

## Stem Cells: A Primer

This primer presents background information on stem cells. It includes an explanation of what stem cells are; what pluripotent stem cells are; how pluripotent stem cells are derived; why pluripotent stem cells are important to science; why they hold such great promise for advances in health care; and what adult stem cells are.

Recent published reports on the isolation and successful culturing of the first human pluripotent stem cell lines have generated great excitement and have brought biomedical research to the edge of a new frontier. The development of these human pluripotent stem cell lines deserves close scientific examination, evaluation of the promise for new therapies, and prevention strategies, and open discussion of the ethical issues.

In order to understand the importance of this discovery as well as the related scientific, medical, and ethical issues, it is absolutely essential to first clarify the terms and definitions.

### What is a stem cell?

Stem cells have the ability to divide for indefinite periods in culture and to give rise to specialized cells. They are best described in the context of normal human development. Human development begins when a sperm fertilizes an egg and creates a single cell that has the potential to form an entire organism. This fertilized egg is **totipotent**, meaning that its potential is total. In the first hours after fertilization, this cell divides into identical totipotent cells. (Figure 1) This means that either one of these cells, if placed into a woman's uterus, has the potential to develop into a fetus. In fact, identical twins develop when two totipotent cells separate and develop into two individual, genetically identical human beings. Approximately four days after fertilization and after several cycles of cell division, these totipotent cells begin to specialize, forming a hollow sphere of cells, called a blastocyst. The blastocyst has an outer layer of cells and inside the hollow sphere, there is a cluster of cells called the inner cell mass.

The outer layer of cells will go on to form the placenta and other supporting tissues needed for fetal development in the uterus. The inner cell mass cells will go on to form virtually all of the tissues of the human body. Although the inner cell mass cells can form virtually every type of cell found in the human body, they cannot form an organism because they are unable to give rise to the placenta and supporting tissues necessary for development in the human uterus. These inner

### Definitions

**DNA** - abbreviation for deoxyribonucleic acid which makes up genes.

**Gene** - a functional unit of heredity which is a segment of DNA located in a specific site on a chromosome. A gene directs the formation of an enzyme or other protein.

**Somatic cell** - cell of the body other than egg or sperm.

**Somatic cell nuclear transfer** - the transfer of a cell nucleus from a somatic cell into an egg from which the nucleus has been removed.

**Stem cells** - cells that have the ability to divide for indefinite periods in culture and to give rise to specialized cells.

**Pluripotent** - capable of giving rise to most tissues of an organism.

**Totipotent** - having unlimited capability. Totipotent cells have the capacity to specialize into extraembryonic membranes and tissues, the embryo, and all postembryonic tissues and organs.

cell mass cells are **pluripotent** — they can give rise to many types of cells but not all types of cells necessary for fetal development. Because their potential is not total, they are not totipotent and they are not embryos. In fact, if an inner cell mass cell were placed into a woman's uterus, it would not develop into a fetus.

The pluripotent stem cells undergo further specialization into stem cells that are committed to give rise to cells that have a particular function. Examples of this include blood stem cells which give rise to red blood cells, white blood cells and platelets; and skin stem cells that give rise to the various types of skin cells. These more specialized stem cells are called **multipotent**. (Figure II)

While stem cells are extraordinarily important in early human development, multipotent stem cells are also found in children and adults. For example, consider one of the best understood stem cells, the blood stem cell. Blood stem cells reside in the bone marrow of every child and adult, and in fact, they can be found in very small numbers circulating in the blood stream. Blood stem cells perform the critical role of continually replenishing our supply of blood cells — red blood cells, white blood cells, and platelets — throughout life. A person cannot survive without blood stem cells.

### **How are pluripotent stem cells derived?**

At present, human pluripotent cell lines have been developed from two sources<sup>1</sup> with methods previously developed in work with animal models.

(1) In the work done by Dr. Thomson, pluripotent stem cells were isolated directly from the inner cell mass of human embryos at the blastocyst stage. Dr. Thomson received embryos from IVF (In Vitro Fertilization) clinics—these embryos were in excess of the clinical need for infertility treatment. The embryos were made for purposes of reproduction, not research. Informed consent was obtained from the donor couples. Dr. Thomson isolated the inner cell mass (see Figure III) and cultured these cells producing a pluripotent stem cell line.

(2) In contrast, Dr. Gearhart isolated pluripotent stem cells from fetal tissue obtained from terminated pregnancies. Informed consent was obtained from the donors after they had independently made the decision to terminate their pregnancy. Dr. Gearhart took cells from the region of the fetus that was destined to develop into the testes or the ovaries. Although the cells developed in Dr. Gearhart's lab and Dr. Thomson's lab were derived from different sources, they appear to be very similar. (Figure III)

The use of somatic cell nuclear transfer (SCNT) may be another way that pluripotent stem cells could be isolated. In studies with animals using SCNT, researchers take a normal animal egg cell and remove the nucleus (cell structure containing the chromosomes). The material left behind in the egg cell contains nutrients and other energy-producing materials that are essential for embryo development. Then, using carefully worked out laboratory conditions, a somatic cell - any cell other than an egg or a sperm cell - is placed next to the egg from which the nucleus had been removed, and the two are fused. The resulting fused cell, and its immediate descendants, are believed to have the full potential to develop into an entire animal, and hence are totipotent. As described in Figure I, these totipotent cells will soon form a blastocyst. Cells from the inner cell mass of this blastocyst could, in theory, be used to develop pluripotent stem cell lines. Indeed, any method by which a human blastocyst is formed could potentially serve as a source of human pluripotent stem cells (Figure IV).

## Potential Applications of Pluripotent Stem Cells

There are several important reasons why the isolation of human pluripotent stem cells is important to science and to advances in health care (Figure V). At the most fundamental level, pluripotent stem cells could help us to understand the complex events that occur during human development. A primary goal of this work would be the identification of the factors involved in the cellular decision-making process that results in cell specialization. We know that turning genes on and off is central to this process, but we do not know much about these "decision-making" genes or what turns them on or off. Some of our most serious medical conditions, such as cancer and birth defects, are due to abnormal cell specialization and cell division. A better understanding of normal cell processes will allow us to further delineate the fundamental errors that cause these often deadly illnesses.

Human pluripotent stem cell research could also dramatically change the way we develop drugs and test them for safety. For example, new medications could be initially tested using human cell lines. Cell lines are currently used in this way (for example cancer cells). Pluripotent stem cells would allow testing in more cell types. This would not replace testing in whole animals and testing in human beings, but it would streamline the process of drug development. Only the drugs that are both safe and appear to have a beneficial effect in cell line testing would graduate to further testing in laboratory animals and human subjects.

Perhaps the most far-reaching potential application of human pluripotent stem cells is the generation of cells and tissue that could be used for so-called "cell therapies." Many diseases and disorders result from disruption of cellular function or destruction of tissues of the body. Today, donated organs and tissues are often used to replace ailing or destroyed tissue. Unfortunately, the number of people suffering from these disorders far outstrips the number of organs available for transplantation. Pluripotent stem cells, stimulated to develop into specialized cells, offer the possibility of a renewable source of replacement cells and tissue to treat a myriad of diseases, conditions, and disabilities including Parkinson's and Alzheimer's diseases, spinal cord injury, stroke, burns, heart disease, diabetes, osteoarthritis and rheumatoid arthritis. There is almost no realm of medicine that might not be touched by this innovation. Some details of two of these examples follow.

- o Transplant of healthy heart muscle cells could provide new hope for patients with chronic heart disease whose hearts can no longer pump adequately. The hope is to develop heart muscle cells from human pluripotent stem cells and transplant them into the failing heart muscle in order to augment the function of the failing heart. Preliminary work in mice and other animals has demonstrated that healthy heart muscle cells transplanted into the heart successfully repopulate the heart tissue and work together with the host cells. These experiments show that this type of transplantation is feasible.
- o In the many individuals who suffer from Type I diabetes, the production of insulin by specialized pancreatic cells, called islet cells, is disrupted. There is evidence that transplantation of either the entire pancreas or isolated islet cells could mitigate the need for insulin injections. Islet cell lines derived from human pluripotent stem cells could be used for diabetes research and, ultimately, for transplantation.

While this research shows extraordinary promise, there is much to be done before we can realize these innovations. Technological challenges remain before these discoveries can be incorporated into clinical practice. These challenges, though significant, are not insurmountable.

First, we must do the basic research to understand the cellular events that lead to cell specialization in the human, so that we can direct these pluripotent stem cells to become the type (s) of tissue needed for transplantation.

Second, before we can use these cells for transplantation, we must overcome the well-known problem of immune rejection. Because human pluripotent stem cells derived from embryos or fetal tissue would be genetically different from the recipient, future research would need to focus on modifying human pluripotent stem cells to minimize tissue incompatibility or to create tissue banks with the most common tissue-type profiles.

The use of somatic cell nuclear transfer (SCNT) would be another way to overcome the problem of tissue incompatibility for some patients. For example, consider a person with progressive heart failure. Using SCNT, the nucleus of virtually any somatic cell from that patient could be fused with a donor egg cell from which the nucleus had been removed. With proper stimulation the cell would develop into a blastocyst: cells from the inner cell mass could be taken to create a culture of pluripotent cells. These cells could then be stimulated to develop into heart muscle cells. Because the vast majority of genetic information is contained in the nucleus, these cells would be essentially identical genetically to the person with the failing heart. When these heart muscle cells were transplanted back into the patient, there would likely be no rejection and no need to expose the patient to immune-suppressing drugs, which can have toxic effects.

### **Adult Stem Cells**

As noted earlier, multipotent stem cells can be found in some types of adult tissue. In fact, stem cells are needed to replenish the supply cells in our body that normally wear out. An example, which was mentioned previously, is the blood stem cell.

Multipotent stem cells have not been found for all types of adult tissue, but discoveries in this area of research are increasing. For example, until recently, it was thought that stem cells were not present in the adult nervous system, but, in recent years, neuronal stem cells have been isolated from the rat and mouse nervous systems. The experience in humans is more limited. In humans, neuronal stem cells have been isolated from fetal tissue and a kind of cell that may be a neuronal stem cell has been isolated from adult brain tissue that was surgically removed for the treatment of epilepsy.

### **Do adult stem cells have the same potential as pluripotent stem cells?**

Until recently, there was little evidence in mammals that multipotent cells such as blood stem cells could change course and produce skin cells, liver cells or any cell other than a blood stem cell or a specific type of blood cell; however, research in animals is leading scientists to question this view.

In animals, it has been shown that some adult stem cells previously thought to be committed to the development of one line of specialized cells are able to develop into other types of specialized cells. For example, recent experiments in mice suggest that when neural stem cells were placed into the bone marrow, they appeared to produce a variety of blood cell types. In addition, studies with rats have indicated that stem cells found in the bone marrow were able to produce liver cells. These exciting findings suggest that even after a stem cell has begun to specialize, the stem cell may, under certain conditions, be more flexible than first thought. At this time, demonstration of the flexibility of adult stem cells has been only observed in animals and limited to a few tissue types.

## Why not just pursue research with adult stem cells?

Research on human adult stem cells suggests that these multipotent cells have great potential for use in both research and in the development of cell therapies. For example, there would be many advantages to using adult stem cells for transplantation. If we could isolate the adult stem cells from a patient, coax them to divide and direct their specialization and then transplant them back into the patient, it is unlikely that such cells would be rejected. The use of adult stem cells for such cell therapies would certainly reduce or even avoid the practice of using stem cells that were derived from human embryos or human fetal tissue, sources that trouble many people on ethical grounds.

While adult stem cells hold real promise, there are some significant limitations to what we may or may not be able to accomplish with them. First of all, stem cells from adults have not been isolated for all tissues of the body. Although many different kinds of multipotent stem cells have been identified, adult stem cells for all cell and tissue types have not yet been found in the adult human. For example, we have not located adult cardiac stem cells or adult pancreatic islet stem cells in humans.

Secondly, adult stem cells are often present in only minute quantities, are difficult to isolate and purify, and their numbers may decrease with age. For example, brain cells from adults that may be neuronal stem cells have only been obtained by removing a portion of the brain of epileptics, not a trivial procedure.

Any attempt to use stem cells from a patient's own body for treatment would require that stem cells would first have to be isolated from the patient and then grown in culture in sufficient numbers to obtain adequate quantities for treatment. For some acute disorders, there may not be enough time to grow enough cells to use for treatment. In other disorders, caused by a genetic defect, the genetic error would likely be present in the patient's stem cells. Cells from such a patient may not be appropriate for transplantation. There is evidence that stem cells from adults may have not have the same capacity to proliferate as younger cells do. In addition, adult stem cells may contain more DNA abnormalities, caused by exposure to daily living, including sunlight, toxins, and by expected errors made in DNA replication during the course of a lifetime. These potential weaknesses could limit the usefulness of adult stem cells.

Research on the early stages of cell specialization may not be possible with adult stem cells since they appear to be farther along the specialization pathway than pluripotent stem cells. In addition, one adult stem cell line may be able to form several, perhaps 3 or 4, tissue types, but there is no clear evidence that stem cells from adults, human or animal, are pluripotent. In fact, there is no evidence that adult stem cells have the broad potential characteristic of pluripotent stem cells. In order to determine the very best source of many of the specialized cells and tissues of the body for new treatments and even cures, it will be vitally important to study the developmental potential of adult stem cells and compare it to that of pluripotent stem cells.

## Summary

Given the enormous promise of stem cells to the development of new therapies for the most devastating diseases, it is important to simultaneously pursue all lines of research. Science and scientists need to search for the very best sources of these cells. When they are identified, regardless of their sources, researchers will use them to pursue the development of new cell therapies.