for the body, replenishing adult tissues. In a developing embryo, stem cells can differentiate into all the specialized cells ectoderm, endoderm and mesoderm. But also maintain the normal turnover of regenerative organs, such as blood, skin, or intestinal tissues.

There are three accessible sources of autologous adult stem cells in humans are, Bone marrow, which requires extraction by harvesting, that is drilling into bone, Adipose tissue, which requires extraction by liposuction, and Blood, which requires extraction through apheresis, where in blood is drawn from the donor (similar to a blood donation), passed through a machine that extracts the stem cells and returns other portions of the blood to the donor.

Highly plastic adult stem cells are routinely used in medical therapies, for example in bone marrow

Corresponding Author

Shankar M
Email:- shankarmanichellappa@gmail.com

transplantation. Stem cells can now be artificially grown and transformed into specialized cell types with characteristics consistent with cells of various tissues such as muscles or nerves through cell culture. Embryonic cell lines and autologous embryonic stem cells generated through therapeutic cloning have also been proposed as promising candidates for future therapies [2].

Self-renewal

Two mechanisms exist to ensure that a stem cell population is maintained. Obligatory asymmetric replication: a stem cell divides into one mother cell that is identical to the original stem cell, and another daughter cell that is differentiated. When one stem cell develops into two differentiated daughter cells, another stem cell undergoes mitosis and produces two stem cells identical to the original was shown in figure 2.

Pluripotent, embryonic stem cells originate as inner cell mass cells within a blastocyst. These stem cells can become any tissue in the body, excluding a placenta. Only cells from an earlier stage of the embryo, known as the morula are totipotent, able to become all tissue in the body and the extra embryonic placenta [3].

Stem cells are the foundation cells for every organ and tissue in our bodies. The highly specialized cells that make up these tissues originally came from an initial pool of stem cells formed shortly after fertilization. Throughout our lives, we continue to rely on stem cells to replace injured tissues hair, blood and the lining of our gut. Stem cells have two key properties. The ability to self-renew, dividing in a way that makes copies of themselves and the ability to differentiate, giving rise to the mature types of cells that make up our organs and tissues.

Tissue-specific stem cells

Tissue-specific stem cells, which are sometimes referred to as adult or somatic stem cells, are already somewhat specialized and can produce some or all of the mature cell types found within the particular tissue or organ in which they reside. Because of their ability to generate multiple, organ-specific, cell types, they are described as multi potent. For example, stem cells found within the adult brain are capable of making neurons and two types of glial cells, astrocytes and oligodendrocytes. Tissue-specific stem cells have been found in several organs that need to continuously replenish themselves, such as the blood, skin and gut and have even been found in other, less regenerative, organs such as the brain. These types of stem cells represent a very small population and are often buried deep within a given tissue, making them difficult to identify, isolate and grow in a laboratory setting. Tissue-specific stem cells have been found in several organs that need to continuously replenish themselves, such as the blood, skin and gut and have even been found in other, less regenerative, organs such as the brain. These types of stem cells represent a very small population and are often buried deep within a given tissue, making them difficult to identify, isolate and grow in a laboratory setting.

Embryonic stem cells

Embryonic stem cells have been derived from a variety of species, including humans, and are described as pluripotent , meaning that they can generate all the different types of cells in the body. Embryonic stem cells can be obtained from the blastocyst, a very early stage of development that consists of a mostly hollow ball of approximately 150-200 cells and is barely visible to the naked eye. The fertilized egg and the cells that immediately arise in the first few divisions are totipotent. This means that, under the right conditions, they can generate a viable embryo (including support tissues such as the placenta). Within a matter of days, however, these cells transition to become pluripotent. None of the currently studied embryonic stem cell lines are alone capable of generating a viable embryo was shown in figure 3[4].

Identification of stem cells

Although there is not complete agreement among scientists of how to identify stem cells was shown in figure 4, most tests are based on making sure that stem cells are undifferentiated and capable of self-renewal. Tests are often conducted in the laboratory to check for these properties. One way to identify stem cells in a lab, and the standard procedure for testing bone marrow or hematopoietic stem cell, is by transplanting one cell to save an individual without HSCs.

To test whether human embryonic stem cells are pluripotent, scientists allow the cells to differentiate spontaneously in cell culture, manipulate the cells so they will differentiate to form specific cell types, or inject the cells into an immune suppressed mouse to test for the formation of a teratoma [5].

Hematopoietic stem cell transplantation remains a dangerous procedure with many possible complications. It is reserved for patients with life-threatening diseases. As the survival of the procedure increases, its use has expanded beyond cancer, such as autoimmune diseases.

STEM CELLS CULTURE

Stem cells are either extracted from adult tissue or from a dividing zygote in a culture dish. Once extracted, scientists place the cells in a controlled culture that prohibits them from further specializing or differentiating but usually allows them to divide and replicate. The process of growing large numbers of embryonic stem cells has been easier than growing large numbers of adult stem cells, but progress is being made for both cell types.

Stem cell therapy

Stem cell therapy is an intervention strategy that introduces new adult stem cells into damaged tissue in order to treat disease or injury. Many medical researchers believe that stem cell treatments have the

potential to change the face of human disease and alleviate suffering. The ability of stem cells to self-renew and give rise to subsequent generations with variable degrees of differentiation capacities, offers significant potential for generation of tissues that can potentially replace diseased and damaged areas in the body, with minimal risk of rejection and side effects [6]. Medical researchers anticipate that adult and embryonic stem cells will soon be able to treat cancer, type- 1 diabetes mellitus, Parkinson disease, Huntingtons disease, Celiac disease, Cardiac failure, muscle damage and neurological disorders, and many others. Nevertheless, before stem cell therapeutics can be applied in the clinical setting, more research is necessary to understand stem cell behavior upon transplantation as well as the mechanisms of stem cell interaction with the diseased injured microenvironment [7]. It is this side effect of conventional chemotherapy strategies that the stem cell transplant attempts to reverse a donor's healthy bone marrow reintroduces functional stem cells to replace the cells lost in the host's body during treatment [8].

POTENTIAL TREATMENTS

Brain damage

Stroke and traumatic brain injury lead to cell death, characterized by a loss of neurons and oligodendrocytes within the brain. Healthy adult brains contain neural stem cells which divide to maintain general stem cell numbers, or become progenitor cells. In healthy adult animals, progenitor cells migrate within the brain and function primarily to maintain neuron populations for olfaction. In pregnancy and after injury, this system appears to be regulated by growth factors and can increase the rate at which new brain matter is formed. Although the reparative process appears to initiate following trauma to the brain, substantial recovery is rarely observed in adults, suggesting a lack of robustness. Stem cells may also be used to treat brain degeneration, such as in Parkinson's and Alzheimer's disease [9].

Stem Cell Technologies

Stem cell technology gives hope of effective treatment for a variety of malignant and non malignant diseases through the rapid developing field that combines the efforts of cell biologists, geneticists and clinicians. Stem cells are defined as totipotent progenitor cells capable of self renewal and multi-lineage differentiation. Stem cells survive well and show steady division in culture which then causes them the ideal targets for vitro manipulation [10].

Cancer

The development of gene therapy strategies for treatment of intracranial tumors offer much promise, and has shown to be successful in the treatment of some dogs although research in this area is still at an early stage. Using conventional techniques, brain cancer is difficult to treat because it spreads so rapidly. Researchers at the Harvard Medical School transplanted human neural stem cells into the brain of rodents that received intracranial tumours. Within days, the cells migrated into the cancerous area and produced cytosine deaminase, an enzyme that converts a non-toxic pro-drug into a chemotheraputic agent. As a result, the injected substance was able to reduce the tumor mass by 81 percent. The stem cells neither differentiated nor turned tumorigenic [11].

Spinal-cord injury

That they had transplanted multi potent adult stem cells from umbilical cord blood to a patient suffering from a spinal-cord injury and that following the procedure, she could walk on her own without difficulty. The patient had not been able to stand up for roughly 19 years. For the unprecedented clinical test, the scientists isolated adult stem cells from umbilical cord blood and then injected them into the damaged part of the spinal cord [12].

Transformation of blastocyst stem cells into motor neurons had eluded researchers for decades. While Zhang's findings were a significant contribution to the field, the ability of transplanted neural cells to establish communication with neighboring cells remains unclear. Accordingly, studies using chicken embryos as a model organism can be an effective proof of concept experiment. If functional, the new cells could be used to treat diseases like Lou Gehrig's disease, muscular dystrophy, and spinal cord injurie [13].

Heart Damage

Among several clinical trials that have reported that adult stem cell therapy is safe and effective, powerful effects have been reported from only a few laboratories, but this has covered oldand recent infarcts as well as heart failure not arising from myocardial infarction. While initial animal studies demonstrated remarkable therapeutic effects, later clinical trials achieved only modest, though statistically significant, improvements Possible reasons for this discrepancy are patient age, timing of treatment and the recent occurrence of a myocardial infarction It appears that these obstacles may be overcome by additional treatments which increase the effectiveness of the treatment or by optimizing the methodology although these too can be controversial. Current studies vary greatly in cell procuring techniques, cell types, cell administration timing and procedures, and studied parameters, making it very difficult to make comparisons. Comparative studies are therefore currently needed [14].

Possible mechanisms of recovery include

- Generation of heart muscle cells
- Stimulation of growth of new blood vessels to repopulate damaged heart tissue
- Secretion of growth factors
- Assistance via some other mechanism

It may be possible to have adult bone marrow cells differentiate into heart muscle cells. The first

successful integration of human embryonic stem cell derived cardiomyocytes in guinea pigs. The contraction strength was measured four weeks after the guinea pigs underwent simulated heart attacks and cell treatment. The cells contracted synchronously with the existing cells, but it is unknown if the positive results were produced mainly from paracrine as opposed to direct electromechanical effects from the human cells. Future work will focus on how to get the cells to engraft more strongly around the scar tissue. Whether the treatments from embryonic or adult bone marrow stem cells will prove more effective remains to be seen.

Other reports required negative standard deviations in subsets of patients, or contained fractional patients, negative NYHA classes. Overall there were many more patients published as having receiving stem cells in trials, than the number of stem cells processed in the hospital's laboratory during that time [15].

Hematopoiesis (blood-cell formation)

The specificity of the human immune-cell repertoire is what allows the human body to defend itself from rapidly adapting antigens. However, the immune system is vulnerable to degradation upon the pathogenesis of disease, and because of the critical role that it plays in overall defense, its degradation is often fatal to the organism as a whole. Research using both hematopoietic adult stem cells and embryonic stem cells has provided insight into the possible mechanisms and methods of treatment for many of these ailments.

Fully mature human red blood cells may be generated *ex vivo* by hematopoietic stem cells (HSCs), which are precursors of red blood cells. In this process, HSCs are grown together with stromal cells, creating an environment that mimics the conditions of bone marrow, the natural site of red-blood-cell growth. Erythropoietin, a growth factor, is added, coaxing the stem cells to complete terminal differentiation into red blood cells. Further research into this technique should have potential benefits to gene therapy, blood transfusion, and topical medicine [16].

Baldness

Hair follicles also contain stem cells, and some researchers predict research on these follicle stem cells may lead to successes in treating baldness through an activation of the stem cells progenitor cells. This treatment is expected to work by activating already existing stem cells on the scalp. Later treatments may be able to simply signal follicle stem cells to give off chemical signals to nearby follicle cells which have shrunk during the aging process, which in turn respond to these signals by regenerating and once again making healthy hair.

Missing teeth

In theory, stem cells taken from the patient could be coaxed in the lab into turning into a tooth bud which, when implanted in the gums, will give rise to a new tooth, and would be expected to grow within two months. It will fuse with the jawbone and release chemicals that encourage nerves and blood vessels to connect with it. The process is similar to what happens when humans grow their original adult teeth. Many challenges remain, however, before stem cells could be a choice for the replacement of missing teeth in the future. Research is ongoing in different fields, alligators which are polyphyodonts grow up to 50 times a successional tooth (a small replacement tooth) under each mature functional tooth for replacement once a year [17].

Deafness

Heller has reported success in re-growing cochlea hair cells with the use of embryonic stem cells.

Blindness and vision impairment

The corneal stem cells into damaged eyes to restore vision. Sheets of retinal cells used by the team are harvested from aborted fetuses, which some people find objectionable. When these sheets are transplanted over the damaged cornea, the stem cells stimulate renewed repair, eventually restore vision. A woman who was blinded in one eye when acid was thrown in her eye at a nightclub. The cornea, which is the transparent window of the eye, is a particularly suitable site for transplants. In fact, the first successful human transplant was a cornea transplant. The absence of blood vessels within the cornea makes this area a relatively easy target for transplantation. The majority of corneal transplants carried out today are due to a degenerative disease called keratoconus [18].

Amyotrophic lateral sclerosis

Stem cells have resulted in significant locomotor improvements in rats with an Amyotrophic lateral sclerosis-like disease. In a rodent model that closely mimics the human form of ALS, animals were injected with a virus to kill the spinal cord motor nerves which mediate movement. Animals subsequently received stem cells in the spinal cord. Transplanted cells migrated to the sites of injury, contributed to regeneration of the ablated nerve cells, and restored locomotor function.

Graft vs. host disease and Crohn's disease

Phase III clinical trials expected to end in second-quarter 2008 were conducted by Osiris Therapeutics using their in-development product Prochymal, derived from adult bone marrow. The target disorders of this therapeutic are graft-versus-host disease and Crohn's disease. It was approved by Canada in May 2012 [19].

Neural and behavioral birth defects

This was done by direct neural stem cell transplantation into the brains of the offspring. The recovery was almost 100 percent, as shown both in behavioral tests and objective brain chemistry tests.

Behavioral tests and learning scores of the treated mice showed rapid improvement after treatment, providing results that rivaled non-treated mice. On the molecular level, brain chemistry of the treated animals was also restored to normal. Through the work, which was supported by the US National Institutes of Health, the US-Israel Binational Science Foundation and the Israel anti-drug authorities, the researchers discovered that the stem cells worked even in cases where most of the cells died out in the host brain. Researchers also plan to work on developing methods to take cells from the patient's own body, turn them into stem cells, and then transplant them back into the patient's blood via the blood stream. Aside from decreasing the chances of immunological rejection, the approach will also eliminate the controversial ethical issues involved in the use of stem cells from human embryos [20].

Diabetes

Diabetes patients lose the function of insulin-producing beta cells within the pancreas.

TRANSPLANTATION

Human embryonic stem cells may be grown in cell culture and stimulated to form insulin-producing cells that can be transplanted into the patient.

However, clinical success is highly dependent on the development of the following procedures: Transplanted cells should proliferate

- Transplanted cells should differentiate in a site-specific manner
- Transplanted cells should survive in the recipient (prevention of transplant rejection)
- Transplanted cells should integrate within the targeted tissue
- Transplanted cells should integrate into the host circuitry and restore function [21].

Stem cell educator therapy

Stem Cell Educator Therapy induces immune balance by using Cord Blood-Derived Multipotent Stem Cells (CB-SCs). A closed-loop system that circulates a patient's blood through a blood cell separator, briefly co-cultures the patient's lymphocytes with adherent CB-SCs *in vitro*, and returns the educated lymphocytes (but not the CB-SCs) to the patient's circulation. The clinical trial (NCT01350219) reveals that a single treatment with the Stem Cell Educator provides lasting reversal of autoimmunity that allows regeneration of islet beta cells and improvement of metabolic in subjects with long-standing type 1 diabetes [22].

Orthopaedics

Clinical case reports in the treatment orthopaedic conditions have been reported. To date, the focus in the literature for musculoskeletal care appears to be on mesenchymal stem cells. The MRI evidence of increased cartilage and meniscus volume in individual human subjects. The results of trials that include a large number of subjects are yet to be published. However, a published safety study conducted in a group of 227 patients over a 3-4 year period shows adequate safety and minimal complications associated with mesenchymal cell transplantation. Wakitani has also published a small case series of nine defects in five knees involving surgical transplantation of mesenchymal stem cells with coverage of the treated chondral defects [23].

Wound healing

Stem cells can also be used to stimulate the growth of human tissues. In an adult, wounded tissue is most often replaced by scar tissue, which is characterized in the skin by disorganized collagen structure, loss of hair follicles and irregular vascular structure. In the case of wounded fetal tissue, however, wounded tissue is replaced with normal tissue through the activity of stem cells. A possible method for tissue regeneration in adults is to place adult stem cell seeds inside a tissue bed soil in a wound bed and allow the stem cells to stimulate differentiation in the tissue bed cells. This method elicits a regenerative response more similar to fetal wound healing than adult scar tissue formation. Researchers are still investigating different aspects of the soil tissue that are conducive to regeneration [24].

Infertility

Culture of human embryonic stem cells in mitotically inactivated porcine ovarian fibroblasts (POF) causes differentiation into germ cells, as evidenced by gene expression analysis. Human embryonic stem cells have been stimulated to form Spermatozoon like cells, yet still slightly damaged or malformed. It could potentially treat azoospermia. In oogonial stem cells were isolated from adult mouse and human ovaries and demonstrated to be capable of forming mature oocytes. These cells have the potential to treat infertility.

Clinical trials

The US Food and Drug Administration gave clearance to Geron Corporation for the initiation of the first clinical trial of an embryonic stem cell-based therapy on humans. The trial aimed evaluates the drug GRNOPC1, embryonic stem cell-derived oligodendrocyte progenitor cells, on patients with acute spinal cord injury. The trial was discontinued in November 2011 so that the company could focus on therapies in the current environment of capital scarcity and uncertain economic conditions. The biotechnology and regenerative medicine company BioTime acquired Geron's stem cell assets in a stock transaction, with the aim of restarting the clinical trial. As of mid 2010 hundreds of phase III clinical trials involving stem cells have been registered [25].

Fig 1. Adult stem cell displaying typical ultrastructural characteristics

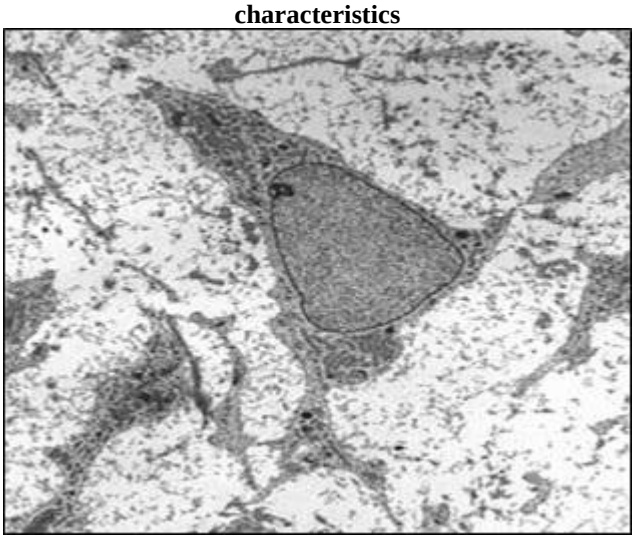


Fig 2. Different daughter cells

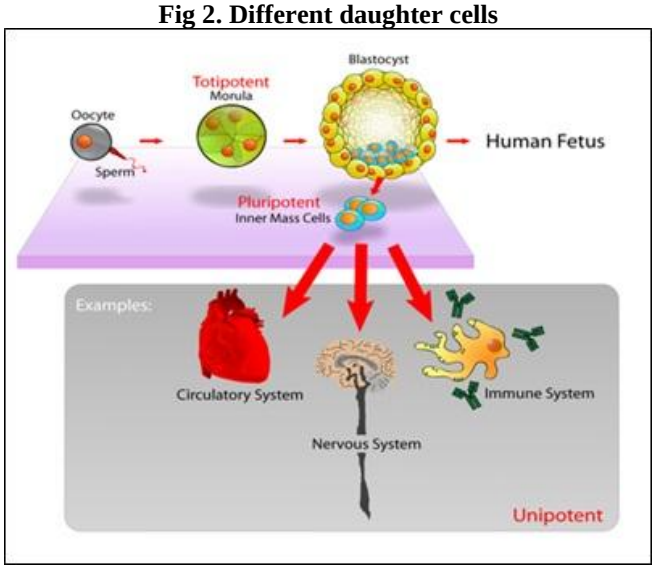


Fig 3. Fertilization of cell

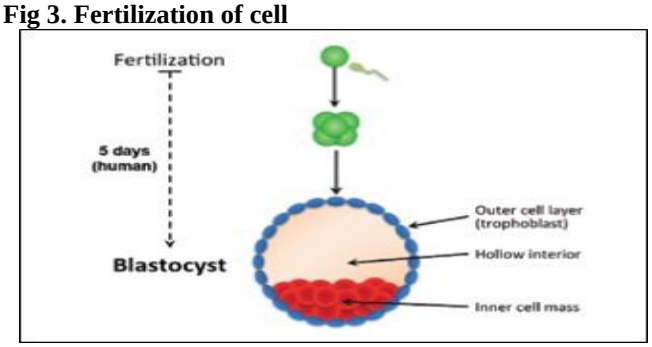


Fig 4. Division of stem cell

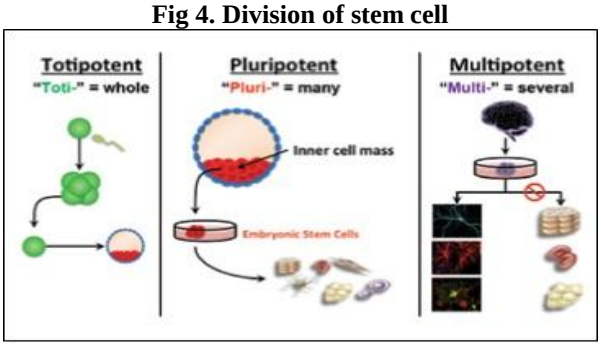
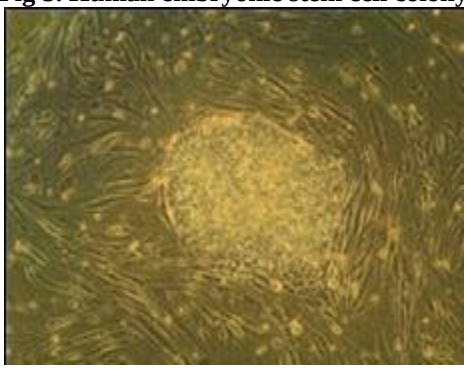


Fig 5. Human embryonic stem cell colony



CONCLUSION

Research provides increasing scope for the application of new knowledge. International agreements and national legislation should anticipate developments and respond quickly to the challenges of the time. Finland, like many other countries, has opposed reproductive cloning, but our legislation on therapeutic cloning is unclear. Sooner or later Finland will have to state its views and formulate rules concerning embryonic stem cell research. The present generation must safe-guard their own existence and make it is good as possible for their own sakes and then for the sakes of those who might follow. Of course, the potential people of the future are not morally irrelevant - many people want to procreate and most people want to see the human race continue to thrive and though they do not yet exist, future generations will exist, and the most immediate generations are the most likely to exist. Future generations will remember us and use what we have now to build on to progress. Without this optimistic continuity, there is little reason to be concerned about the future.

Future generations are a sort of "afterlife" for us present people, they are to live in a future we hope to be better. People are willing to work hard and even die for that vision of a better future, a future that they do not expect to be living in.

REFERENCES

1. The official National Institutes of Health resource for stem cell research.
2. Becker AJ, McCulloch EA, Till JE. (1963). Cytological demonstration of the clonal nature of spleen colonies derived from transplanted mouse marrow cells. *Nature*, 197(4866), 452-454.
3. Siminovitch L, McCulloch EA, Till JE. (1963). The distribution of colony forming cells among spleen colonies. *Journal of Cellular and Comparative Physiology*, 62 (3), 327-336.
4. Schöler, Hans R. (2007). The Potential of Stem Cells: An Inventory. In kolaus Knoepffler, Dagmar Schipanski, and Stefan Lorenz Sorgner. *Humanbiotechnology as Social Challenge*. Ashgate Publishing, 28.
5. Written by peter crosta for medical news. (2008).
6. Lindvall O, Kokaia Z. (2006). Stem cells for the treatment of neurological disorders. *Nature*, 441(7097), 1094-1096.
7. Singec I, Jandial R, Crain A, Nikkhah G, Snyder EY. (2007). The leading edge of stem cell therapeutics. *Annu. Rev. Med*, 58, 313-328.
8. Ghosh, Pallab. (2013). Stroke patients see signs of recovery in stem cell trial BBC News health.
9. Fontes, Paolo. (1999). Stem Cell Technology. PMC, 13.
10. Stem Cells Tapped to Replenish Organs the scientist. (2000). By Douglas Steinberg.
11. Kang KS, Kim SW, Oh YH, et al. (2005). A 37-year-old spinal cord-injured female patient, transplanted of multipotent stem cells from human UC blood, with improved sensory perception and mobility, both functionally and morphologically a case study. *Cytotherapy* 7 (4), 368-373.
12. Cummings BJ, Uchida N, Tamaki SJ, et al. (2005). Human neural stem cells differentiate and promote locomotor recovery in spinal cord-injured mice. *Proc. Natl. Acad. Sci. U.S.A*, 102, 39.
13. Strauer BE, Yousef M, Schannwell CM. (2010). The acute and long-term effects of intracoronary Stem cell Transplantation in 191 patients with chronic heart failure: the STAR-heart study. *European journal of heart failure*, 12 (7), 721–729.
14. Yousef M, Schannwell CM, Kostering M, Zeus T, Brehm M, Strauer BE. (2009). The BALANCE Study, clinical benefit and long-term outcome after intracoronary autologous bone marrow cell transplantation in patients with acute myocardial infarction". *Journal of the American College of Cardiology*, 53(24), 2262–2269.
15. Giarratana MC, Kobari L, Lapillonne H, et al. (2005). Ex vivo generation of fully mature human red blood cells from hematopoietic stem cells. *Nat. Biotechnol*, 23 (1), 69–74.
16. Yen AH, Sharpe PT (2008). Stem cells and tooth tissue engineering. *Cell Tissue Res*, 331 (1), 359–72.
17. Fetal tissue restores lost sight Medical News Today, 2004.
18. Querida Anderson (2008). Osiris Trumpets Its Adult Stem Cell Product. *Genetic Engineering & Biotechnology News*, 13.
19. Israeli scientists reverse brain birth defects using stem cells. (2008).
20. Cell Basics. (2009). What are the potential uses of human stem cells and the obstacles that must be overcome before these potential uses will be realized. In Stem Cell Information World Wide Web site. Bethesda, MD: National Institutes of Health, U.S. Department of Health and Human Services, 26.
21. Yong Zhao, Zhaoshun Jiang, Tingbao Zhao, Mingliang, et al. (2012). Reversal of type 1 diabetes via islet β cell regeneration following immune modulation by cord blood-derived multipotent stem cells". *BMC Medicine*, 10, 1-11.
22. Centeno CJ, Busse D, Kisiday J, Keohan C, Freeman M, Karli D. (2008). Regeneration of meniscus cartilage in a knee treated with percutaneously implanted autologous mesenchymal stem cells. *Med. Hypotheses*, 71 (6), 900-908.
23. Wakitani S, Nawata M, Tensho K, Okabe T, Machida H, Ohgushi H. (2007). Repair of articular cartilage defects in the pathello-femoral joint with autologous bone marrow mesenchymal cell transplantation: three case reports involving nine defects in five knees. *J Tissue Eng Regen Med*, 1 (1), 74-79.
24. White YAR, Woods DC, Takai Y, Ishihara O, Seki H, Tilly JL. (2012). Oocyte formation by mitotically active germ cells purified from ovaries of reproductive-age women. *Nature Medicine*, 18 (3), 413-421.
25. Chen J, Li Y, Wang L, *et al.* (2001). Therapeutic benefit of intravenous administration of bone marrow stromal cells after cerebral ischemia in rats. *Stroke*, 32 (4), 1005-1011.