

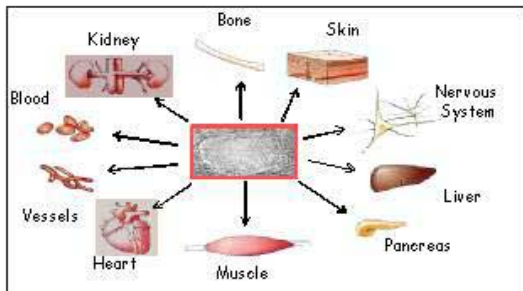
Stem Cell Primer*

*Adapted from materials from the ISSCR web site.
(<http://www.isscr.org/public/index.htm>)

Embryonic Stem Cells

The most flexible type of stem cell is the embryonic stem cell. This cell is distinct from the stem cells found within the adult body, because it comes from cells found in the 5-6 days old embryo, called the blastocyst. Embryonic stem cells are made from left-over blastocysts that have developed from fertilized eggs produced by assisted reproduction technologies, such as in vitro fertilization. These left-over blastocysts, if not donated to another couple or used for research, are usually discarded.

Once embryonic stem cells have been established in culture, large numbers of cells can be grown for a long time, without losing the stem cell character. The most remarkable feature of embryonic stem cells is their ability to generate all functional adult cell types. Culture methods have been developed to turn embryonic stem cells into brain, heart, muscle cells, blood cells, blood vessels, skin, pancreatic islet cells and bone cells.



Potential of human embryonic stem cells. Embryonic stem cell colonies shown in the middle could potentially one day be grown in culture to the illustrated organs and more. While to date many cell types have been grown from embryonic stem cells, culture conditions need more refinement before entire organs could possibly be grown in culture.

It is therefore anticipated that research with embryonic stem cells will help produce cells and tissue for replacement therapies for treating disorders such as Parkinson's disease, heart attacks, blood disorders and diabetes.

Adult Stem Cells

Adult stem cells are stem cells that come from different parts of the body and, depending on where they are from, have different properties. They exist in several different tissues including bone marrow, blood and the brain. Although a wealth of information on adult stem cells has already been collected, scientists still do not understand their specific properties well. It had for example been suggested that adult stem cells from one organ can turn into cells of another organ. Recent rigorous studies have however not proven such claims.

Hematopoietic stem cells, the blood forming stem cells, are adult stem cells found mainly in the bone marrow and they provide the blood cells required for daily blood turnover and for fighting infections. Compared to adult stem cells from other tissues, hematopoietic stem cells are easy to obtain and have been studied by scientists for many years. They were the first stem cells to be used successfully in blood disorder therapies.

More recently, their use in treatment of breast cancer and coronary artery diseases is also being explored.

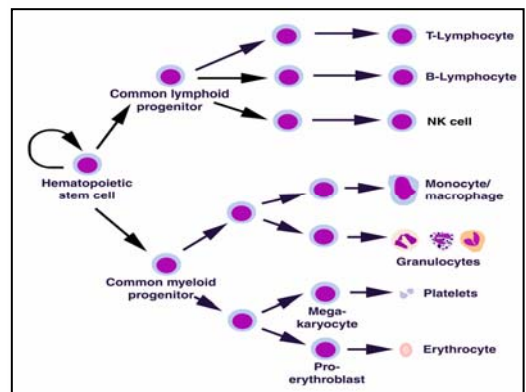


Illustration of the different cells within the blood that are generated by the hematopoietic stem cells. In murine transplantation experiments it has been demonstrated that one single hematopoietic stem cell can reconstitute an entire mouse which had its blood wiped out entirely, including its own hematopoietic stem cells. This mouse can then live healthily with the new blood generated by one single stem cell.

Mesenchymal stem cells are another well-characterized population of adult stem cells. These cells, also found in the bone marrow, can form a variety of cell in the laboratory, including fat cells, cartilage, bone, tendon and ligaments, muscles cells, skin cells and even nerve cells.

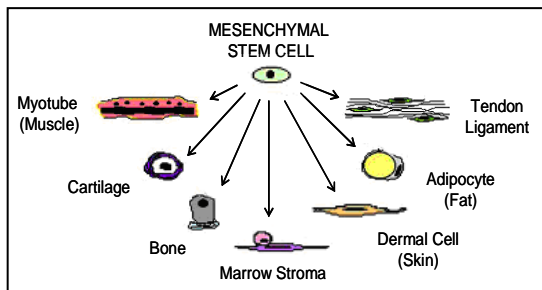


Illustration of the different cell types generated by human mesenchymal stem cells.

Unlike most other human adult stem cells, mesenchymal stem cells can be obtained in quantities appropriate for clinical applications, making them good candidates for use in tissue repair. Perhaps one of the important considerations for human applications is that mesenchymal stem cells can be obtained from a small bone marrow sample from a given patient, expanded in culture, and given back to the patient. This would avoid the problems associated with immune rejection of foreign transplanted cells or tissues.

Umbilical cord blood stem cells can be obtained from the umbilical cord immediately after birth. Like bone marrow, umbilical cord blood is another rich source of hematopoietic stem cells. These hematopoietic stem cells are usually referred to as neonatal stem cells and are less mature than the hematopoietic stem cells found in the bone marrow of adults.

The advantages of using cord blood as a source of stem cells are that umbilical cord blood is easily obtained and is abundant; thousands of babies are born each day and, until recently, umbilical cord blood was discarded after birth. Now, cord blood is collected and stored by public or private cord blood banks.

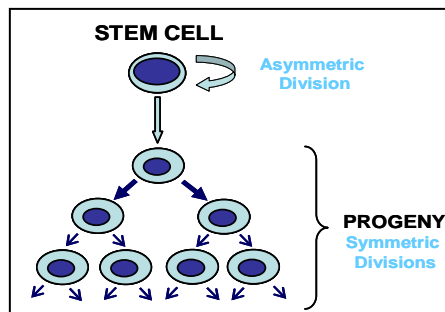
Cord blood has recently emerged as an alternative source of hematopoietic stem cells

for the treatment of leukemia and other blood disorders. However, there is a limited number of stem cells in any given cord which prevents its generalized use for the treatment of blood disorders in adults.

The use of umbilical cord blood stem cells for other uses, such as organ and tissue repair, is under also investigation.

Self-Renewal and Stem Cells Expansion for Therapy

A stem cell is defined as a cell that can renew itself indefinitely, while producing progeny that mature into more specialized, organ-specific cells. To accomplish this task, the stem cell has to divide continuously. These divisions are asymmetric. One of the two daughter cells retains the stem cell characteristics, while the other is destined for a limited number of future divisions and will produce the more organ-specific cells. Although scientists are learning more and more about this complicated cell division mechanism, they are still a long way from understanding it.



Stem Cell Division. The stem cell divides asymmetrically, generating one cell that repeats the feat indefinitely, and one cell that continues to divide symmetrically, dividing each time into two equal daughter cells.

For successful therapeutic application most of the adult stem cells isolated from the body will have to be amplified in culture. While the embryonic stem cell can be coaxed to amplify in culture without losing its capabilities, adult stem cells (with exception of the mesenchymal stem cell) can not be amplified in culture to meaningful cell numbers. Only once scientists

will be able to grow adult stem cells in culture will they be able to work with the small numbers of adult stem cells that can be obtained from a patient, and produce sufficient cells for therapy.

Nuclear Transfer (NT)

Contrary to adult stem cells, the embryonic stem can be propagated and even amplified for a long time in culture, without losing its stem cell character. The major problem facing widespread use of embryonic stem cells in cell therapies and organ replacement however is their anticipated rejection by the patient's immune system, which will recognize them as foreign and destroy them. Treatment with drugs that lessen the severity of the immune reaction usually do not stop an already launched immune response.

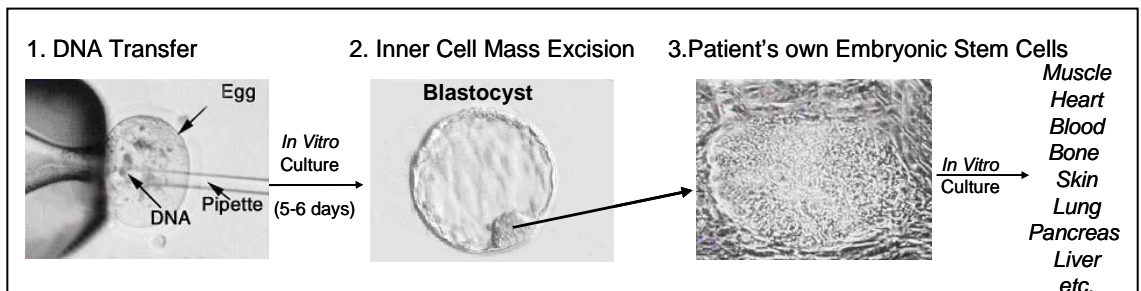
The only way to overcome an immune reaction, or rather to avoid it, is to perfectly match the donor and the recipient. A perfect match, however, exists only between identical twins. For all other patients, donor and recipients are matched as well as possible, and the patients must remain on drugs for the rest of their lives, to prevent the occurrence of an immune reaction with deadly outcome. For the vast majority of patients in need of transplants or tissue repair there are however no appropriate donors.

One way around this problem would be to produce "custom" embryonic cells, by nuclear transfer, matching the patient's immunologic

profile. This approach, would lead to the production of cells and tissue matching the patient and that would not cause an immune reaction when the cells are transplanted into the patient.

For nuclear transfer, the DNA from any one cell in the body of a patient (usually a skin or muscle cell) could be removed and transferred through a microscopic glass tube into an unfertilized egg that previously had its own DNA removed, as shown below (first image below).

In a culture dish, the egg is then coaxed into developing as if it had been fertilized. The one egg cell divides rapidly and generates the blastocyst. The inner cell mass, a part of the blastocyst (middle image below), is then removed and embryonic stem cells grown out of it. These embryonic stem cells, containing the patient's DNA, now match the patient's profile and will not be rejected by the patient's immune system. These embryonic stem cells can now be used to generate cells and tissues for the patient. While this procedure sounds straightforward and is being performed successfully in animal models, it was only very recently (Feb 2004) performed successfully with human eggs and human DNA. However, many technological, legal and ethical hurdles need to be overcome before this procedure can be used for human therapy.



Nuclear Transfer Procedure: Image 1: under the microscope, the DNA of the patient is introduced into the egg, through a microscopic glass tube. Image 2: After 5-6 days, the egg has developed into a ball of cells, the blastocyst, of which is removed the inner cell mass. Image 3: After culture in a plastic dish, the inner cell mass has grown to aggregates which contain the embryonic stem cells that match the patients immunologic profile.



Reproductive Cloning

In reproductive cloning, the DNA of an unfertilized egg is removed and replaced by DNA obtained from another individual and the egg is coaxed into dividing. Up to this point, the procedure is similar to nuclear transfer to make stem cells. The resulting embryo is then implanted into the uterus of a substitute mother, where it can develop until birth.

In contrast to natural reproduction, reproductive cloning does not create a genetically new and unique individual. Only the DNA from the DNA donor is present, which makes the newborn a genetic replicate of the DNA donor, a clone.



Clone #1: Dolly the sheep, was born to stardom on July 5, 1996. After a short life of six years (sheep live up to 12 years) Dolly had to be euthanized on Feb. 14, 2003, because of progressive lung disease. Examination following her death confirmed a virus-induced lung tumor. (Image kindly provided by the Roslin Institute, Edinburgh, UK.)

While this procedure may sound relatively easy, reproductive cloning is full of with profound technical and, more importantly, biological problems. The birth of normal animal clones is very rare. Despite cloning and implantation of hundreds of embryos, very few live cloned animals have been obtained thus far, around 1 percent of all the eggs that received donor DNA. In addition, the clones that do survive are in very poor health with many problems, including obesity, arthritis, infection, breathing problems and death at young ages. It is not known why

reproductive cloning is so inefficient. Scientists are actively working in this area and possible reasons are beginning to emerge.

Every cell of the body contains exactly the same set of genes. Certain genes are either turned on or off during development, which allows a cell to become specialized as a brain cell or a heart cell, or any other cell type in the body. Normally, after the embryonic stage is over, genes that are crucial to orchestrated embryonic development are turned off, and remain that way, for the rest of the life of the individual. This is called 'epigenetic' control of gene expression.

During reproductive cloning, the donor DNA that is introduced into the egg is "reprogrammed" by the egg, which turns some of the embryonic genes back on and others off, as appropriate in an egg cell at that stage of development. However, the donor DNA is obtained from the cell of an adult and is "old." Important embryonic genes have been silenced forever in the past of the "old" DNA. The smooth and orchestrated embryonic ballet of turning genes on and off therefore cannot take place, resulting in extremely high rates of pre-term abortions, miscarriages and birth defects.

There are also significant risks for the mother, associated with carrying a grossly overweight fetus and having an abnormal pregnancy. Cloned newborn animals are often significantly overweight as compared to normal pups and have a large and dysfunctional placenta (this is often referred to as "Large Offspring Syndrome"). For all of these reasons, human reproductive cloning is considered unsafe and therefore opposed adamantly by the scientific community.