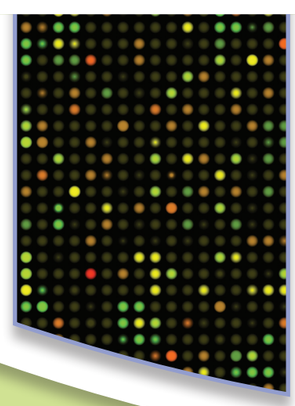

PUBLIC UNDERSTANDING OF BIOTECHNOLOGY

Biotechnology in Medical Research

What is biotechnology?

The term 'biotechnology' was first used in 1919 by the Hungarian engineer Karl Ereky, but mankind has been using biotechnology for many centuries, primarily to produce food. Biotechnology does not only involve the manipulation of DNA (or the hereditary material of an organism) by means of genetic engineering. Rather, the technology encompasses the use of living organisms (plants, animals, bacteria or viruses) or biological processes to make useful products. We utilise the biological process of fermentation that takes place in yeast to make bread, wine and beer. The earliest farmers have been using the principles of biotechnology to improve their crops or livestock by selecting plants or animals with desirable traits and using them for propagation (plants) or breeding (animals).

Similarly, biotechnology has been used in medical science for many hundreds of years, with mankind's discovery that diseases can be cured by using products derived from living organisms. The first known use of antibiotics dates back 2500 years, when the Ancient Chinese used mouldy curds made from soybeans to fight infection. One of the pioneers in the development of modern antibiotics is Louis Pasteur. In 1877, he discovered that he could prevent the growth of anthrax bacteria (*Bacillus anthracis*) by using a saprophytic *Bacillus* (a bacterium that feeds on dead matter). In 1928, Alexander Fleming discovered penicillin, which is an antibiotic that is produced by the fungus *Penicillium*. But it was not until the 1940s that penicillin was produced on a large scale.

Following on Oswald Avery's finding in 1944 that DNA (deoxyribonucleic acid) was the means by which bacteria pass on their hereditary material, came James Watson and Francis Crick's breakthrough. In 1953, they found that DNA has the structure of a double-helix, consisting of three types of chemical units. One of these classes of building blocks, called nitrogenous bases, is found in four types in DNA (namely Adenine, Thymine, Guanine and Cytosine); these chemicals form paired rungs across the helix (A pairs with T and G with C). The linear sequence of these building blocks is arranged in various combinations, unique to every living thing on earth. DNA is tightly coiled and packaged to form structures that are called chromosomes. The hereditary information is encoded in the DNA and each piece of DNA that codes for one protein is called a gene. The information that is carried by each gene is determined by the sequence of the nitrogenous bases.

The 'modern age' of biotechnology dawned in 1973, when Herb Boyer and Stanley Cohen developed a technique to introduce foreign DNA into an *Escherischia coli (E. coli)* bacterium, to create a transgenic (or 'genetically engineered') bacterium. This technology, also referred to as recombinant DNA technology, was used four years later to successfully introduce the human insulin gene into *E. coli*. The transgenic bacterium was then able to produce synthetic human insulin. Central to Boyer and Cohen's recombinant DNA technique was the discovery of restriction endonucleases, which allow precise cutting and joining of DNA from different sources. A finding for which Werner Aber, Daniel Nathans and Hamilton Smith received the 1978 Nobel Prize for Medicine.

Why use biotechnology?

Biotechnology offers another avenue through which disease-causing genes can be identified and thereafter therapies and treatments can be developed. Through the development and use of biotechnology, we have been able to determine the DNA sequence of the human genome (the total complement of genetic material) and also to identify the thousands of genes encoded within this DNA. The sequence of nitrogenous bases that make up the genes of an organism is responsible for the individual traits of that organism. Scientists have discovered that certain inherited human diseases are caused by faulty genes. For example, Huntington's disease (a degenerative disorder of nerve cells, resulting in loss of coordination, intellectual abilities and emotional disturbance) is caused by a single gene that in affected people produces a defective protein. This protein results in nerve cell death. Many South African scientists have played major roles within research groups searching for genes responsible for a variety of genetic diseases. For example, one of the genes responsible for the degeneration of the retina in Retinitis pigmentosa, a disease resulting in progressive loss of sight, and a gene involved in the faulty relay of electrical messages that control heart beats were identified by University of Cape Town and University of Stellenbosch groups, respectively. Knowledge of the gene responsible for causing a specific disease allows study of the biological mechanisms underlying the condition, which in turn may lead to the development of new drug therapies. Another reason for using biotechnology is that large biological molecules (for example synthetic insulin) cannot be synthesised in a laboratory using traditional techniques of chemistry. They can only be made by living cells in processes using biotechnological techniques. Other large molecules that are produced by means of biotechnology are human growth hormone, blood clotting factors for haemophiliacs, fertility drugs, and so on.

The role of biotechnology in diagnostics

Biotechnology plays a vital role in modern diagnostic science. During diagnostic DNA testing, scientists scan patients' DNA for errors, also known as mutations. These mutations can be large (for example a piece of chromosome missing, or added) or small (a difference in one of the nucleotide bases making up the double helix of DNA). Sometimes pieces of chromosomes can become 'switched', so that genes end up in the wrong positions on the chromosome.

- Through our current knowledge of DNA and genes, we can examine a person's DNA (usually taken from a blood sample) to do the following:

scientific discoveries, for example gene therapies, that are made at publicly financed institutions, such as universities.

Points to ponder

- There is still no cure for many of the diseases that can be diagnosed or predicted by means of biotechnology. Knowledge of carrying a gene for an adult-onset untreatable disease might place a psychological burden on an individual. Also, genetic tests cannot always accurately predict future disease. Parents of unborn children could face a difficult choice of ending a pregnancy, or not.
- The ownership of genetic information – who will own the information gleaned from a genetic test? Diseases that are found only among certain groups (for example Tay-Sach's disease that is common to people from Jewish East European descent) can lead to stigmatisation and discrimination. The United States Senate has recently unanimously passed legislation that will only allow patients and their doctors access to data from genetic testing, thereby banning genetic discrimination.
- Any error made when germline therapy is carried out will be passed on to future generations. Although this therapy is not being performed yet, many have raised concerns that this might change the basic nature of human beings by altering their genetic make-up.
- Pharmacogenomics will make the development and testing of new pharmaceuticals more effective because participants in clinical trials could be pre-screened to determine if the pharmaceutical being tested would be harmful or ineffective because of their particular genetic makeup. This could result in smaller, faster and therefore cheaper trials.
- Economics play a vital role in the development of new treatments. Three important diseases for which a vaccine would be of immense benefit, HIV, malaria and tuberculosis, exist mainly in poor countries. Biotechnology and pharmaceutical companies have little incentive to develop vaccines, since there is minimal financial return.
- Despite the Human Genome Project, we still only have a limited knowledge of the functions of genes. It is not known exactly whether genes have more than one function. So if genes are replaced during gene therapy, this might influence other body processes.
- Most genetic disorders involve more than one gene, as well as interaction with the environment. Diet, lifestyle and other environmental factors play an important role. For example, genetic tests can show whether a woman carries a mutation in the BRCA1 gene, which puts her at risk of developing breast cancer. But not everybody that carries this mutation develops breast cancer. Conversely, if genetic tests reveal that a person, for example, does not have a gene putting him at risk for cardiovascular disease, this might lead to "carelessness" and unhealthy life style choices.

Examples of treatments developed with the aid of biotechnology

• Cancer treatments

Several monoclonal antibody treatments against various forms of cancer are used today. A monoclonal antibody is a molecule, manufactured with the use of biotechnology, which attaches itself to the cancer cell; once attached to the cancer cell, it kills these rogue cells in various ways. **Rituximab** is an antibody that makes the cancer cells more visible to the immune system, so that the body'simmune system destroys them. It is used to treat non-Hodgkin's lymphoma.

Cetuximab blocks the growth signals of the cancer cells, so that they do not grow, and is used to treat colon cancer. In the case of **Ibritumomab**, a radioactive particle is combined with the monoclonal antibody so that radiation can be delivered directly to the cancer cells, without harming surrounding normal tissue. It is used to treat non-Hodgkin's lymphoma. **Gemtuzumab** is a monoclonal antibody combined with powerful chemotherapeuticals that only become active once they enter the cancer cell, limiting harm to surrounding normal tissue. It is used to treat acute myelogenous leukaemia. **Herceptin** is used to treat breast cancer in women whose cancer cells express the protein HER2. The herceptin specifically binds to those cells and stop them from proliferating.

• Blood clotting factors for haemophiliacs

Haemophilia is a hereditary genetic disease affecting the body's ability to control blood coagulation. The disease only manifests in males, although females are carriers. In the most common form of haemophilia, blood clotting factor VIII is absent. To treat the disorder, haemophiliacs must get regular infusions of the missing clotting factor. The replacement factor can be isolated from 'normal' blood serum, or can be manufactured through biotechnology. However, sometimes a patient can develop antibodies (inhibitors) against the replacement clotting factor, rendering the replacement ineffective. A recombinant human factor VIIa has been manufactured to successfully treat uncontrollable bleeding in patients with circulating inhibitors.

• Bone marrow transplantation

Bone marrow transplants have been performed since the 1950s to treat patients suffering from disorders of the blood, for example leukaemia. Before a transplant, a patient first receives chemotherapy to destroy cancerous cells. Subsequently the patient receives a transplant from their own healthy bone marrow, or from another person with a matching genetic make-up. To find an exact match, blood samples from possible donors are analysed to determine their human leukocyte antigens (HLAs). If the donor and recipient do not have matching HLAs, graft-versus-host disease will follow, where the donated bone marrow cells attack the recipient's tissue.

• Xenotransplantation

Currently more than 3 500 South Africans are waiting to receive organ transplants and 35 000 are waiting for tissue transplants. Only 1 000 of them can be helped due to the chronic shortage of donors. Albeit still only on a relatively small scale, biotechnology solves this problem by means of xenotransplantation (where the donor organs come from other species, for example pigs). Approximately 60 000 heart valves transplants, using heart valves from pigs, are performed in the USA annually.

• Preventing rejection after an organ transplant

To prevent donor organs being rejected by the recipient's body, doctors used to use powerful immunosuppressant medication. This prevented the organ from being rejected, but also weakened patients' immune systems, increasing their vulnerability to various infections. Cyclosporine, a natural product derived through biotechnology from a fungus that grows in soil, only suppresses the part of the immune system that involves rejection, with a less severe impact on the rest of the immune system.

This fact sheet has been reviewed by independent experts and has followed SAASTA's Scientific Editorial Process.
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NRF National Research Foundation | SAASTA South African Agency for Science and Technology Advancement

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**The PUB programme is an initiative of the Department of Science and Technology and is implemented by SAASTA.
The mandate of PUB is to promote a clear, balanced understanding of the potential of biotechnology and to ensure broad public awareness, dialogue and debate about biotechnology and its current and potential applications.
For more information visit www.pub.ac.za or contact info@pub.ac.za, Tel: 012 392 9300 or Fax: 012 320 7803**

- Determine the sex of an unborn baby;
- Carrier screening: the identification of unaffected individuals who carry one copy of a faulty gene for a disease that requires two copies of the faulty gene to manifest, for example haemophilia, or Tay-Sachs disease (disorder of the body's ability to metabolise a specific fatty substance);
- Prenatal diagnostic screening: for example screening for Down syndrome;
- Newborn screening: phenylketonuria (an inborn metabolic disorder, leading to abnormally high levels of the amino acid phenylalanine, which if left untreated can cause severe mental retardation);
- Presymptomatic testing for predicting adult-onset disorders: for example familial high blood cholesterol;
- Presymptomatic testing for estimating the risk of developing adult-onset cancers (for example colon cancer and bladder cancer). Persons with a mutation in the BRCA1 gene have a 65% cumulative chance of developing breast cancer in their life time;
- Confirmational diagnosis of symptomatic individuals: for example cystic fibrosis and Huntington's disease;
- Forensic/identity testing: for example analysis of semen for prosecution of sexual offenders, analysis of blood, bone and hair of murder victims as well as paternity testing.

The role of biotechnology in therapeutics

Biotechnology contributes to the development of treatments against disease in two ways: gene therapy and pharmacogenomics.

Gene therapy

Although gene therapy is still in its infancy, there have been cases where patients were treated with this technique. Essentially, gene therapy involves the treatment of disease by changing the genetic message or instructions of body cells.

Gene therapy can follow one of three approaches: the replacement of a faulty gene with a normal gene; the inactivation or 'knocking out' of the faulty gene; or introducing a completely new gene into the body to fight the disease. At the moment, only somatic gene therapy is performed. This is gene therapy involving the body cells of patients. Any change in genetic instructions remains within this individual and will not be passed on to his or her children. Germline therapy is gene therapy that involves the reproductive cells (sperm in men, and ova in women), in which changes in genetic instructions will be passed on to the patients' children. However, at this stage, this type of gene therapy is still experimental and it is not being performed on any patients (see the section on 'Points to ponder'). Scientists use a carrier, or vector, to transport a gene into the cells of a patient. Viruses are often used as vectors, but only after their disease-causing genes have been removed so that they cannot cause disease. The vector virus carrying the new gene can be introduced into the patient in a number of ways, for example, intra-muscular injection, intravenously or by nasal aerosol spray. The vector then 'infects' the target cells and delivers the new gene into the cell.

The first case of gene therapy being used was reported in 1990, when doctors at the National Institutes of Health (USA) treated a toddler girl suffering from severe combined immune deficiency (SCID). The movie "*The boy in the bubble*" tells the story of David Vetter who suffered from the same disease. SCID is caused by an abnormal ADA gene, which is the gene that regulates the production of an enzyme, adenosine deaminase. This enzyme is vital to the normal functioning of the immune system. The doctors removed bone marrow cells from the little girl, treated them with a vector carrying a normal ADA gene, and then returned the treated bone marrow cells to the young patient. After the treatment, her immune system started to function normally.

Because a faulty gene is also the cause of cystic fibrosis, the approach of replacing the faulty gene with a normal, healthy one could also be used to treat this disease. Although clinical trials have been conducted using gene therapy to combat cystic fibrosis, as this stage there still appear to be many barriers to overcome to achieve efficacy.

Scientists are also investigating how gene therapy can be used against cancer. Various possibilities under study range from replacing missing or altered genes that can cause cancer, to introducing new genes into cancer cells, making them more vulnerable to treatment.

Pharmacogenomics

Pharmacogenomics is the study of how a person's body reacts to pharmaceuticals, given that person's specific genetic make-up. Widespread application of pharmacogenomics is not done at present, but medical scientists believe that it has great potential to improve current therapies. By knowing an individual's genetic profile, a doctor would be able to prescribe the correct medication, at the correct dosage. The risk of adverse reactions, side effects and overdosage would therefore be minimal. The basis of pharmacogenomics is the identification of SNPs (pronounced 'snips', derived from the abbreviation for 'single-nucleotide polymorphisms'). SNPs are differences between individual humans of a single nucleotide base in a particular position in their DNA. In the past, the sequencing of a person's DNA was a lengthy and expensive procedure, but with the development of the DNA microarray (or DNA chip, as it is also called) the sequencing can be done quickly. SNPs can be used to map and identify specific genes that play a role in diseases such as diabetes, cancer and arthritis. The proteins that these genes encode can become targets for new therapies.

As such, pharmacogenomics can play an important role in many conditions, for example, oncology, treatment of high blood cholesterol levels, tailoring treatment for people with psychiatric disorders and treatment for people with cardiovascular diseases. However, the use of pharmacogenomics to 'individualise' treatment is still in its infancy. Currently, pharmacogenetic testing, that is looking at variation in one gene, to determine an individual's possible reaction to treatment is used to a limited extent.

The role of biotechnology in developing vaccines

The word 'vaccine' was derived from the Latin for 'cow' (vacca) – referring to Edward Jenner's discovery in 1796 that milkmaids, who were in frequent contact with cowpox, were immune to the dreaded smallpox.

A vaccine is a harmless biological preparation that is given to humans to make them immune to a specific disease. The human body's immune system recognises the vaccine as being 'foreign', destroys it, but also 'remembers' what this foreign matter looked like. When the body then actually encounters the 'real' disease (or virulent form), the immune system recognises it and will be ready to fight off the infection. Scientists may take one of several routes to develop a vaccine, depending on how the disease-causing microbe infects body cells, how the body's immune system reacts, the physical characteristics of the microbe and also where the vaccine is going to be used. The various approaches are the following:

• Live, attenuated vaccines

These vaccines contain a version of the disease-causing microbe that has been weakened (attenuated), so that it cannot cause disease but only prompt the immune system to remember it. Live, attenuated vaccines cause a very strong immune reaction, so that only one or two doses generally give lifelong immunity. It is mostly used against viral diseases, such as measles, mumps and chickenpox. It is not safe to use a live, attenuated vaccine on a person with a weakened immune system (HIV-positive individuals or patients receiving chemotherapy). These vaccines also need to be refrigerated to stay potent, limiting their use in some developing countries. There is also the remote possibility that the weakened microbe might mutate back to its virulent form and cause disease.

• Inactivated vaccines

In this case, the disease-causing microbe is killed with chemicals, heat or radiation, and not just weakened. The microbe can therefore not mutate back to its virulent form. However, this type of vaccine does not produce such a strong immune reaction, so additional immunisation ('booster shots') are necessary. Inactivated vaccines are freeze-dried, so they can be stored easily – making them better for use in developing countries. Examples of inactivated vaccines are those against cholera, bubonic plague and hepatitis A.

• Subunit vaccines

These vaccines do not include the entire disease-causing microbe, but only the antigens that stimulate the immune system the most. Antigens are 'markers' on the surface of a microbe, and this is the part that is recognised by the immune system's T-cells, and to which the T-cells bind. T cells are a group of white blood cells that play an important role in the body's immune defence system. Scientists can make the antigens from the microbes in the laboratory using recombinant DNA technology. In this case the vaccine is called a recombinant subunit vaccine, such as the vaccine against the hepatitis B virus.

• Toxoid vaccines

These are vaccines that are used against bacteria that secrete toxins, for example diphtheria and tetanus. A toxoid vaccine is made by treating the toxin with formalin, rendering the toxin harmless. The vaccine causes the immune system to produce antibodies against the toxin, which bind to the toxin and block its action.

• Conjugate vaccines

Many harmful bacteria have an outer coating of sugar molecules known as polysaccharides. This coating hides the antigens (markers) on the surface of the bacteria so that the immature immune system of a child or baby cannot recognise it. Scientists link antigens or toxoids, from a microbe that an immature immune system can recognise, to the polysaccharides, thereby making a conjugate vaccine. The vaccine against *Haemophilus influenzae* type B (Hib) is a conjugate vaccine.

• DNA vaccines

These vaccines are still in the experimental phase, but several types are already being tested in humans. A DNA vaccine only uses the genes of the microbe that code for the antigens of that microbe. When those genes enter the body, they are taken up by body cells, and then instruct the body cells to produce antigens. The antigens then stimulate the immune response. DNA vaccines are easy to produce and store. Vaccines against herpes and influenza are currently being tested.

• Recombinant vector vaccines

Recombinant vector vaccines are similar to DNA vaccines, but they use an attenuated virus or bacterium as a vector to carry the DNA of the disease-causing microbe into the body. The vector virus then 'infects' body cells, thereby delivering the DNA to the body cells. Researchers are working on viral-based and bacterium-based recombinant vector vaccines against HIV, rabies and measles. A vaccine can be monovalent (immunises against one disease), or multivalent (immunises against more than one disease, or two or more strains of the same disease-causing microbe).

Biotechnology and the Omics Era

Biotechnologists find themselves in the Omics era. Omics refers to a total understanding, so Genomics and Proteomics refer to understanding the whole genome and the proteome (i.e. how all the proteins in a cell interact with one another) in a specific cell. This has developed from the approach that one single gene does not act in isolation and that mutation of a gene results in a mutated protein that in turn affects an entire cell, hence causing disease. Understanding howow this single mutant gene/protein causes a cascading effect through the entire proteome is the eventual goal; allowing biomedical biotechnologists to understand disease better for the development of targeted therapeutics.

Regulation and legislation of biotechnology

The regulatory process ruling the use and development of biotechnology started soon after Boyer and Cohen's discovery of the recombinant DNA technique. In mid-1974, scientists called for a voluntary moratorium on certain experiments with recombinant DNA.

This was followed by the Asilomar Conference, during which scientists from all over the world, lawyers and government officials debated the way forward. The conference concluded that research on recombinant DNA should be ruled by strict guidelines, which were then issued by the National Institutes of Health. According to these guidelines, all experiments and trials concerning human gene transfer ('gene therapy trials') making use of recombinant DNA technology must be reviewed by the Recombinant DNA Advisory Committee of the NIH. Researchers who receive funding from the NIH for their work (many South African scientists receive funding from this source), or who conduct their research at facilities receiving funding from the NIH, are bound by these guidelines. However, researchers who receive private funding, and who conduct their research at privately funded institutions, are not bound by the guidelines.

On the local front, the Medical Research Council (www.mrc.ac.za) has an Ethics Committee that is registered with the Office for Human Research Protection in the USA. The mandate of the MRC's Ethics Committee is to review all applications for funding of medical research to ensure that the goals of the project do not violate the sanctity of life and obey all rules. The Committee has developed a set of research guidelines on various forms of research, including human genetic research. In addition, every tertiary institution where such research is conducted also has its own ethics committee that oversees research experiments. South Africa has no specific law that governs the biotechnology industry or research field, but the country has legislation that governs different aspects of the subject. The South African Department of Science and Technology published a National Biotechnology Strategy in 2001, which outlines the government's plans to build the biotechnology industry in South Africa.

Legislation mainly covers safety and ethical issues; as well as intellectual property rights. Safety and ethical issues are dealt with in the National Health Act No 61 of 2003 (Department of Health), which contains a chapter on the use of blood, blood products, tissue and human reproductive cells (sperm and ova) during medical research. The Act also addresses the issue of human cloning. Intellectual property rights are covered from two fronts. The Companies and Intellectual Properties Registration Office (CIPRO), which forms part of the South African Department of Trade and Industry has laws that are applicable to biotechnology (for example Section 25 of the Patent Act, Act 57 of 1978 applies to scientific discoveries). The Department of Science and Technology's Intellectual Property Rights from Publicly Financed Research and Development Bill deals with

Next Generation DNA Sequencing in Biotechnology

In the 1997 science fiction film GATTACA, a future was proposed where one could have your entire genome sequenced in a very short period of time. This is now becoming science fact, as the human genome project drove biotechnological advancement, pulling in bioscientists, chemists, engineers and computer scientists to build instruments that would sequence DNA faster and more efficiently. Current instruments are capable of sequencing entire human genomes in a matter of weeks and the drive is on to introduce disruptive technologies that would deliver "a genome in a day". Combined with rapidly advancing computing power, Bioinformaticians (computer scientists who analyse biological information) are pushing our understanding of the human genome to its limits.

Stem Cell Biotechnology and Regenerative Medicine

The promise of stem cell biotechnology is great. Stem cells are at the ground state of mammalian development. Our understanding of stem cell biology will allow for the development of our ability to manipulate the cells in vitro (i.e. outside the body). Two major groupings of stem cells have been identified to date i.e. pluripotent stem cells and adult stem cells. Pluripotent stem cells include the controversial embryonic stem cells, which possess the ability to form any tissue type in the body.Single embryonic stem cells cannot form whole organisms. Pluripotent simply translates from Latin meaning "very many, power". That power is harnessed in stem cell laboratories worldwide to understand how we can make specific heart cells, for example, in a cell culture dish. On the other end of the scale adult stem cells lack the potency of pluripotent stem cells but can still form defined cell types within a specific bodily tissue. Classic examples of this are the haematopoietic stem cells that are found in bone marrow; these cells can drive the formation of the entire blood and immune cell system and are the key to bone marrow transplants. Adult stem cells are termed multipotent, oligopotent or unipotent depending on the level of potency, or more simply how many cell types can be formed from one single adult stem cell. Naturally, understanding how proteins and genes communicate within the stem cell to either maintain a stem cell state (termed stemness) or change (differentiate) has allowed for a veritable revolution in stem cell biology. Prof Shinya Yamanaka and coworkers at Kyoto University described a simple, elegant method of turning normal skin cells into pluripotent stem cells, effectively rewinding the cell to a stem cell. These cells are called induced pluripotent stem cells (iPS) and represent a driving force in both drug discovery and regenerative medicine. Currently iPS cells are being explored in drug discovery as a means of making any cell type to test new therapeutic drugs for toxicity. Since iPS cells can be made from patients with hereditary diseases, we can now make disease specific iPS cells to further understand how a disease progresses and to test new therapeutics against these disease model cells. Regenerative medicine is the next step, where stem cells will be used to replace damaged tissues and organs in the human body. Tissue and organ engineering is still in its infancy due to the complexity of human organs.

South Africa has a small but growing network of stem cell biologists working towards furthering our understanding of pluripotent and adult stem cell biology. Recently, researchers at the Council for Scientific and Industrial Research in Pretoria working with collaborators Prof Sue Kidson at the University of Cape Town announced the first iPS cells generated on African soil, bringing South Africa into the cutting edge of stem cell biotechnology.