### Removal of stem cells

In terms of Chapter 8, ministerial authorisation is required for the removal of stem cells, placenta, embryonic or foetal tissue and umbilical cord. The regulations relating to the use of human biological material, in contrast, require that human biological material, which includes stem cells, may be removed or withdrawn from living adult donors with their informed consent (i.e. no ministerial authorisation is required). This is clearly an oversight that causes unnecessary uncertainty as far as routine procedures, such as bone marrow transplantations, are concerned.

### Stem cell research

In terms of chapter 8, permission of the Minister is required for research on stem cells after informed consent of the donor of the cells has been obtained. The application to do this research must be in writing and the applicant must undertake to document the research for record purposes. The regulations, in addition, provide that genetic health research may be carried out on withdrawn biological material, which includes embryos and progenitor stem cells, provided that the research has been approved by a registered health research ethics committee.

As far as research involving embryos is concerned, chapter 8 provides that the Minister may permit research on zygotes not more than 14 days old, provided that the prior consent is obtained from the donor of such zygotes and that the applicant undertakes to document the research for record purposes. In addition, the regulations provide that excess embryos obtained from in vitro fertilisation may be used to produce embryonic stem cell lines for the purposes of research, provided that the written informed consent from the embryo donor is obtained. Research may also be carried out on primordial germ cells (e.g. stem cells found in the gonad of a fetus which are capable of becoming ova or sperm) obtained from aborted fetuses. The prior written informed consent from the donor of the aborted fetus is required for this purpose.

### Cloning

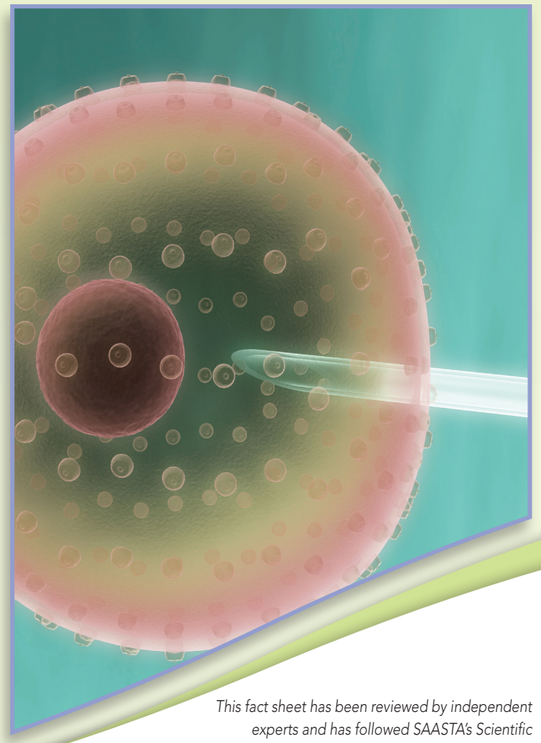
Chapter 8 expressly prohibits reproductive cloning of a human being. The Minister may permit therapeutic cloning utilising adult or umbilical cord stem cells.

### Unaddressed issues

Although the regulations (with changes that came into effect in 2012) contain further prescriptions with regard to who may carry out the harvesting of the stem cells, the documentation of stem cell research and places where the harvesting may be carried out, they are silent on certain issues. These include where stem cell research may take place, the issue of ownership of human biological material (including stem cells derived from such material), as well as the issue of intellectual property rights on the information derived from and products emanating from such research.

## New research on stem cells

- Scientists have shown that a new therapeutic cloning (SCNT) technique does not require unfertilised eggs. They discovered that if the fertilised 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 circumvent 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 foetus lives) called amniotic-derived stem cells (AFS cells). 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 from them. Although scientists do not 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 found no tumours produced when the cultured neural stem cells were transplanted into rats. This means that scientists may be one step closer to using stem cell-derived neurons in the treatment of stroke patients.
- Scientists have treated muscular dystrophy (MD) in mice with muscle derived from mouse embryonic stem cells. MD is an inherited disease characterised by degeneration of skeletal muscle and progressive weakness. Scientists 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 human 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. Scientists may in the future be able to use MAPCs to treat diseases of the blood.
- Substantial research effort has been directed at making iPS technology safer and more efficient. Recent publications indicate that it is now possible to substantially increase the efficiency of the re-programming process underlying iPS generation. Likewise, the development of alternative re-programming techniques not requiring transfection techniques and oncogenes, has led to a lower cancer risk associated with iPS technology. Currently, a major research focus in this field is to establish whether iPS are equivalent to naturally occurring stem cells in the organism, both in their developmental characteristics and potential for therapeutic applications.



*This fact sheet has been reviewed by independent experts and has followed SAASTA's Scientific Editorial Process.*
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PUBLIC UNDERSTANDING OF BIOTECHNOLOGY

# Cloning and Stem Cells

## What is cloning?

Cloning is a term used to broadly to 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 a general term, and covers various types of cloning: for example, DNA cloning, reproductive cloning, and therapeutic cloning.

- **DNA cloning** is a technique whereby recombinant DNA technology is used to create multiple copies of a gene (the molecular blueprint for the synthesis of proteins that perform specific functions in an organism) so that its function can be studied. In contrast, **cloning of organisms** involves the creation of genetically identical duplicates of these organisms.
- **Reproductive cloning** is used to generate copies of an organism. Many animals have been cloned this way. However, human cloning is illegal in every country in the world.
- **Somatic Cell Nuclear Transfer (SCNT)** for therapeutic medicine is used to create stem cells that can potentially be used as treatments illnesses and disabilities. The cells are maintained and harvested using cell culture techniques before they give rise to a developed organism. However, these cells have not been used yet in any human treatments, since there are still many scientific, technical and ethical problems that must be solved. In recent years, a more promising (and less controversial) approach towards obtaining stem cells with therapeutic potential has been the generation of **induced pluripotent stem cells (iPS)**, which can be obtained without reproductive cloning.

In the National Bill of Health (2003) of South Africa, cloning is defined as the manipulation (transfer) of genetic material (contained in the nucleus) from adult, zygote or embryonic cells into an enucleated donor egg cell (i.e. from which the nucleus has been removed) in order to make an identical copy (clone) of the donor. This process is more correctly called somatic cell nuclear transfer (SCNT). SCNT can be used for reproductive or therapeutic purposes.

## What are stem cells?

Stem cells are essentially self-renewing, undifferentiated (or "unspecialised") cells that have the ability to turn into other types of cells. For instance, a stem cell can turn into a liver cell, a skin cell, a nerve cell, 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 can provide instructions to the stem cells to tell them what to become. Because of their unique qualities, stem cells can potentially be used to regenerate new tissue and organs and they play a role in replacing old, dying cells in existing organs and tissues.

There are many types of stem cells.

- **Embryonic stem cells** (ES cells) are found in early embryos (five to six days old). The cells of four to eight-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 that they receive from the specific culture they are placed in. This ability to turn into any cell type makes them very valuable for treatment of illnesses, called therapeutic applications.
- **Adult stem cells** have been found in specialised tissues: in bone marrow, brain, skin, eyes, heart, kidneys, lungs, gastrointestinal tract, pancreas, liver, breast, ovaries, prostate, and testis. Adult stem cells are more specific and, until recently, it was thought that they were only capable of differentiating into a few types of cells. More recent research shows that adult stem cells 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 with these cells are far less controversial than those using 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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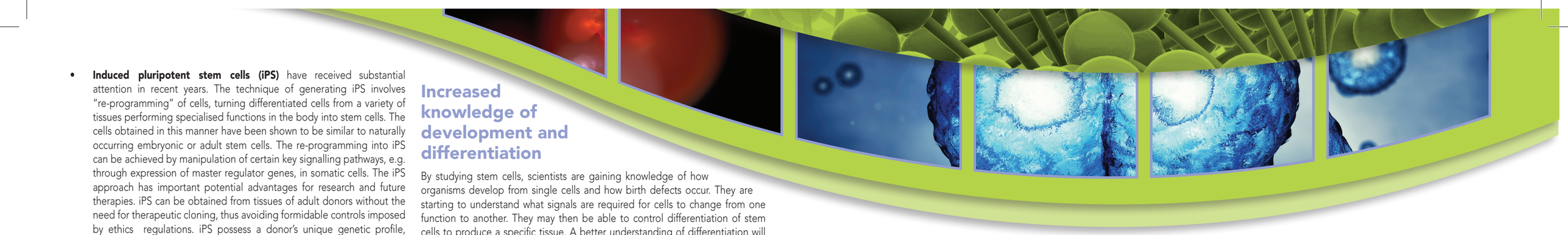

- **Induced pluripotent stem cells (iPS)** have received substantial attention in recent years. The technique of generating iPS involves "re-programming" of cells, turning differentiated cells from a variety of tissues performing specialised functions in the body into stem cells. The cells obtained in this manner have been shown to be similar to naturally occurring embryonic or adult stem cells. The re-programming into iPS can be achieved by manipulation of certain key signalling pathways, e.g. through expression of master regulator genes, in somatic cells. The iPS approach has important potential advantages for research and future therapies. iPS can be obtained from tissues of adult donors without the need for therapeutic cloning, thus avoiding formidable controls imposed by ethics regulations. iPS possess a donor's unique genetic profile, permitting a powerful approach to personalised medicine.

## 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 occurs in diabetes, heart disease and Parkinson's disease. 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 into diabetic patients. 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.

Parkinson's disease is a common neurodegenerative disease (disease caused by abnormal deterioration of the nervous system) which affects over 2% of people over the age of 65 years. 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 allow nerve signals to bridge the gap between nerve cells. Dopamine deficiency causes symptoms of tremors, rigidity and abnormal reduced mobility. There are three sources of stem cells currently being tested for treatment of Parkinson's disease: embryonic stem cells, neural stem cells, and mesenchymal stem cells (from bone marrow). Stem cell transplantation in animals as models of Parkinson's disease has shown that it can restore damaged brain function and relieve symptoms.

However, recent clinical trials in humans aimed at therapies for neurodegenerative disorders like Parkinson's disease have not yet yielded consistent and convincing results. Due to the overwhelming complexity of the human central nervous system, much more research furthering our understanding of factors that govern the successful functional integration of stem cell-derived neurons into the damaged nervous system is required.

## 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 a possible 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 in healing that organ, although 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. Traumatic injuries to the spinal cord cause permanent neurological damage. Stem cell-based therapies for spinal cord injuries are moving closer to clinical application, as scientists gain a better understanding of stem cell biology and applicability. Recent studies in animals have shown that stem cell transplantation may improve recovery and help neurological tissue to regain function after spinal cord injury.

## Increased knowledge of development and differentiation

By studying stem cells, scientists are gaining knowledge of 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 of how old, damaged cells are replaced by healthy cells generated from stem cells. This knowledge has application in the field of ageing. The possibility of generating iPS from individual patients suffering from inherited disorders facilitates the study of pathological processes at the cellular and molecular level that lead to the disease or disorder, in the context of the affected individual.

## 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 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* fertilisation (IVF) 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 "left over". Only some research-based clinics keep these unused embryos, while other clinics leave them to "die" if they are not implanted. Embryos can also be created through IVF specifically for research purposes.
- Embryonic stem cells can also be obtained from abortion clinics, after extraction from aborted foetuses. To obtain the stem cells, some cells from the embryo (the inner mass cells) are cultured in a dish in conditions that enable them to grow.
- Stem cells can be obtained from the inner mass cells 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 "turns off" 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 cell 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.

- Adult stem cells can be obtained from specialised tissues (such as bone marrow and fat tissues) and from the umbilical cord at birth. At present almost all the therapeutic treatments using adult stem cells come from bone marrow stem cells and umbilical stem cells. Stem cells from highly specialised tissues (such as liver or skin) can be difficult to isolate, and the culture conditions can cause unwanted changes in the nature of the cells.
- Induced pluripotent stem cells (iPS) are obtained by re-programming somatic cells into stem cells by manipulation of certain key signalling pathways involved in cell growth and differentiation. This is frequently achieved by forcing somatic cells into expressing a defined set of master regulator genes, or transcription factors, by transfection (a process whereby nucleic acids are introduced into cells). Because this approach is associated with an increased risk of tumour development, and because it is rather inefficient regarding the numbers of re-programmed cells generated and may give rise to incompletely re-programmed cells, alternatives to this approach are being developed. These involve mimicking the action of transcription factors with small chemical compounds and drugs, manipulating gene expression by introducing micro-RNA (small non-coding nucleic acid that regulates gene expression), as well as using alternative vectors for gene expression in the target cells.

## Obstacles to stem cell therapy

- Immune rejection: Stem cells which do not have an identical immune match may cause the recipient to react and reject the transplanted cells. 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. The iPS approach avoids the problem of immune rejection, because a donor's own somatic cells are used for re-programming into stem cells.
- Cost: It may cost too much and take too long to produce a sufficient number of well-characterised stem cells from an individual patient. 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 a medium containing animal products such as bovine serum. This aspect also applies to stem cells induced by transfection with viral vectors.
- 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 that contaminating undifferentiated cells are removed before transplant. Re-programming of somatic cells with the aim of obtaining iPS often involves transfection of cells with oncogenes (genes involved in cancer formation), thus increasing cancer risk.
- 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

There is much ethical debate around stem cell research and therapy. This is due mostly to the creation and the destruction of embryos to acquire embryonic stem cells.

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 (iPS).

| Pro SCNT Research | Anti SCNT 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 beings either 14 days after conception or even in a new born baby at birth and that 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 in cloning technology. Embryos created for stem cell research by SCNT could theoretically be used to generate a human life genetically identical (a clone) to its donor.<br><br>Creating embryos means potential life is created by man, and many believe this represents humans playing God. |

## Regulation of stem cells and stem cell research

Most countries have legislation controlling human embryonic stem cell research. 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. A few countries such as Austria, Germany and Italy do not allow HESCs to be derived in any way.

In South Africa, chapter 8 of the National Health Act (NHA), as well as regulations promulgated in terms of this chapter, regulates research on stem cells withdrawn from living and dead persons. The most relevant regulations promulgated in terms of chapter 8 governing stem cell research are the regulations relating to the use of human biological material, which deal with the withdrawal of human biological material, including stem cells, their subsequent use and research on such material. Other relevant regulations governing stem cells promulgated and published in terms of chapter 8 are the regulations relating to stem cell banks and those relating to the import and export of human tissue, blood, blood products, cultured cells, stem cells, embryos, foetal tissue, zygotes and gametes.