SMGroup

Medical Biotechnology

Jalaluddin M Ashraf*

School of Biotechnology, Yeungnam University, Republic of South Korea.

***Corresponding author:** Jalaluddin M Ashraf, Department of Biotechnology, School of Biotechnology, Yeungnam University, Gyeongsan, Republic of South Korea, Email: jmashraf@gmail.com

Published Date: February 15, 2015

INTRODUCTION

Contrary to its name, biotechnology is not an only technology, but a group of technologies that share common characteristics; working with living cells and their molecules and having a wide range of practical applications to develop our lives [1]. Being the most exciting and revolutionary science of the century, It involves the use of living organisms and bioprocesses in and around engineering, technology, medicine and other fields [2,3]. In general, biotechnology has expanded its wing and covers the wide areas: health care (medical), crop production and agriculture, non food (industrial) uses of crops and other products (e.g. biodegradable plastics, vegetable oil, biofuels etc.), animal husbandry and bioremediation (environmental biotechnology) [4]. The most promising application of biotechnology is found to be in medical field by generations of bio-therapeutics (insulin, growth hormones, antibiotics etc.) and as diagnostics tools (PCR, FISH, micro-array technique).

As part of the historical aspects of biotechnology, it emerged long ago, but the term "biotechnology" was first coined in Karl Ereky book in 1919. Biotechnology is application of scientific and technical methods in the transformation of some substances through biological

agents (microorganisms, plant and animal cells and enzymes) to be used in agriculture, animal husbandry, food production and medicine industries [2,3]. Although industrial process for mass production of acetone by fermentation was reported in the same year by Dr. Chaim Wiezman at University of Manchester, definition is still consistent as coined by Erkey. Historical period of biotechnology starts emerging as human by his consciousness used biological processes to produce fermentation products such as bread, alcoholic beverages, dairy products, pickles, vinegar, etc. Sumerians and Babylonians used wine making six thousand years before Christ birth (6000BC). Four thousand years ago Egyptians baked bread using yeast and ferment [6,7]. Modern biotechnology allows achieving the technology required to fight rare and disabling diseases, reduce environmental impacts, to obtain a cleaner and safer energy use and more efficient manufacturing processes [history of biotechnology, http://www.bio.org]. According to time classification, three phases of technology development can be recognized [5]; first period of present century in which human by his realization used techniques for fermentation and cultivation of microorganisms. Subsequently, human used fermentors in the production of antibiotics, enzymes, nutrient components, organic chemicals and other materials that led to the development of this science. Finally, Modern biotechnology period of development in which genetic science is used to make changes in human life. This time period started since 1976 with the transfer of genes from one microorganism to another microorganism. Up until that time, scientists were using natural and inherent properties of microorganisms. Afterwards, scientists start modifying and alter the properties of microorganisms. They created micro-organisms with entirely new characteristics to produce new compounds with much higher levels of efficiency [8,9].

The most important beneficial legacy of the early 20th century biotechnology was the discovery of penicillin by Alexander Fleming in 1928, an antibiotic derived from the mold *penicillium*. Large scale production of penicillin was achieved in 1940s. Discovery of penicillin starts the age of making antibiotics. Currently, a variety of microorganisms have been used to generate thousand liters of antibiotic by using advanced biotechnology tools. The field of biotechnology took a jump with the discovery of the double-helix structure of the deoxyribose nucleic acid (DNA) by two famous scientists called Watson and Crick [9]. They shared Nobel Prize along with Wilkins for cracking one of the most important biological puzzles.

The 'modern age' of biotechnology dawned in 1973, when Herb Boyer and Stanley Cohen developed a technique to introduce DNA into an *Escherichia coli* to create a transgenic (or 'genetically engineered') bacterium [10]. Central to Boyer and Cohen's recombinant DNA technique led to the discovery of restriction endonucleases by Werner Aber, Daniel Nathans and Hamilton Smith. They received the 1978 Nobel Prize for Medicine for their work. Biotechnologists use restriction endonucleases enzymes to cut and remove DNA segments from one organism and recombine it with DNA in another organism. This is called recombinant DNA (rDNA) technology, and it is one of the basic tools of modern biotechnology [11]. rDNA is usually used synonymously with genetic engineering and allows researchers to move genetic information between

unrelated organisms to produce desired products or characteristics or to eliminate undesirable characteristics [4].

Over the past few years rDNA technology has been broadly utilized to generate therapeutic products (insulin, growth hormones and monoclonal antibody) for treating human diseases. With a fast increase in the number of patient's worldwide, there has been a tremendous scope to identify and create medicine for various human diseases.

With the development of biotechnology, the modern biotechnologists have broadly divided it into following branches:

1. **Bioinformatics** is an interdisciplinary field which addresses biological problems using computational techniques, and makes the rapid organization of analysis of biological data possible. Bioinformatics plays a key role in various areas, such as functional genomics, structural genomics, and proteomics and forms a key component in the biotechnology and pharmaceutical sector.

2. **Blue biotechnology** is a term that has been used to describe the marine and aquatic applications of biotechnology.

3. **Green biotechnology** is applied to agricultural processes, for example domestication of plants via micropropagation, designing of transgenic plants etc.

4. **White biotechnology**, also known as industrial biotechnology is biotechnology applied to industrial processes.

5. **Red biotechnology** is applied to medical processes. Some examples are the designing of organisms to produce antibiotics, and the engineering of genetic cures through genetic manipulation [12,13].

BIOTECHNOLOGY IN MEDICINE

Medical biotechnology, red biotechnology, is the application of biological techniques in research and development in healthcare and medicine. Biotechnology is facilitating the development of new medicines, producing them faster, cheaper, safe and more efficient [14-17]. One of the outstanding characteristics of medical biotechnology is the contribution of arrays of products, which include treatment of bacterial infections, diabetes, immune disorders, cancer, and degenerative conditions such as heart infarction and neurodegenerative diseases (i.e., Parkinson's disease, Alzheimer's disease, or stroke diseases).

In future, conventional medicine will be gradually replaced by molecular medicine. By year 2020, drugs based on pharmogenomics will be routine part of common diseases such as diabetes and high blood pressure and by year 2040, individualized medicines will be produced [18]. No disease will remain unknown and no disease mechanism will be uncontrollable in near future. We have briefly discussed below some of the major biotechnology-based products.

Gene Therapy

Gene therapy is a collection of methods that allows correction of a gene defect in hereditary disease that has been diagnosed in an individual/embryo. Gene therapy can follow one of three approaches: the replacement of a defective gene with a normal gene; the inactivation or 'knocking out' of the defective gene; or introducing a completely new gene into the body to fight the disease, so that the human body can make the correct protein and consequently eliminate the root cause of the disease [19].

A genetic disease is the consequence of structural or functional alteration in the DNA in which the DNA sequence is changed is called a gene mutation. Many genes that cause the development of diseases have been identified so far including cancer, cardiovascular, respiratory, mental disease that by mutations in multiple genes. Furthermore, diseases can be genetically inherited from parents to children, known as Mendelian disorders, and can also result from structural or functional changes in DNA in the body cells [20]. It has been reported that approximately one out of every 10 people has a manifestation of genetic disorder. Disease including cystic fibrosis, Duchenne muscular dystrophy, Huntington's disease of nervous system, thalassemia, hemophilia, sickle cell anemia, Lesh Nayhan syndrome, phenylketonuria, etc. which are caused by single gene mutation. These diseases are candidates for gene therapy [21,22].

Gene therapy may be classified into (i) germ line gene therapy and (ii) somatic cell gene therapy. i) In germ line gene therapy, germ cells, i.e., sperms or eggs are modified by introduction of functional gene, which are ordinarily integrated into their genomes. Therefore, change due to therapy would be heritable. Ii) In somatic cell gene therapy the gene is introduced only in somatic cells, especially of those tissues in which expression of the concerned gene is critical for health. Somatic gene therapy does not affect any descendants of the person being treated.

There are basically two ways of implementing a gene therapy treatment:

(1) *Ex vivo,* this means "outside the body"- Afflicted cells from the patients are removed and grown in the laboratory. They are then exposed to a virus carrying the desired gene. The virus enters the cells, and the desired gene becomes part of the DNA of the cells. The cells are allowed to grow in the laboratory before being returned to the patient by injection into a vein.

(2) *In vivo,* which means "inside the body"- No cells are removed from the patient's body. Instead, desired genes with vectors are delivered to cells in the patient's body to replace the defective or missing genes with healthy genes.

However, gene therapy is still under developmental stages and various clinical trials are on going around the world. Its success will be based on the successful cure of genetic diseases by gene therapy.

Monoclonal Antibodies

Another landmark attained in the beginning of the 20th century was the making of monoclonal antibodies, which was proposed by Paul Ehrlich. He proposed that drug compounds can be accurately, precisely, and specifically delivered along with monoclonal antibodies. Monoclonal Antibodies are the antibodies that are identical because they are produced by one type of immune cells [23].

In 1984, Kohler and Milstein were honoured with the Nobel Prize in recognition of the importance of their contribution to the development of means for the production of monoclonal antibodies. Their breakthrough occurred in 1975, when they fused normal antibody-producing B lymphocytes with myeloma tumor cells; resulting in clones of cells they termed hybridomas [24]. The production of monoclonal antibodies involves human and mouse hybrid cells and this technology is known as hybridoma technology [25].

Monoclonal antibodies are playing a valuable role in biomedical research, microbiological research, in diagnosis of Hepatitis, AIDs, influenza, herpes simplex, and in treatment of such diseases as infections and cancer [26,27]. They are used to determine the concentration of hormone and specific proteins in the blood or urine. For example, monoclonal antibodies against a hormone can detect pregnancy only 10 days after conception, and an unusually high blood level of a prostate-specific antigen, which is measured by its interaction with a monoclonal antibody, provides an early warning that a man may have developed prostate cancer.

Moreover, they can be coupled with radioisotopes to generate *in vivo* diagnostic imaging. Currently a large number of therapeutic antibodies have been introduced into human medicine e.g. to suppress immune system, against inflammatory diseases, to kill or inhibit malignant cells etc [28,29].

Vaccines

Vaccine technology is around thousand years old when Chinese used to collect the scabs from smallpox patients; crushed them into powder, which they either inhaled or rubbed into pricked skin. Modern vaccination dates back to 1798, when Edward Jenner used non-lethal cowpox virus to induce immunity against structurally similar but deadly variola virus that causes smallpox in humans [30].

A vaccine is a innocuous 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 actually encounters the 'real' disease (or virulent form), the immune system recognises it and will be ready to fight off the infection [31]. Advance in novel technologies such as bioinformatics, microarrays, proteomics and recombinant DNA technology have revolutionized the development of new vaccines [32,33]. 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 etc [30]. Available vaccines include: Live, attenuated vaccines; contain a version of the disease-causing microbe that has been attenuated (weakened). It is mostly used against viral diseases, such as measles, mumps and chickenpox. Inactivated vaccines; contain chemicals, heat or radiation killed disease-causing microbes. Examples of inactivated vaccines are those against cholera, bubonic plague and hepatitis A. Subunit vaccines or recombinant subunit vaccine; do not include the entire disease-causing microbe, but only the part of microbes (antigens), such as the vaccine against the hepatitis B virus. Toxoid vaccines; are used against toxins secreting bacteria, for example, diphtheria and tetanus. A toxoid vaccine is made by treating the toxin with formalin, rendering the toxin harmless. Conjugate vaccines: are used against the bacteria having an outer coating of polysaccharides. The vaccine against Haemophilus influenzae type B (Hib) is a conjugate vaccine. DNA vaccines; only use the genes of the microbe that code for the antigens of that microbe. Vaccines against herpes and influenza are currently being tested. 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. Researchers are working on viral-based and bacterium-based against HIV, rabies and measles [34].

Recombinant Insulin

Recombinant DNA technology has completely revolutionized disease treatments. Diabetes mellitus (DM) is a metabolic disorder resulting from a defect in insulin secretion, insulin action, or both. Insulin deficiency in turn leads to chronic hyperglycaemia with disturbances of carbohydrate, fat and protein metabolism [35-38]. Insulins are the mainstay of treatment in type I diabetes and also in later stages of type II diabetes. Left uncontrolled diabetes can lead to severe diabetic complications [39-48]. Therefore, compliance with the insulin therapy is important in preventing the adverse clinical effects of the disease. In 1922, Banting and Best discovered the hormone, insulin, which is produced by pancreas. One of the best treatments of diabetes mellitus is insulin injections that regulate the blood-sugar level. Previously, insulin derived from the pancreas glands of slaughtered cattle and pigs were used in treatment of diabetes [49,50]. However, insulin obtained from animal pancreas, causes allergy or other types of reactions in some patients such as insulin allergy, insulin resistance and insulin lipodisatrophy [51].

After more than half a century of treating diabetics with animal insulins, recombinant DNA technologies and advanced in medical research has made a great effort to reproduce the physiological insulin. As the next step, over the last decade, insulin analogs were constructed by changing the structure of the native protein with the goal of improving the therapeutic properties of it [17]. Currently, two formulations of human insulin, synthesized by recombinant DNA technique, are into the market, namely Regular Human Insulin (RHI) and the intermediate-acting Neutral Protamine Hagedorn (NPH). RHI's slow onset and relatively long duration of action represent important limitations to its use and NPH insulin shows limited duration of action [52,53].

In addition there are Insulin analogs, i.e. modified recombinant human insulins, were developed to overcome limitations of RHI and NPH [54,55], such as rapid acting analogs (RAAs); are characterized by faster absorption, and shorter duration of action compared to RHI, Short-Acting Analogues; have rapid onset and shorter duration of action [56,57]. Long-Acting Insulin Analogs; the shorter duration of action of RAAs with respect to RHI has unveiled the need of long-acting insulin preparations. It is used especially in insulin-deficient individuals [58], Premixed Insulin Analogs; consisting of a fixed combination of NPH and RHI or RAA, in different ratios. Premixed insulin analogs may represent a more convenient insulin regimen, especially for patients who need a simplified approach [59,60], Novel Insulins; search of novel insulins is ongoing. There are faster insulins with a more rapid onset of action, potentially even faster than the currently available RAAs, and others, which are aimed to ameliorate current LAAs' properties, [61]. In recent years, an attempt has been made to develop insulin which can be safely given through the oral route or sublingually.

Human Genome Project

The human genome project (HGP) was initiated as a multinational collaborative research project to map and sequence human genome [62]. It has been reported that the $3-billion project was founded in 1990 by the United States Department of Energy and the US National Institutes of Health. Later, other countries also took part in the HGP, including the United Kingdom, France, Germany, Japan, China, and India.The primary aim of the human genome project was to study the genetic and health effects of radiation and chemical products of energy production and it was determined that the best way was to study the DNA directly. Human genome is now estimated to contain around 30-40 K protein coding genes in which only about 2% of the human genome code for proteins and at least 50% of the genomes does not code for proteins (the so called "junk DNA").

Although the genetic basis of diseases has been known for millennia, genetic diseases were perceived as rare diseases for the most of the 20th century, and the field of genetics was studied as a speciality different from medicine. There are reports that suggest more than 3000 genetic disorders known to be caused by genetic mutations. The number of diseases known to have a genetic component has increased significantly in recent years, and includes the neurodegenerative diseases - Alzheimer's disease, multiple sclerosis and Parkinson's disease [63], asthma [64], cystic fibrosis, diabetes [65], a variety of cancers [66,67], hypertension [68] and obesity etc [69]. In the same way, genetic variations have been linked with differing susceptibilities to many other diseases, including the infectious diseases, [70,71] and HIV/AIDS [72].

The impact of that human genome project has now and will be, that it will help the scientists in better understanding of the molecular mechanisms of diseases; markers for disease can be discovered; will help in understanding the biology of genome organization and gene regulation; will be useful in developing new technologies and finally HGP can increase the public awareness of development and application of biotechnology [73].

RNA Interference

The discovery of RNA interference (RNAi) in the late 1990s heralded a new era for loss-of-function experiments. RNAi, as commonly defined, is a phenomenon leading to post-transcriptional gene silencing after endogenous production or artificial introduction into a cell of small interfering double strand RNA with sequences complementary to the targeted gene [74]. Whereas the transcription of the gene is normal, the translation of the protein is prevented by selective degradation of its encoded mRNA. RNAi is operative in cells of organisms ranging from plants, to nematodes and flies, and to mammals attests to its fundamental importance in the selective suppression of protein translation by targeted degradation of the encoding mRNA.

RNAi technology has been in use in various fields of biotechnology, particularly in the food plant engineering that produces lower levels of natural toxins. No plant that uses RNAi technology has yet passed the experimental stage; however, research work has been known to effectively reduce the allergen levels in tomato plants and also is known to cut the cancer-causing agents in tobacco plants. Also, other plant characters that have also been bioengineered include the production of nonnarcotic natural products by using the opium plant, development of resistance to common plant viruses, and protection of plants with dietary antioxidants. Therefore, based upon these and other findings initially made in studies of plants, it seems very likely that RNAi evolved as a mechanism to defend plant cells against viral infections [75].

RNAi technology in the field of biomedical science holds great promise for the development of therapeutics directed against silencing the specific gene involved in the development of diseases. Recent studies have demonstrated the clinical potential of RNAi in the treatment of viral infections, cancers, renal disorders, and neurodegenerative diseases etc [76]. Cancer, for example, is frequently caused by over-excited genes in the cells, and retarding their activities could stop the disease progression. In addition, viral infections can also be treated using RNAi-based therapies and this can be done by reducing the activity of key viral genes. It has been shown in the laboratory that human cells have successfully stopped the growth of HIV, polio, hepatitis C, and other viruses in human cell culture and RNAi-based therapies against HIV are under clinical trial stages. Moreover, the importance of RNAi technology has some beneficial effects in finding out the cause of the disease.

Phage Therapy

The worldwide surfacing of 'superbugs' and a dry antibiotic pipeline threaten modern society with a return to the preantibiotic era [77]. Phage therapy is generally to treat pathogenic infections caused by microorganisms and bacteriophages. Phages – the viruses of bacteria – could help fight antibiotic-resistant bacteria. Phages enter bacterial cells and disturb bacterial metabolism causing the bacterium to lyse. Phage therapy was first attempted in 1919 by Felix d'Herelle and was commercially developed in the 1930s before being replaced by antibiotics in most of the western world. Now it has been reported that phage therapy is very effective in special clinical conditions

and is known to have some unique advantages over antibiotic treatments, for example, antibodies cannot be used as localized drugs but phases can be used as localized drugs as they can infiltrate deeper in the infected area and remove the infection from the source. Another interesting aspect of bacteriophage is that these phages stop reproducing once the specific bacteria they target are destroyed. It has been reported that phages do not develop secondary resistance, which happens quite often in antibiotic treatments.

Over the last few years, it has been reported that phages are being used to treat bacterial infections in those patients who do not respond to antibiotics. In addition, bacteriophages are found to be much more specific than antibiotics and do not cause any harmful effects on the host organism, but instead sustain a healthy relationship with advantageous bacteria in the body [31]. On the contrary, there are some disadvantages of phage therapy because a phage will only kill a bacterium if it is a match to the specific strain of the bacterium and it will not kill non-specific strain of bacterium. However, phage therapy has many potential applications that include medicine, veterinary science, dentistry, and agricultural science.

Bio-Nanotechnology

Bionanotechnology is unique fusion of biotechnology and nanotechnology. Nanotechnology is a novel scientific approach that involves materials and equipments capable of manipulating physical as well as chemical properties of a substance at molecular levels. On the other hand, biotechnology uses the knowledge and techniques of biology to manipulate molecular, genetic and cellular processes to develop products and services and is used in diverse fields from medicine to agriculture. Fascinatingly, new nanotools are often made by refining the applications of the nanotools that are already being used. The imaging of native biomolecules, biological membranes, and tissues is also a major topic for the nanobiology researchers. Other topics concerning nanobiology include the use of cantilever array sensors and the application of nanophotonics for manipulating molecular processes in living cells,

Applications of bionanotechnology are enormously extensive and is best described as helping modern medicine progress from treating symptoms to generating cures and regenerating biological tissues [31]. Applications of nanotechnology in medicine currently being developed involve employing nano-particles to deliver drugs, heat, light or other substances to specific cells in the human body. Engineering particles to be used in this way allows detection and/or treatment of diseases or injuries within the targeted cells, thereby minimizing the damage to healthy cells in the body [78].

Furthermore, the pathophysiological conditions and anatomical changes of diseased or inflamed tissues can potentially trigger a great deal of scopes for the development of various targeted nanotechnological products. This development is like to be advantageous in the following ways: 1. Drug targeting can be achieved by taking advantage of the distinct pathophysiological features of diseased tissues [79,80]; 2. Various nanoproducts can be accumulated at higher

concentrations than normal drugs [80]; 3. Increased vascular permeability coupled with an impaired lymphatic drainage in tumors improve the effect of the nanosystems in the tumors or inflamed tissues through better transmission and retention [81,82]. 4. Nanosystems have capacity of selective localization in inflammed tissues [83]. 5. Nanoparticles can be effectively used to deliver/transport relevant drugs to the brain overcoming the presence of blood–brain barrier (meninges) [84,85]. 6. Drug loading onto nanoparticles modifies cell and tissue distribution and leads to a more selective delivery of biologically active compounds to enhance drug efficacy and reduces drug toxicity [86,87].

Stem Cell Therapy

Stem cell therapy is emerging as a potentially revolutionary new way to treat disease and injury, with wide-ranging medical benefits. It aims to repair damaged and diseased body-parts with healthy new cells provided by stem cell transplants.

Stem cells are primal cells which are considered to be progenitor of more than 200 cell types present in adult body. All stem cells are unspecialized (undifferentiated) cells that are characteristically of the same lineage. They maintain the ability to divide throughout life and give rise to cells that can become highly specialized and take the place of cells that die or are lost [88]. Under the precise conditions, stem cells have the potential to develop into mature cells that have characteristic shapes and specialized functions, such as heart cells, skin cells, or nerve cells [89,90].

Two broad types of stem cells exist: adult stem cells and embryonic stem cells.

Adult stem cells: are undifferentiated cells found among differentiated cells in a tissue or organ that can renew itself and can differentiate to yield the major specialized cell types of the tissue or organ [91]. They are also known as somatic stem cells which can be found in children as well as adults [88].

Embryonic Stem Cells: are pluripotent cells derived from the early embryo that are characterized by the ability to proliferate over prolonged periods of culture while remaining undifferentiated and maintaining a stable karyotype, but with the potential to differentiate into derivatives of all three germ layers. Because embryonic stem cells have the potential to differentiate into normal tissues of all types, the ability to derive and maintain embryonic stem cells in culture has captured the imagination of scientists and the lay public in terms of the possibility of having an unlimited supply of normal differentiated cells to engineer diseased tissues to regain normal function [92-100].

There are many ways in which human adult and embryonic stem cells can be used in clinical application in disease treatment including: Cancer [101], adult stem cells have been in use for many years predominantly in the treatment of cancer (such as leukemia and related bone and blood cancers) [102,203]; Spinal cord injury, adult stem cells are injected at damaged part of

the spinal cord [104]; Muscle damage; adult stem cells are also apparently able to repair muscle damaged after heart attacks.

Embryonic stem cells have been used to study the specific signals and differentiation steps required for the development of many tissues including: Genetic therapy, embryonic stem cells could be genetically manipulated to introduce the therapeutic gene [105] and may additionally be indirectly beneficial for cellular gene therapy; Drug Testing, embryonic stem can provide materials for drug testing that may improve the safety and efficacy of human drugs [106,107].

References

1. North Carolina Biotechnology Center: About Biotech.
2. Mahbodi F. Biotechnology bulletin. 2001.
3. Sharifi G. Botechnology principle and concept, 1st edn. Kerman: Kerman University. 2009.
4. Principles of Biotechnology.
5. Glick BR, Pasternak JJ. Molecular Recombinant DNA. Washington DC: ASM press. 1994.
6. Coombs JM. Macmillan Dictionary of Biotechnology. Hants: Macmillan Publishers. 1992.
7. Zaid A, Hughes HG, Porceddu E, Nicholas FW. Glossary of biotechnology and genetic engineering. Rome: FAO. 1999.
8. Mahbodi F. Biotechnology bulletin. 2002.
9. Emtiazi G. Principle of genetic engireeing and molecular biology, 2nd edition. Giti press. 2011.
10. Johnson IS. Human insulin from recombinant DNA technology. Science. 1983; 219: 632-637.
11. Bio's guide to Biotechnology.
12. U.S. department of energy human genome project program. Supra note 6.
13. Nehal MM, Patel PM, Patel NM. A review on regulatory aspects of biotechnology derived products. IJRAP. 2011; 2: 1495-1500.
14. Drews J. Stategic trends in the drug industry. Drug Discov Today. 2003; 8: 411-420.
15. Knowles J, Gromo G. A guide to drug discovery: Target selection in drug discovery. Nat Rev Drug Discov. 2003; 2: 63-69.
16. Castle AL, Carver MP, Mendrick DL. Toxicogenomics: a new revolution in drug safety. Drug Discov Today. 2002; 7: 728-736.
17. Vajo Z, Fawcett J, Duckworth WC. Recombinant DNA technology in the treatment of diabetes: insulin analogs. Endocr Rev. 2001; 22: 706-717.
18. Shastry BS. Pharmacogenetics and the concept of individualized medicine. Pharmacogenomics J. 2006; 6: 16-21.
19. Genetics Home.
20. Mohammad Z, Narasu ML. A review article Biotechnology Applications in Medicine. International Research Journal of Applied and Basic Sciences 4: 2557-2563.
21. Capecchi B, Serruto D, Adu-Bobie J, Rappuoli R, Pizza M. The genome revolution in vaccine research. Curr Issues Mol Biol. 2004; 6: 17-27.
22. Gulzar A Niazi, S Riaz-ud-Din, National Centre of Excellence in Biotechnology and Genomics in Medicine. A Review World Journal of Medical Sciences. 2006; 1: 72-78.
23. Pandey S. Hybridoma technology for production of monoclonal antibodies. International Journal of Pharmaceutical Sciences Review and Research. 2010; 2: 88-95.
24. Köhler G, Milstein C. Continuous cultures of fused cells secreting antibody of predefined specificity. Nature. 1975; 256: 495-497.
25. Franklin WA, Shpall EJ, Archer P, Johnston CS, Garza-Williams S, et al. Immunocytochemical detection of breast cancer cells in marrow and peripheral blood of patients undergoing high dose chemotherapy with autologous stem cell support. Breast Cancer Res Treat. 1996; 4: 1-13.
26. Hawkers N. Patients angered as watchdog refuses to allow bowel cancer drugs on NHS. The Time. 2006; 21: 1-6.

27. Karp G. Cell and Molecular Biology. New York: John Wiley and Sons. 1996.

28. Funaro A, Horenstein AL, Santoro P, Cinti C, Gregorini A. Monoclonal antibodies and therapy of human cancers. Biotechnol Adv. 2000; 18: 385-401.

29. Lewis R. Herceptin earns recognition in breast cancer arsenal. The Scientist. 2001; 15: 10.

30. Vaccines. National Institute of Allergy and Infectious Diseases.

31. Khan FA. Biotechnology in medical sciences. Florida: CRC Press. 2014.

32. Capecchi B, Serruto D, Adu-Bobie J, Rappuoli R, Pizza M. The genome revolution in vaccine research. Curr Issues Mol Biol. 2004; 6: 17-27.

33. Grandi G. Antibacterial vaccine design using genomics and proteomics. Trends Biotechnol. 2001; 19: 181-188.

34. Mäkelä PH. Vaccines, coming of age after 200 years. FEMS Microbiol Rev. 2000; 24: 9-20.

35. Kumar PJ, Clark M. Textbook of Clinical Medicine. London: Saunders. 2002.

36. Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Diabetes Care. 1997; 20: 1183-1197.

37. Beverley B, Eschwège E. The diagnosis and classification of diabetes and impaired glucose tolerance. In: John C Pickup, Gareth Williams, editors. Textbook of Diabetes. 2003; 2.1-2.1.

38. Lindberg G, Lindblad U, Melander A. Sulfonylureas for treating type 2 diabetes mellitus. Cochrane Database Systemic Reviews. 2004.

39. Bearse MA Jr, Han Y, Schneck ME, Barez S, Jacobsen C. Local multifocal oscillatory potential abnormalities in diabetes and early diabetic retinopathy. Invest Ophthalmol Vis Sci. 2004; 45: 3259-3265.

40. Hove MN, Kristensen JK, Lauritzen T, Bek T. The prevalence of retinopathy in an unselected population of type 2 diabetes patients from Arhus County, Denmark. Acta Ophthalmol Scand. 2004; 82: 443-448.

41. Seki M, Tanaka T, Nawa H, Usui T, Fukuchi T, et al. Involvement of brain-derived neurotrophic factor in early retinal neuropathy of streptozotocin-induced diabetes in rats: therapeutic potential of brain-derived neurotrophic factors for dopaminergic amacrine cells. Diabetes. 2004; 53: 2412-2419.

42. Moran A, Palmas W, Field L, Bhattarai J, Schwartz JE. Cardiovascular autonomic neuropathy is associated with microalbuminuria in older patients with type 2 diabetes. Diabetes Care. 2004; 27: 972-977.

43. Huang C, Kim Y, Caramori ML, Fish AJ, Rich SS. Cellular basis of diabetic nephropathy: II. The transforming growth factor-beta system and diabetic nephropathy lesions in type 1 diabetes. Diabetes. 2002; 51: 3577-3581.

44. Shukla N, Angelini GD, Jeremy JY. To: Looker HC, Fagot-Campagna A, Gunter EW et al. (2003) Homocysteine as a risk factor for nephropathy and retinopathy in Type 2 diabetes. Diabetologia 46:766-772. Diabetologia. 2004; 47: 140-141.

45. Svensson M, Eriksson JW, Dahlquist G. Early glycemic control, age at onset, and development of microvascular complications in childhood-onset type 1 diabetes: a population-based study in northern Sweden. Diabetes Care. 2004; 27: 955-962.

46. Saely CH, Aczel S, Marte T, Langer P, Drexel H. Cardiovascular complications in type 2 diabetes mellitus depend on the coronary angiographic state rather than on the diabetes state. Diabetologia. 2004; 47: 145-146.

47. Wallace C, Reiber GE, LeMaster J, Smith DG, Sullivan K. Incidence of falls, risk factors for falls, and fall-related fractures in individuals with diabetes and a prior foot ulcer. Diabetes Care. 2002; 25: 1983-1986.

48. Centers for Disease Control and Prevention (CDC). History of foot ulcer among persons with diabetes--United States, 2000-2002. MMWR Morb Mortal Wkly Rep. 2003; 52: 1098-1102.

49. Hayward G. Applied Genetics. Edinburgh: Thomas Nelson and Sons Ltd. 1991.

50. Hilson R. Diabetes, a Beyond Basics Guide. Melbourne: Methuen. 1987.

51. Hirsch IB. Insulin analogues. N Engl J Med. 2005; 352: 174-183.

52. DeWitt DE, Hirsch IB. Outpatient insulin therapy in type 1 and type 2 diabetes mellitus: scientific review. JAMA. 2003; 289: 2254-2264.

53. Gerich JE. Novel insulins: expanding options in diabetes management. Am J Med. 2002; 113: 308-316.

54. Holleman F, Schmitt H, Rottiers R, Rees A, Symanowski S. Reduced frequency of severe hypoglycemia and coma in well-controlled IDDM patients treated with insulin lispro. The Benelux-UK Insulin Lispro Study Group. Diabetes Care. 1997; 20: 1827-1832.

55. Setter SM, Corbett CF, Campbell RK, White JR. Insulin aspart: a new rapid-acting insulin analog. Ann Pharmacother. 2000; 34: 1423-1431.

56. Bolli GB, Di Marchi RD, Park GD, Pramming S, Koivisto VA. Insulin analogues and their potential in the management of diabetes mellitus. Diabetologia. 1999; 42: 1151-1167.

57. Cucinotta D, Russo GT. Biphasic insulin aspart in the treatment of type 2 diabetes mellitus. Expert Opin Pharmacother. 2009; 10: 2905-2911.

58. Davidson J, Vexiau P, Cucinotta D, Vaz J, Kawamori R. Biphasic insulin aspart 30: literature review of adverse events associated with treatment. Clin Ther. 2005; 27 Suppl B: S75-88.

59. Danne T, Bolinder J. New insulins and insulin therapy. Int J Clin Pract Suppl. 2011; : 26-30.

60. Steiner S, Hompesch M, Pohl R, Simms P, Flacke F. A novel insulin formulation with a more rapid onset of action. Diabetologia. 2008; 51: 1602-1606.

61. Simon AC, DeVries JH. The future of basal insulin supplementation. Diabetes Technol Ther. 2011; 13 Suppl 1: S103-108.

62. Roberts L. The human genome. Controversial from the start. Science. 2001; 291: 1182-1188.

63. Mathisen PM. Gene discovery and validation for neurodegenerative diseases. Drug Discov Today. 2003; 8: 39-46.

64. Van Eerdewegh P, Little RD, Dupuis J, Del Mastro RG, Falls K. Association of the ADAM33 gene with asthma and bronchial hyperresponsiveness. Nature. 2002; 418: 426-430.

65. Walder K, Segal D, Jowett J, Blangero J, Collier GR. Obesity and diabetes gene discovery approaches. Curr Pharm Des. 2003; 9: 1357-1372.

66. Egland KA, Vincent JJ, Strausberg R, Lee B, Pastan I. Discovery of the breast cancer gene BASE using a molecular approach to enrich for genes encoding membrane and secreted proteins. Proc Natl Acad Sci U S A. 2003; 100: 1099-1104.

67. Suzuki T, Shen H, Akagi K, Morse HC, Malley JD. New genes involved in cancer identified by retroviral tagging. Nat Genet. 2002; 32: 166-174.

68. Morris BJ, Benjafield AV, Lin RC. Essential hypertension: genes and dreams. Clin Chem Lab Med. 2003; 41: 834-844.

69. Boutin P, Froguel P. Genetics of human obesity. Best Pract Res Clin Endocrinol Metab. 2001; 15: 391-404.

70. Omi K, Ohashi J, Naka I, Patarapotikul J, Hananantachai H. Polymorphisms of CD36 in Thai malaria patients. Southeast Asian J Trop Med Public Health. 2002; 33 Suppl 3: 1-4.

71. Omi K, Ohashi J, Patarapotikul J, Hananantachai H, Naka I. CD36 polymorphism is associated with protection from cerebral malaria. Am J Hum Genet. 2003; 72: 364-374.

72. Michael NL. Host genetic influences on HIV-1 pathogenesis. Curr Opin Immunol. 1999; 11: 466-474.

73. van Ommen GJ. The Human Genome Project and the future of diagnostics, treatment and prevention. J Inherit Metab Dis. 2002; 25: 183-188.

74. Bosher JM, Labouesse M. RNA interference: genetic wand and genetic watchdog. Nat Cell Biol. 2000; 2: E31-36.

75. Ratcliff F, Harrison BD, Baulcombe DC. A similarity between viral defense and gene silencing in plants. Science. 1997; 276: 1558-1560.

76. Pushparaj PN, Melendez AJ. Short interfering RNA (siRNA) as a novel therapeutic. Clin Exp Pharmacol Physiol. 2006; 33: 504-510.

77. Walsh C. Where will new antibiotics come from? Nat Rev Microbiol. 2003; 1: 65-70.

78. http://www.nanotechproject.org

79. Vasir JK, Labhasetwar V. Targeted drug delivery in cancer therapy. Technol Cancer Res Treat. 2005; 4: 363-374.

80. Vasir JK, Reddy MK, Labhasetwar V. Nanosystems in drug targeting: opportunities and challenges. Curr Nanosci. 2005; 1: 47–64.

81. Maeda H, Wu J, Sawa T, Matsumura Y, Hori K. Tumor vascular permeability and the EPR effect in macromolecular therapeutics: a review. J Control Release. 2000; 65: 271-284.

82. Matsumura Y, Maeda H. A new concept for macromolecular therapeutics in cancer chemotherapy: mechanism of tumoritropic accumulation of proteins and the antitumor agent smancs. Cancer Res. 1986; 46: 6387-6392.

83. Allen TM, Cullis PR. Drug delivery systems: entering the mainstream. Science. 2004; 303: 1818-1822.

84. Alyautdin RN, Tezikov EB, Ramge P, Kharkevich DA, Begley DJ, et al. Significant entry of tubocurarine into the brain of rats by

adsorption to polysorbate 80-coated polybutylcyanoacrylate nanoparticles: an in situ brain perfusion study. J Microencapsul. 1998; 15: 67–74.

85. Garcia-Garcia E, Gil S, Andrieux K, Desmaële D, Nicolas V. A relevant in vitro rat model for the evaluation of blood-brain barrier translocation of nanoparticles. Cell Mol Life Sci. 2005; 62: 1400-1408.

86. Feng SS, Mu L, Win KY, Huang G. Nanoparticles of biodegradable polymers for clinical administration of paclitaxel. Curr Med Chem. 2004; 11: 413-424.

87. de Kozak Y, Andrieux K, Villarroya H, Klein C, Thillaye-Goldenberg B. Intraocular injection of tamoxifen-loaded nanoparticles: a new treatment of experimental autoimmune uveoretinitis. Eur J Immunol. 2004; 34: 3702-3712.

88. Avasthi S, Srivastava RN, Singh A, Srivastava M. Stem Cell: Past, Present and Future- A Review Article Internet Journal of Medical Update. 2008; 3: 22-30.

89. [89] Chandross KJ, Mezey E. Plasticity of adult bone marrow stem cells. Mattson MP and Van Zant G editors. JAI Press: Greenwich, CT; 2001.

90. Slack JM1. Stem cells in epithelial tissues. Science. 2000; 287: 1431-1433.

91. Kumar S, Singh NP. Stem cells: A new paradigm. Indian Journal of Human Genetics. 2006; 12: 4-10.

92. Amit M, Itskovitz-Eldor J. Derivation and spontaneous differentiation of human embryonic stem cells. J Anat. 2002; 200: 225-232.

93. Carpenter MK, Rosler E, Rao MS. Characterization and differentiation of human embryonic stem cells. Cloning Stem Cells. 2003; 5: 79-88.

94. Hoffman LM, Carpenter MK. Characterization and culture of human embryonic stem cells. Nat Biotechnol. 2005; 23: 699-708.

95. Martin GR. Isolation of a pluripotent cell line from early mouse embryos cultured in medium conditioned by teratocarcinoma stem cells. Proc Natl Acad Sci U S A. 1981; 78: 7634-7638.

96. Moon SY, Park YB, Kim DS, Oh SK, Kim DW. Generation, culture, and differentiation of human embryonic stem cells for therapeutic applications. Mol Ther. 2006; 13: 5-14.

97. Polak JM, Bishop AE. Stem cells and tissue engineering: past, present, and future. Ann N Y Acad Sci. 2006; 1068: 352-366.

98. Rodríguez CI, Galán A, Valbuena D, Simón C. Derivation of clinical-grade human embryonic stem cells. Reprod Biomed Online. 2006; 12: 112-118.

99. Skottman H, Dilber MS, Hovatta O. The derivation of clinical-grade human embryonic stem cell lines. FEBS Lett. 2006; 580: 2875-2878.

100. Thomson JA, Itskovitz-Eldor J, Shapiro SS, Waknitz MA, Swiergiel JJ. Embryonic stem cell lines derived from human blastocysts. Science. 1998; 282: 1145-1147.

101. Tuch BE. Stem cells--a clinical update. Aust Fam Physician. 2006; 35: 719-721.

102. Beachy PA, Karhadkar SS, Berman DM. Tissue repair and stem cell renewal in carcinogenesis. Nature. 2004; 432: 324-331.

103. Liu S, Dontu G, Wicha MS. Mammary stem cells, self-renewal pathways, and carcinogenesis. Breast Cancer Res. 2005; 7: 86-95.

104. Rolletschek A, Blyszczuk P, Wobus AM. Embryonic stem cell-derived cardiac, neuronal and pancreatic cells as model systems to study toxicological effects. Toxicol Lett. 2004; 149: 361-369.

105. Mitsui K, Tokuzawa Y, Itoh H, Segawa K, Murakami M. The homeoprotein Nanog is required for maintenance of pluripotency in mouse epiblast and ES cells. Cell. 2003; 113: 631-642.

106. Evans MJ, Kaufman MH. Establishment in culture of pluripotential cells from mouse embryos. Nature. 1981; 292: 154-156.

107. Martin GR. Isolation of a pluripotent cell line from early mouse embryos cultured in medium conditioned by teratocarcinoma stem cells. Proc Natl Acad Sci U S A. 1981; 78: 7634-7638.