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.

Locally, research institutions such as 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 according to Prof. Tony Bunn and Dr. Michelle Mulder of the MRC's Innovation Centre, the country has legislation that governs different aspects of the subject. The 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 (CIPLO), 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 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. Knowing that you carry a gene for a future 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 about 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 cheaper, because participants to clinical trials could be pre-screened to show if the pharmaceutical being tested would be harmful or ineffective to them. This will make the trials smaller, faster and therefore cheaper.
- Economics play a vital role in the development of new treatments. Three important diseases against 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. This puts her at risk for 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.





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, that attaches itself to the cancer cell. Once it's attached to the cancer cell, it kills the cancerous cells in various ways. Rituximab is an antibody that makes the cancer cell more visible to the immune system, so that the body's immune 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 the carriers. In the most common form of haemo-philia, 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 from the blood, for example leukaemia. Before a transplant, a patient first receives chemotherapy to destroy cancerous cells. Subsequently the patient receives a transplant of 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 or 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.

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.

PROMOTING A CLEAR, BALANCED UNDERSTANDING OF BIOTECHNOLOGY

PUBLIC UNDERSTANDING OF BIOTECHNOLOGY

BIOTECHNOLOGY AND 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 the medical science for many hundreds of years, with mankind's discovery that they could cure diseases by using products derived from living organisms. The first known use of antibiotics dates back to 2500 years ago, 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 passed on their hereditary material, came James Watson and Francis Crick's breakthrough. In 1953 they found that DNA had the structure of a double-helix and consisted of four types of building blocks.

These building blocks are arranged in various combinations, unique to every living thing on earth.

DNA is tightly coiled to form structures that are called chromosomes. The instructions encoded in the DNA, also referred to as 'genes', carry the hereditary information. Each piece of DNA that codes for one protein, is a gene. The information that is carried by each gene is determined by the sequence of the building blocks.

The 'modern age' of biotechnology dawned in 1973, when Herb Boyer and Stanley Cohen developed a technique to introduce DNA into an *Escherichia coli* bacterium, to create a trans-

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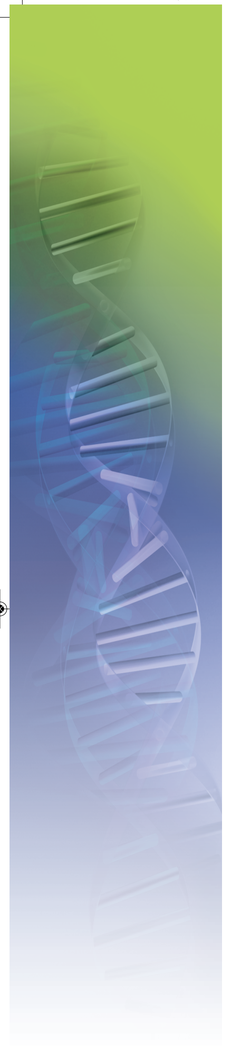

genic (or 'genetically engineered') bacterium. This technology, also referred to as recombinant DNA technology, was used four years later to successfully introduce the human insulin gene into *E. coli*. The transgenic bacterium was then able to produce synthetic human insulin.

Central to Boyer and Cohen's recombinant DNA technique was the discovery of restriction endonucleases by Werner Aber, Daniel Nathans and Hamilton Smith. They received the 1978 Nobel Prize for Medicine for their work.

Why use biotechnology?

Biotechnology offers another avenue through which therapies and treatments can be developed. Through the development and use of biotechnology techniques, we have been able to determine the structure of DNA and also the coded message in the gene that is contained within the DNA. The sequence of genes on an organism's DNA is responsible for the individual traits of that organism.

Scientists have discovered that certain diseases of the human body 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 produces a defective protein. This protein results in nerve cell death.

Retinitis pigmentosa is a disease of the human eye, resulting in progressive loss of sight. South African scientists, Dr Soraya Bardien and her colleagues at the University of Cape Town, identified one of the genes responsible for the degeneration of the retina.

Since we now know which genes cause specific diseases, scientists can use them as targets to develop specific treatments. Because the target is known, the discovery process is easier (and cheaper). Also, these gene targets cannot be reached by means of conventional pharmaceuticals.

Another reason for using biotechnology, is that large biological molecules (for example synthetic insulin) cannot be made 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. 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:

- Determine the sex of an unborn baby
- Carrier screening: the identification of unaffected individuals who carry one copy of a gene for a disease that requires two copies of a gene to manifest, for example haemophilia, or Tay-Sachs disease (disorder of the body's ability to metabolise fats)
- Prenatal diagnostic screening: for example screening for Down syndrome
- Newborn screening: testing newborn babies for HIV, 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 (GeneCare, a Cape Town based company designed a comprehensive genetic test for cardiovascular disease, incorporating other risk factors such as lipid metabolism, blood clotting, folate/homocystein metabolism, and iron metabolism
- 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 has a 65% cumulative chance of developing breast cancer
- 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, paternity testing

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

'building blocks' or chemical 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.

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 have been 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). Changes in genetic instructions will be passed on to the patient's children. However, at this stage, this type of gene therapy is still experimental and it is not being performed on any patients (see the sections 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 their disease-causing genes are first removed so that they cannot cause disease. The vector virus carrying the new gene can be injected into the patient, or given intravenously. 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 little girl. 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. A phase I clinical trial is currently being conducted on gene therapy against cystic fibrosis. Because scientists were not convinced of the efficacy of the therapy, it is now being reformulated before further testing takes place.

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

Pharmacogenomics

The term pharmacogenomics (or pharmacogenetics, the two terms can be used interchangeably) is derived from pharmacology (study of pharmaceuticals) and genetics, so it is the study of how a person's body reacts to pharmaceuticals, given that person's specific genetic make-up.

Widespread application of pharmacogenetics 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 as 'snips', derived from the abbreviation for 'single-nucleotide polymorphisms'). SNPs are differences between individual human beings of a single base pair 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 for can become targets for new therapies.

As such, pharmacogenomics can play an important role in oncology, treatment of high blood cholesterol levels, tailoring treatment for people with psychiatric disorders, treatment for people with cardiovascular diseases.

However, the use of pharmacogenomics to 'individualise' treatment is still in its infancy. Pharmacogenetic testing to determine an individual's possible reaction against treatment is currently most advanced in Scandinavian countries. Here it is mainly used for dose selection to treat psychiatric illness.

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 against the dreaded smallpox.

A vaccine is a harmless biological preparation that is given to humans to make them immune against 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, 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. 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 (immunizes against one disease), or multivalent (immunizes against more than one disease, or two or more strains of the same disease-causing microbe).

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.