Pluripotent Stem Cells and Their Future Potential

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Summary

Stem cells show great future promise for use in cell therapy and drug screening, since they have the ability to proliferate extensively while maintaining pluripotency. Embryonic stem cells are one type of stem cell which has been investigated for these purposes. The big promise of these cells, however, has been questioned by an ethical discussion associated with their embryonic origin, technical hurdles and the risk of teratoma formation. Research has therefore partly shifted focus towards induced stem cells, a cell type that can be formed by reprogramming somatic cells to a pluripotent state and has the same properties as embryonic stem cells. This way the ethical discussion associated with cells derived from embryos are avoided. There are three different approaches to generate induced stem cells; reprogramming by nuclear transfer, reprogramming by fusion with embryonic stem cells and reprogramming by defined factors. So far, only the two last have so far been successfully used on human cells.

Today there is no cell therapy used in clinic that is based on either embryonic stem cells or induced stem cells. However, research with focus on embryonic stem cells has advanced furthest, and several therapies are under development and currently waiting for approval from the US regulatory authority (Food and Drug Administration) to begin clinical trials. The hurdles that have to be overcome before embryonic stem cells and induced stem cells can be used in clinic application are partly the risk of teratoma formation and the low reprogramming efficiency. The low reprogramming efficiency to obtain induced stem cells is a problem associated with all potential applications, also drug screening.

Drug screening is the other major area where pluripotent stem cells potentially can be used in. In drug development for heart medicine for example, the candidate drug could be tested for toxicity and efficacy on heart cells generated from differentiated pluripotent cells before administration to humans. This is a relatively new research area and it is of great importance that scientists learn how to control cell differentiation for this to be a reality.
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1 Introduction

Since their discovery, stem cells have been holding a future clinical potential for several applications. There are two different types of stem cells. The early stem cell research had embryonic stem cells (ES) as the silver bullet, since they have the ability to proliferate extensively while maintaining pluripotency (Yu & Keller 2006). The big promise of these cells, however, has been questioned by an ethical discussion associated with their embryonic origin, technical hurdles and the risk of tumor formation (C. Annerén, personal communication, Guenin 2005). Therefore, research has therefore partly shifted focus towards adult stem cells and induced stem cells. Adult stem cells are the second type of stem cells and they are derived from mature tissue. They are characterized by a limited proliferative capacity, and in general can only differentiate into the cell type they are derived from (National Institute of Health 2008a). Induced stem cells, on the other hand, are similar to embryonic stem cells, due to the fact that they are somatic cells reprogrammed to a pluripotent state that have the same properties as embryonic stem cells. This way the ethical discussion associated with cells derived from embryos are avoided (Yamanaka 2008).

The properties associated with stem cells make them highly interesting for cell therapy and drug screening. Cell therapy is based on the concept of using cells as the therapeutic agent in order to rebuild or replace damaged or dysfunctional tissue (National Institute of Health 2008b). Drug screening is another area where stem cells possibly hold big future promise, partly because of their use in drug toxicity and efficacy tests but also for the opportunity to study human diseases. Stem cells open the possibility to test new drugs in vitro on human cells instead of only in animal models before administration to humans, thus avoiding toxic affects associated with species-specific differences and the use of experimental animals (Yu & Thompson 2006). So far, there is no use of human embryonic or induced stem cells either in clinic or in drug screening (Nischikawa et al. 2008). However, there are currently some cell therapies in clinic applications based on adult stem cells (Organogenesis Inc 2008, MEDINET 2008).

The purpose of this report was to investigate the formation, current research and future potential regarding embryonic and induced stem cells, using published articles.
2 Embryonic Stem Cells and Their Properties

Embryonic stem cells are unspecialized cells with the ability to self-renew indefinitely and differentiate into all three germ lines; ectoderm, endoderm and mesoderm (Figure 1). They are derived from the inner cell mass of the blastocyst from *in vitro* fertilization (Yu & Thompson 2006). Mouse ES cells were the first ES cell to be discovered in 1981 (Evens et al. 1981) and it was not until 1998 scientists were able to derive human ES cells (Thomson et al. 1998).

![Figure 1 Embryonic stem cells are pluripotent cells derived from the blastocyst that can differentiate to all cell types.](image)

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2.1 Formation of Embryonic Stem cells

Human embryonic stem cells (hES) are derived from the blastocyst of *in vitro* fertilized egg, a technique where an oocyte and sperm are united in the laboratory, instead of in the female body. The purpose of *in vitro* fertilized (IVF) embryos is to treat some forms of infertility. A process that sometimes results in IVF excessive embryos no longer needed and instead of discarding them they can be donated to research (Yu & Thompson 2006). The hES cells are then isolated from the donated blastocyst by transferring the inner cell mass into a cell culture containing cell medium and feeder cells. The cells start to divide and when the culture dish begins to be crowded the cells are re-plated to several dishes. After six or more months the cells are referred to a hES cell line if the cells appear genetically normal (Bethasda 2008).

Since hES cells are derived from human embryonic cells they have been, and still are, subject to an ethical debate. The debate is focused on whether human embryos can be destroyed for research purpose, even though the results stemming from them may be used for human therapies (Guenin 2005). Today there are many different opinions on the subject and the establishment of hES cell lines are strictly regulated in many countries (Luong et al. 2008).
3 Induced Stem Cells and Their Properties

Induced stem cells are somatic cells reprogrammed to a pluripotent state, which results in cells with the capacity to self-renew and differentiate into all cell types, just as embryonic stem cells. Induced stem cells and ES cells are thought to be identical and have similar properties, morphologies and gene and protein expressions (Wering et al. 2007). Currently there are three different methods to generate induced stem cells: reprogramming by nuclear transfer, reprogramming by fusion with ES cells and reprogramming with defined factors, each method with its pros and cons (Yamanaka 2008).

3.1 Reprogramming by Nuclear Transfer

Reprogramming by nuclear transfer, or somatic cell nuclear transfer as it also is called, is a process where the DNA from an oocyte is removed and replaced by DNA from a somatic cell (Figure 2). The egg containing the somatic cell DNA is stimulated and cell division follows. (Houghton Mifflin Company 2008)

Figure 2 Stem cell formation by nuclear transfer. This method is based on the replacement of the egg cell nucleus by the somatic cell’s nucleus, which results in clones.

This method was first successfully carried out in frogs by Briggs and King in 1952 (Briggs & King 1952). The frog’s relatively large egg made the process easier, which resulted in that the process was demonstrated in mammal cells several years later (Yamanaka 2008). However, in 1975, nuclear transfer of rabbit morula cell nuclei into nucleated rabbit eggs was reported (Bromhall 1975). A similar technique was later also used to produce the famous sheep “Dolly” (Wilmut et al. 2002). So far no scientist has successfully generated human ES clones (Yamanaka 2008).

The advantage with this technique is that it is possible to avoid immune rejection when used in patient-specific cell therapy, but it is subjected to strong regulations and an intensive ethical debate associated with human cloning (Yamanaka 2008).
3.2 Reprogramming by Fusion with Embryonic Stem Cell

Reprogramming by fusion with embryonic stem cells is a different approach to generate induced stem cells (Figure 3). This technique has been used to obtain human induced stem cells and was first demonstrated 2005 by Cowan and colleges. In this experiment human embryonic stem cells were fused with human fibroblasts, which resulted in hybrid cells maintaining a stable tetraploid DNA content and characteristic human ES cell morphology and antigen expression (Cowan et al. 2005).

The disadvantage with fused cells is their tetraploid DNA content, which includes both the somatic cell and the ES cell chromosomes. This may cause an immune rejection upon implantation and is not likely to be allowed in clinical applications (Yamanaka 2008).

![Figure 3 Reprogramming by fusion with embryonic stem cell](image)

3.3 Reprogramming by Defined Factors

Both the above described techniques depend on transcription factors responsible for the reprogramming within the oocyte or the ES cell (Do & Scholer 2004, Strelchenko et al. 2006, Wilmut et al. 2002). Several of these factors have been characterized and by genetic modification it is possible to up-regulate them in the somatic cell, which results in pluripotent reprogramming. The up-regulation is performed by introducing a retroviral vector containing the transcription factors, a straightforward process that can be carried out in most research laboratories. The disadvantage associated with this approach is that the reprogramming efficiency is very low and that the virus might interfere with the genes and lead to cancer (Holdan & Vogal 2008). The induced cells generated from this method are more specifically called inducible pluripotent stem cells (iPS cells), they will in this report, however, be called induced stem cells.

This technique has been used successfully on different human cells. Yamanaka and his colleagues were the first to demonstrate reprogrammed adult human fibroblast in 2007, using the transcription factors Oct ¾, Sox2, Klf4 and c-Myc (Takahashi et al. 2007).

Oct ¾ has been demonstrated to be crucial for maintaining pluripotency in cell culture (Yamanaka 2008). Sox2 disruption results in rapid differentiation (Yamanaka 2008). C-Myc is also important for maintenance of pluripotency and self-renewal. However, it has been found to
be an oncogene and researchers are therefore trying to replace it by other factors or small molecules (Yamanaka 2008, Wering et al. 2007, Huangfu et al. 2008a). Klf4 has been associated with both tumour suppression and oncogenesis. This may cause problems in using cells reprogrammed with this factor in clinical applications (Yamanaka 2008). Huangfu and colleagues are therefore aiming to reprogram cells without Klf4 (Huangfu 2008a).

The major hurdles that have to be overcome to be able to use this technique for clinical applications is the low reprogramming efficiency and the use of viral integration and foreign genes, among them known oncogenes like c-Myc and Klf4 (de Souza 2008, Huangfu et al. 2008a). Many scientists are now focusing on overcoming these hurdles. Recent papers describing experiments performed on mouse have shown that some of the transcription factors can be up-regulated by using small molecules. This also resulted in increased reprogramming efficiency (Shi et al. 2008, Wernig et al. 2008b, Huangfu et al. 2008b). It has also been shown that only the transcription factors Oct4 and Sox2 are essential for generating induced stem cells, the reprogramming efficiency is, however, very low in these cases (C. Annerén, personal communication, Huangfu et al. 2008a). Therefore, other factors such as c-Myc, Klf4, Nanog or Lin28 are used to increase the reprogramming rate (de Souza 2008, Huangfu et al. 2008a, Yu et al. 2007).

3.4 A Comparison of Embryonic Stem Cells and Inducible Pluripotent Stem Cells

Embryonic stem cells and induced stem cells are thought by many scientists to be biologically identical, both being able to differentiate into cells of all three germ layers (Wernig et al. 2007). This has been shown to be true for murine induced stem cells and ES cells but it is hard to demonstrate this for human cells, since it would require generating human embryos (Goldman 2008). The comparison is also limited by the fact that embryonic stem cells are used as a benchmark to characterize induced stem cells, and different ES cell lines have different expression patterns, which makes a comparison difficult (Goldman 2008). Several articles have been published where the two cell types are compared, and it has been demonstrated that the cells have similar morphologies, proliferation patterns and teratoma formations. Teratomas are germ cell tumors containing several different types of tissue (National Cancer Institute 2008). Human ES cells and induced stem cells are, however, different regarding gene expression and DNA methylation pattern (Okita et al. 2007, Wernig et al. 2007). The low reprogramming efficiency and insufficient data on a comparison of the two cell types has raised skepticism among some scientists, and not all scientists are convinced by the recent findings associated with induced stem cells. Some are arguing that the cells instead are rare stem cells co-existing in embryonic fibroblast culture (Liu et al. 2008). It has also been argued to be irrelevant whether the cell types are identical or not, the important focus should be on how they can be used (Goldman 2008).
4 Applications

The pluripotent and proliferative properties of ES- and induced stem cells offer opportunities in cell therapy and drug screening. The cells could be used as therapeutic agents and replace damaged or dysfunctional cells or tissue in cell therapy and offer treatment to traditionally hard-to-treat diseases, such as cardiac diseases and Type 1 Diabetes (Nischikawa et al. 2008). Potentially, they can also be used in drug development for toxicity and efficacy tests. In drug screening for heart medicine, for example, the candidate drug could be tested for toxicity and efficacy on heart cells generated from differentiated pluripotent cells before administration to humans (Yu & Keller 2006). This section summarizes what is possible today and what challenges need to be overcome.

4.1 Embryonic Stem Cells for Cell Therapy

There are currently no available clinical cell therapies using ES but there is considerable preclinical research within the area. The near term challenge for the use of hES cell in cell therapy is to be able to control the differentiation. The cells can not be used in an undifferentiated state, since this may lead to tumor growth. It is therefore necessary to first differentiate the cells into a specific cell type, before administration. This is also important to make sure that the cells differentiate into the cell of interest (Annerén/ personal communication). To date, scientists have been able to generate several cell types from hES cells including neural, cardiac, endothelial, hematopoietic, pancreatic, hepatic, bone, trophoblast, and multilineage cells (Yu & Keller 2006). The next step will be to demonstrate functional utility of these cells, both in vitro and in pre-clinical models for human disease (Murry et al. 2008). The use of hES cell in cell therapy is driven by scientists in universities and pharmaceutical companies.

Pharmaceutical companies have shown interest in this area because of the potential new means to treat various diseases, but also because these cells can be used allogeneically. Allogeneic cells are donated and may therefore be provided as off-the-shelf product, which makes the commercialization process appealing. However, the approach to use differentiated allogeneic cells is not free from hurdles. Mature cells express surface proteins that can be recognized as foreign by the recipient’s immune system and an immune system may react by rejecting the new tissue or cells (C. Annerén, personal communication).

Geron Corporation and Advanced Cell Technologies are two of the companies involved in the hES cell research with the goal to develop clinical applications. Geron Corporation is currently developing hES cell therapies for spinal cord injuries, congestive heart failure and damage caused by heart attack, type 1 diabetes, osteoarthritis, and osteoporosis (Geron Corporation 2008a-e). The therapy for spinal cord injuries has progressed furthest and is based on oligodendrocytes derived from hES (Geron Corporation 2008a). Research tests have shown that injected mouse ES cells differentiate to oligodendrocytes resulting in significant recovery of the animal’s ability to move and bear weight. These results led to focus on human cells, and neural progenitor cells derived from hES cells have been administrated to a rat model of spinal cord injury. The effect was improved locomotor functional behavior after administration. The company is now waiting for regulatory approval to proceed with the therapy into clinical trials (Geron Corporation 2008b).

Advanced cell Technologies are developing a Hemangioblast program, which involves hemangioblast precursor cells derived from hES cells used to achieve vascular repair (Advanced
The cells have been generated in large numbers by using an in vitro differentiation system and have shown capacity to expand, be cryopreserved and differentiate into multiple hematopoietic lineages as well as into endothelial cells. Functional utility has further been analyzed in animal models in mice with ischemia-reperfusion injury of the retina, in rats with diabetes and in mice after myocardial infarction. In all cases the cells were demonstrated to localize to the site of injury in the damaged vasculature and appeared to participate in repair. When used after myocardial infarction the cells also reduced the mortality rate and restored blood flow. Overall the data suggest that human embryonic stem cells could be important for vascular repair (Lu et al. 2007).

These are just a few examples of how hES cells can be used as therapeutic agents, which illustrate how far science has come as of today. Human ES cells can be differentiated into specific cell types, but these cells need to be tested in vitro and in animal models to prove functional utility and safety. So far, no cell therapies based on hES cells have been approved for trials on humans, which indicates that more research is needed in order to prove patient safety and proof of concept.

### 4.2 Induced Stem Cells in Cell Therapy

Induced stem cells can be the ideal source for patient-specific therapy, since reprogrammed somatic cells are genetically identical to other cells in the host, and therefore do not induce an immune rejection. This research area is still new and the first human induced stem cell was generated in 2007 (Takahashi et al. 2007). Today, scientists are able to reprogram different human somatic cell types into a pluripotent state but the efficiency of the induced stem cell technology is still very low (Nishikawa et al. 2008, Zhou et al. 2008).

Just as for hES cells, it is crucial to be able to control the cell differentiation. Human induced cells have been differentiated into cardiac cells and neural cells (Takahashi et al. 2007, Dimos et al. 2008) and in teratomas cells from all three germ layers have been identified (Takahashi et al. 2007, Huangfu et al. 2008a). However, the majority of research is still performed with animal cells and only a few experiments have been done in animal models to show functional utility. Hanna and colleagues have reprogrammed and differentiated autologous skin cells, which then have been administered to a humanized sickle cell anemia mouse model. Transplanting hematopoietic progenitor cells resulted in rescue of the mouse (Hanna et al. 2007). The same group also has been able to derive neurons from reprogrammed mouse fibroblasts that had been implanted in a model for Parkinson’s disease. This resulted in improved symptoms and behavior (Wernig et al. 2008).

### 4.3 Drug Screening

In 2004 AstraZeneca launched Extanta, an oral blood thinner, which was a result of 20 years R&D and several million dollars. However, as early as 2006 the company had to pull the drug back from 12 European countries, due to reports showing that the drug increased the risk for severe sudden onset liver injury (Wadman 2007). This is unfortunately not the only story where promising compounds go through the whole drug development process without discovering potential lethal side affects.
The drug development process is costly and therefore much interest is focused on finding an in vitro toxicity test, used to discover inter-species differences that are not discovered in animal models (Yu & Thomson 2006). Stem cells could potentially be used for such an application. Pluripotent stem cells can be directed to differentiate into different cell types often associated with severe side affects (National Institute of Health 2008a). Researchers now hope that in vitro toxicity tests, based on stem cells, can be developed, avoiding inter-species differences and using animals for experimental purposes (Baker 2008).

### 4.4 Embryonic Stem cells for Drug Screening

Stem cells for safer medicine (SC4SM 2008) are collaboration between the UK government and the three large pharmaceutical companies (AstraZeneca, GlaxoSmithKline and Roche). Together they are working towards using human stem cells to catch drug-safety problems in petri dishes, instead of discovering them in patients (SC4SM 2008, Wadman 2008).

The difference between using stem cells for drug screening and using them in cell therapy is that the challenge lies in developing as many cell lines as possible, not a few highly characterized lines (Baker 2008). Stem cells can be used to test toxicity of new drugs but also to test the efficacy on the cell types affected by the disease the drug targets.

The development of an in vitro human ES cell toxicity tests is not yet complete. An in vitro test with mouse ES cell has been developed, which can be used to evaluate inhibition of growth and differentiation of mouse ES cells after exposure of chemicals. This test has demonstrated the potential of using pluripotent stem cells for in vitro testing, but the result can still be questioned since the species-specific differences have not been overcome (Ameen et al. 2007). The technical challenges for achieving this lie in controlling the pluripotent cell differentiation in culture and to be able to keep the cells in that state (Rubin 2008).

Liver metabolism is one area where toxicity tests are needed, since drugs targeting liver cells, hepatocytes, often are subjected to species-specific differences. Human hepatocytes derived from donated organ or tissue can be used, but they are in limited supply and have the disadvantage of being hard to keep functional in cell culture. Pluripotent cells differentiated to hepatocytes therefore would have a large potential for this application (Ameen et al. 2007). Scientists within this field have been able to generate hepatocyte-like cells from hES cell line, that express several important proteins. The protocol for this differentiation is, however, not optimized but work so far shows great promise (Ek et al. 2007).

#### 4.4.1 Induced Stem Cell for Drug Screening

Possibly, induced stem cells can also be used in drug screening. The advantage of using induced stem cells for toxicity test is that cell lines can be established more quickly compared to ES cell lines, and, as earlier mentioned, this is an advantage. The only thing necessary to generate an induced stem cell line with defined factors is a skin biopsy, a retroviral vector, and a technique to identify the reprogrammed cells (Baker 2008). A clinical application within this field is, however, a future long term potential. Better techniques to generate the reprogrammed cells are needed, as well as a more basal understanding of the cell properties.
The use of induced stem cells in drug screening most likely will be important for studying drug efficacy on diseased cells. Today embryonic stem cells lines with Spinal muscular atrophy and Huntingtons disease can be generated from human embryos used for pre-implantation genetic diagnosis (Robin 2008). This can be carried out for several monogenic disorders, but a prerequisite is that the gene causing the disorder is known. Induced stem cells can be an alternative approach and also provide a major potential for establishing cell lines from patient with particular diseases (Rubin 2008). These cell lines will be specific for a particular disease and therefore provide a model for studying human diseases and for testing drug efficacy.
5 Discussion

Induced stem cells can be derived by three different methods, each having different advantages and disadvantages. Reprogramming by defined factors is, however, the approach possessing most clinic potential. This conclusion is based on that the method has been used to generate human reprogrammed cells and that it is relatively simple (Takahashi et al. 2007). A future clinic application based on this method is a long term potential and several challenges regarding the reprogramming have to be overcome. The reprogramming is today based on introducing transcription factors, some of which are known oncogenes by a viral vector. A method which have to change in the future, but several research groups are dedicated to find solutions to these hurdles.

This has to be followed by studies investigating induced stem cells differentiation. So far, only a few articles have been published where human induced stem cells are directed to differentiate. There are today several published articles regarding the large potential of induced stem cells and all their clinical opportunities, but it is important not to forget that human induced stem cells is a new finding. The first time this cell type was generated was in 2007 and it can therefore be assumed that clinical applications using induced stem cells are a long term future opportunities (Takahashi et al. 2007).

The major difference between induced stem cells and ES cells is their origin and that affects the end use of the cells. The major potential of induced stem cells lies in patient-specific therapy and the ability to study human genetic diseases. This can be compared to hES cells, which allow for allogenic cell therapy and therefore treatment of common diseases.

The progress towards a clinical application for hES cells has advanced longer than that for induced stem cells. Studies within the field of cell therapy show great promise and functional utility have been demonstrated in animal models (Lu et al. 2007, Geron Corporation 2008b). However, more reports need to show that there is no risk of tumor growth and that it is safe for the patient. Cell therapy based on hES cells therefore is also a long term opportunity. So far, no clinical trials have started and it can therefore be assumed that it will take another ten years before a clinical application is a reality. The use of hES cells will probably be developed faster because research here has progressed relatively far.

In conclusion, pluripotent stem cells offer great potential, although more basic research is needed in order to understand the basic properties of these cells and the differentiation.
References


