



PUBLIC UNDERSTANDING OF BIOTECHNOLOGY



CLONING AND STEM CELLS

Cloning is a term used to broadly describe any process that produces an identical copy of biological material, from individual genes or cells to even whole organisms. A "clone" is a genetically identical copy of the original. The word cloning is an umbrella term, and covers various types of cloning: recombinant DNA technology or **DNA cloning, reproductive cloning, and therapeutic cloning.**

- DNA cloning is a technique used to create multiple copies of a gene so that its function can be studied. It is defined by the National Bill of Health (2003) of South Africa as the manipulation (transfer) of genetic material (the nucleus) from adult, zygote or embryonic cells into an enucleated donor egg cell in order to make an identical copy (clone) of the donor. This process is called somatic cell nuclei transfer (SCNT). SCNT can be used for reproductive or therapeutic purposes.
- Reproductive cloning is used to generate copies of an organism and many animals have been cloned this way. However, human cloning is illegal and is very controversial, particularly as technology advances to make human cloning a possibility.
- Therapeutic cloning is used to obtain cloned stem cells that can be used for treatments for illnesses. Therapeutic cloning uses the same technology as reproductive cloning (SCNT). It is used to create stem cells which have potential for treating diseases and other disabilities caused by tissue damage.

What are stem cells?

Stem cells are essentially "un-specialised" cells. They do not yet have a specialised function and have the ability to turn into other types of cells. For instance, a stem cell can turn into liver cells, skin cells, nerve cells, etc. In order for stem cells to give rise to cells which can differentiate and become specialised, they need to be given the correct signals. A wide range of chemical signals from nearby cells provide instructions to the stem cells. Because of their unique qualities, stem cells can potentially generate new spe-

cialised tissue and organs. They also play a role in replacing old, dying cells in existing organs and tissues.

There are various types of stem cells.

- **Embryonic stem cells** (ES cells) are found in early embryos (5-6 days old). The cell of 4-8 cell embryos are said to be totipotent. These cells have the ability to form an entirely independent human being if implanted in a uterus, since they are able to give rise to both the embryo and the placenta. Because of the potential of **totipotent** stem cells and the resultant ethical dilemmas, scientists have, in the main, avoided research on this type of stem cell. A later stage of the embryo (blastocyst stage) has an inner cell mass of about 200 cells which are **pluripotent** stem cells. These cells have a limited ability to turn into any type of specialised cell, depending on the information and signals they receive from the specific culture they are placed in. This ability to turn into any cell type makes them so valuable for treatment of illnesses, called therapeutic applications (Ratajczak *et al*, 2007; Mimeault and Batra, 2006; <http://stemcells.nih.gov>; www.genome.gov).
- **Adult stem cells** have been found in specialised tissues: in bone marrow, brain, skin, eyes, heart, kidneys, lungs, gastro-intestinal tract, pancreas liver, breast ovaries, prostate, and testis (Mimeault and Batra, 2006). Adult stem cells are more specific and till recently, it was thought that were only capable of differentiating into a few types of cells. More recent research shows adult stem cells (called induced stem cells or iPS) may be able to form a wider range of tissues than previously thought and it appears that they can become embryonic stem cells with a full range of potential. Because they can be taken from adult tissues experiments in these cells are far less controversial than in embryonic stem cells. For example bone marrow stem cells not only generate blood cells, but can also form neurons. Haematopoietic stem cells can develop into heart muscle. This phenomenon is known as plasticity. Further research is needed to establish exactly what adult stem cells are able to become.



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Why is stem cell research potentially so valuable?

Treatment for disease

Scientists believe that stem cells may, at some point in the future, become the basis for treatment of diseases caused by irreversibly damaged and injured tissue such as diabetes, heart disease and Parkinson's disease (Mimeault and Batra, 2006). They are particularly optimistic in cases where the disease is caused by loss of function of a specific type of cell.

In type 1 diabetes, a person's own immune system destroys their pancreatic cells which normally produce insulin needed to maintain low blood sugar levels. It may be possible to direct stem cells in culture to turn into insulin-producing cells, which may then be transplanted in diabetic patients (Burns et al, 2006). Embryonic stem cells as well as adult stem cells from various tissues including the pancreas, liver, bone marrow and adipose tissue (body fat) have the potential to differentiate into insulin-producing cells (Zulewski, 2008; Soria et al., 2008).

Parkinson's disease is a common neurodegenerative (abnormal deterioration of the nervous system) disease which affects over 2% of people over the age of 65. It is caused by the loss of function of dopamine (DA)-producing neurons. Dopamine acts as a neurotransmitter in the brain. Neurotransmitters are chemicals that permit nerve signals to bridge the gap between nerve cells. When deficient, it causes symptoms of tremors, rigidity and abnormal reduced mobility (difficulty to move). There are three sources of stem cells currently in testing for treatment of Parkinson's disease: embryonic stem cells, neural stem cells, and mesenchymal stem cells (from bone marrow). Stem cell transplantation in animals with the equivalent of Parkinson's disease has shown that it can restore damaged brain function and relieve symptoms (Wang et al, 2007; Trzaska and Rameshwar, 2007.).

Replacing organs or tissues that have been damaged or destroyed

One of the most important potential applications of stem cells is cell-based therapy to replace organs or tissues that are failing or have been destroyed. Today, organs are donated from living or deceased people, but the demand far exceeds the supply. Stem cells, which may be directed to differentiate into specific tissue, may offer the possibility of an alternative. This does not necessarily involve growing entire new organs. A few healthy stem cells or a small amount of tissue inserted into damaged organs such as the liver or heart, could assist to heal that organ, though much investigation is still needed to establish the exact potential.

Another area of promise for therapy using stem cell technology is in the treatment of spinal cord injuries, and stem cell-based therapies for spinal cord injuries are moving closer to clinical applica-

tion as scientists gain a better understanding of stem cell biology and applicability (Coutts and Keirstead, 2008). Traumatic injuries to the spinal cord cause permanent neurological damage. Recent studies in animals have shown that stem cell transplantation may improve the recovery and help regain function after spinal cord injury (Kim et al, 2007).

Increased knowledge of development and differentiation

By studying stem cells, scientists are gaining knowledge in how organisms develop from single cells and how birth defects occur. They are starting to understand what signals are required for cells to change from one function to another. They may then be able to control differentiation of stem cells to produce a specific tissue. A better understanding of differentiation will also lead to a better understanding of abnormalities in differentiation which lead to diseases such as cancer.

Scientists are also gaining knowledge in how old, damaged cells are replaced by healthy cells generated from stem cells. This knowledge has application in the field of ageing.

Screening new drugs and toxins

Stem cells can be used to screen new drugs and toxins. New medications can be tested on differentiated, specific human cells in a controlled, experimental environment, without first being tested for safety in the human body. This may provide a more accurate assessment than that which is derived from tests performed on animals with a different makeup to humans.

How are stem cells obtained?

- Embryos can be obtained from **fertility clinics** where in vitro fertilization is used to fuse eggs with sperm to form embryos. Multiple embryos are made in case the first embryo is unsuccessfully transplanted. Therefore, there are many unwanted embryos leftover. Only some research-base clinics keep these unused embryos while other clinics leave them to "die" if they are not implanted. Embryos can be created through in vitro fertilization (IVF) specifically for research purposes.
- Embryonic stem cells can also be obtained from abortion clinics, extracted from aborted fetuses.
- Stem cells can be obtained from embryos that have been created by a method called Somatic Cell Nuclear Transfer (SCNT).

What is Somatic cell nuclear transfer (SCNT)?

Somatic cell = all cells of the body except gametes (sperm or egg), containing two sets of each chromosome

Nuclear = the nucleus of the somatic cell containing the genetic information

Transfer = moving the nucleus from one cell to another

In this method, the nucleus from a donor somatic cell is transferred to an egg cell (oocyte) from which the nucleus has been removed. An unfertilised egg cell works best, because it is more likely to accept the donor nucleus.

The donor cell must be in a dormant phase, the G0 cell stage, which causes the cell to shut down but not die. To do this, the cell is starved in culture, with just enough nutrients to survive. The cell then shuts down all its active genes to conserve energy and nutrients and enters the dormant state. In this state, the nucleus is ready to be accepted by the egg cell. In order to transfer the nucleus, either the empty egg cell is fused with the donor somatic cell or the donor somatic cell nucleus is injected into the egg cell. An electric current is then applied to the cells to stimulate its division to form an embryo. The resulting cells of the embryo have a nuclear genetic and immune match to the donor individual. All cloning experiments of adult mammals have used a variation of nuclear transfer.

- Parthenogenesis, in which unfertilised egg cells are used to create an embryo. Only women can be the “donors” when embryonic stem cells are created by this method (Revazova et al, 2008).
- Adult stem cells from specialised tissues and from the umbilical cord at birth. Stem cells from specific tissues can be difficult to isolate, and the culture conditions can unwantedly change the nature of the cell.

Obstacles to stem cell therapy

(Rusnak and Chudley, 2006)

- **Immune rejection:** Stem cells which do not have an identical immune match may cause the recipient to react and reject the transplanted cells (Saric et al, 2008). Theoretically, using cloned embryonic stem cells (by SCNT) from an individual patient may avoid the problem of immune rejection. Because somatic cells are used, anyone can be a donor. In reality, the genetic match of stem cells created by SCNT is not 100% identical. Parthenogenesis creates stem cells with an identical immune match to the donor. However, only women can be donors.
- **Cost:** It may cost too much and take too long to produce a sufficient number of well-characterised stem cells from an individual patient (President’s Council on Bioethics, 2003). Due to cost, it may be necessary to use stem cells generated from one individual to treat multiple individuals. This then raises the problem of immune rejection.
- **Safety:** Safety issues include concerns over the transfer of animal pathogens because stem cell lines are usually cultured in medium containing animal products such as bovine serum.
- **Cancer threat:** Stem cells by their nature divide indefinitely and methods need to be developed to ensure that they do not retain this capacity or malfunction in any other way. It is important when cells are transplanted for treatment,

that only differentiated cells are transplanted and contaminated undifferentiated cells are removed before transplant.

- **Technically difficult:** Egg donation for SCNT is difficult. It is an invasive procedure, unlike sperm donation. SCNT is an inefficient technique with a low success rate. Delivering cells to target organs or tissues is also technically difficult.

Ethical issues of stem cell therapy

(The President’s Council on Bioethics, 2003)

There is much ethical debate around stem cells research and therapy. This is due mostly to the creation and the destruction of embryos to acquire embryonic stem cells. (See table below)

The ethical objections to stem cell therapy may be avoided with the development of new technologies other than SCNT, to make use of adult stem cells or other non-embryonic stem cells such as reprogrammed somatic cells.

Regulations on stem cells and stem cell research

(Townsend and Jones, 2004; Australian Stem Cell Centre, 2007)

Most countries have legislation controlling human embryonic stem cell research. Excluded countries include Portugal, Norway, Poland, Slovakia, Thailand and the U.S.A. Many countries (UK, and other EU countries) allow human embryonic stem cells (HESCs) to be derived from human embryos created by IVF and also allow the creation of HESCs using the cloning technology of SCNT for research purposes. Other countries allow the use of IVF embryos to generate stem cells but do not allow the creation of embryos for research purposes using SCNT. Few countries such as Austria, Germany and Italy do not allow HESCs to be derived in any way.

In South Africa, the National Health Bill passed in 2003 makes allowance for HESC research on excess embryos from IVF and also allows embryos specifically created for stem cell research by SCNT. Reproductive cloning is not permitted. Draft regulations to the Bill were published in the Government Gazette in January, 2007, and have not yet been finalised. Regulations regarding places where stem cell research may take place, and ownership of excess embryos before and after harvesting of stem cells are included. Research may be undertaken if prior informed consent is obtained from the donor, if approved by the Minister of Health with recommendation from the National Human Genetics and Stem Cell Research Ethics subcommittee, and if research is documented for record purposes. Any information obtained from the research

Pro Stem Cell Research	Anti Stem Cell Research
What constitutes the beginning of life?	
Some people believe the embryo should not be given the status of a human being. They believe that life only begins either after 14 days after conception or even a new born baby at birth and destroying an embryo does not destroy human life.	Some people believe the embryo has the moral status of a person from the moment of conception. Since stem cell research involves the destruction of an embryo, these people believe that human life is destroyed.
Some believe it would be immoral not to use surplus embryos if they could potentially develop cures to treat humans with disease and debilitating conditions to improve quality of life.	
	Stem cell technology has powerful potential and uses cloning technology. Embryos created for stem cell research by SCNT could theoretically be used to generate a human life genetically identical (a clone) of it donor. Creating embryos means potential life is created by man, and many believe this is humans playing God.

is not subject to intellectual property (patent) rights (Government Gazette, Vol 499).

The USA has no federal legislation regarding human cloning or stem cell research. Federal policy does however restrict the federal funds available for research. Within the individual states there is much variation in laws. California permits HESC research, some states ban it and some states have no laws.

New research on stem cells

- Induced pluripotent stem cells (iPS cells) act like human embryonic stem cells, but they are derived from adult somatic cells. Two groups of researchers have shown that they can induce human skin cells to behave like embryonic stem cells by forcing them to express certain genes that are normally expressed in stem cells (Takahashi et al, 2007; Junying et al, 2007). These cells are not ready for therapeutic use because the methods use viruses to induce expression of the required genes, and they can possibly form cancers. With further understanding of how cells become reprogrammed, this may be avoided (Stadtfeld et al, 2008).
- Scientists have shown that a new therapeutic cloning (SCNT) technique does not require unfertilized eggs (Egli et al. 2007). Scientists have shown that if the fertilized egg (zygote) is stopped from dividing, the reprogramming factors remained and could reprogram adult mouse skin cells. Scientists hope that this technique can make use of abnormal human zygotes which are created in excess after IVF. Abnormal zygotes are believed to be incapable of surviving to birth and so this would relieve some of the ethical objections to using excess IVF embryos.
- Scientists have generated non-embryonic stem cells from cells in human amniotic fluid (liquid in the uterus in which the fetus lives), called amniotic derived stem cells (AFS cells) (De Coppi et al, 2007). AFS cells did not make all the proteins expected in

pluripotent cells, but scientists could produce fat cells, nerve cells, liver cells, and bone-forming cells. Although scientists don't know how many different types of cells they may form, it is possible that one day they may produce a bank of different cell types. Since amniotic fluid is regularly collected from pregnant women during amniocentesis, this source of stem cells would be less of an ethical issue than embryos.

- Scientists can drive human embryonic stem cells to become neurons. Recently scientists have developed a culture method which selects only human neural stem cells, leaving no undifferentiated cells. They have found no tumours produced when transplanted into rats. This means, scientists may be one step closer to using stem cell-derived neurons in treatment of stroke patients (Daadi et al, 2008).
- Scientists have treated muscular dystrophy (MD) in mice with muscle derived from mouse embryonic stem cells (Perlingeiro et al, 2008). MD is an inherited disease characterised by degeneration of skeletal muscle and progressive weakness. They have used a method to sort differentiated muscle cells from potentially carcinogenic undifferentiated cells. When the mouse stem cell-derived muscle cells were injected into mice with a muscle-wasting condition, tests showed that their muscle function improved. Scientists hope to be able to use human embryonic stem cell-derived muscle cells to treat MD.
- Multipotent adult progenitor cells (MAPCs) are a group of non-blood stem cells found in bone marrow. Scientists have successfully used mouse MAPCs to generate the blood-forming system in mice, generating long-term blood stem cells and all types of early blood cells (Serafini et al, 2007). Scientist may in the future be able to use MAPCs to treat diseases of the blood.

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