

# A Study Of Genetic Engineering Techniques In Biotechnology Based Pharmaceuticals

S Zachariah, L Pappachen

## Citation

S Zachariah, L Pappachen. *A Study Of Genetic Engineering Techniques In Biotechnology Based Pharmaceuticals*. The Internet Journal of Nanotechnology. 2008 Volume 3 Number 1.

## Abstract

Modern pharmaceutical biotechnology encompasses gene cloning and recombinant DNA technology. The prime focus of the study was to know in depth of the concepts of Genetic engineering that are implemented in pharmaceutical industry. This study unleashes the paramount significance of Genetic engineering emerged out with outstanding success. Pharmaceutical trends are moving towards two profound technologies the first being Novel Drug Delivery system.. This review work ensures the concepts of Recombinant DNA , and the hybridoma techniques that are implemented in drug discovery and research. Genetic engineering now allows biological synthesis and large-scale production of several proteins with therapeutic potential. The principal challenge in this sphere is to identify new, medically and commercially significant targets. In the future, genetic engineering will surely provide invaluable tools for the study of the molecular basis of cellular control and pathophysiology, which will permit biochemists and medicinal chemists to design novel medicines.

## INTRODUCTION

Biotechnology is the application of scientific and engineering principles to the processing of materials by biological agents to provide goods and service. This review provides an in-depth look at the most promising major biologics (blood, blood components and derivatives, allergenic and vaccines) that include biotechnology based drugs and products (therapeutic proteins, recombinant DNA vaccines, gene therapies, and devices). During the early days of genetic engineering, the availability of this remarkable new technology produced a sense that the world of science was experiencing a momentous occasion. Because it is a technology that is evolving and finding new applications ,it is constantly providing new challenges and impressions.

New biotechnology comprises genetic engineering, protoplast fusion and monoclonal (citation) antibody techniques, powerful new “tools” designed to generate efficient bioprocesses and products for the pharmaceutical industry. The following areas of biotechnology are highlighted: human insulin, interferons and other growth factors, neuroactive peptides, blood products, antibiotics, enzymes, monoclonal antibodies, vaccines and oncogenes. Biotechnology has helped the bio-industries in producing the novel compounds and optimization, and scale up products, including single protein and mycoprotein. The genetic engineering is not a single technique but represents a

collection of interrelated techniques, including recombinant recombinant DNA technology, that together permits scientists to manipulate genetic information; to express this genetic information in a defined manner; and, as a result, to convert microorganisms into minifactories capable of manufacturing desired gene products.

## PRINCIPLES OF TOOLS OF GENETIC ENGINEERING

The fundamental unit of a genome that control heredity is known as gene. The recent work on structure of chromosomes and deoxyribonucleic acid clearly defines the gene as “a small segment of polynucleotide chain consisting of hundreds or thousands of nucleotide”.

There are various biological tools which are used to carry out manipulation of genetic materials and cells as well, for example enzymes, foreign or passenger DNA, cDNA bank and gene bank.

## EXONUCLEASES

These enzymes act upon genome and digest the base pairs on 5' or 3' ends of a single nicks or gaps in double stranded DNA.

## ENDONUCLEASES

They act upon genetic material and cleave the double

stranded DNA at any pointy except the ends, but their action involves only one strand of the duplex.

### **RESTRICTION ENDONUCLEASES**

These enzymes occur naturally in bacteria as a chemical weapon against invading viruses and cut both strands of DNA when certain foreign nucleotides are introduced in the cell.

### **DNA LIGASES**

Mertz and Davis (1972) for the first time demonstrated that cohesive termini of cleaved DNA molecule could be covalently sealed with E.coli DNA ligase and were able to produce recombinant DNA molecules. DNA ligase seals single strand nicks in DNA which has 5'->3'-OH(hydroxyl) termini. There are two enzymes which E.coli and that encoded by T<sub>4</sub> phage, and hence the enzyme is known as T<sub>4</sub> DNA ligase.

### **DNA POLYMERASE**

This enzyme polymerizes the DNA synthesis of DNA template and also catalyses a 5'->3' and 3'-5' exonucleolytic degradation of DNA<sub>1</sub>. The DNA polymerase, investigated by A.Kornberg and coworkers in E.coli is now known as DNA polymerase.

### **CLONING VECTORS**

Vectors are those DNA molecules that can carry a foreign DNA fragment when inserted into it. Vectors are also known as vehicle DNAs. Based on the nature and sources, the vectors are grouped into bacterial plasmids, bacteriophages, and cosmids and plasmids.

### **PLASMIDS**

Plasmids are the extrachromosomal, self-replicating and double stranded closed and circular DNA molecules present in the bacterial cell. Plasmids contain sufficient genetic conformations for their own replication. A plasmid can be considered a suitable cloning vehicle if it possesses three following features:

1. It can be readily isolated from the cells.
2. It possesses a single restriction site for one or more restriction enzyme(s)
3. Insertion of a linear molecule at one of these sites does not alter its replication properties.
4. It can be reintroduced into a bacterial cell and cells carrying the plasmid with or without the insert can

be selected or identified<sup>2</sup>.

### **COSMIDS**

Based on the properties of DNA and col E1 plasmid DNA, a group of Japanese workers<sub>3</sub> (Fukumaki et al ;1976) showed that the presence of a small segment of phage DNA containing cohesive end on the plasmid molecule is a sufficient prerequisite for invitro packing of this DNA into infectious particles. For the first time it was developed by Collins and Hohn<sub>4</sub> (1978)

### **PLASMIDS**

The plasmids may be inserted into a phage  $\lambda$  genome, as a phage  $\lambda$  genome into the bacterial chromosome, during lysogenic cycle. Insertion of plasmid into phage  $\lambda$  is done with a view to have a specific site responsible for recombinational insertion of the phage into bacterial chromosome during lysogenic cycle.

### **GENE CLONING IN PROKARYOTES**

Success in genetic engineering has been possible due to the rapid development in gene cloning<sub>6</sub> methodologies. It is essentially the insertion of a specific fragment of foreign DNA into a cell, through a suitable vector, in such a way that inserted DNA replicates independently and transferred to progenies as result of cell division. Manniatis et al<sub>5</sub> have described the basic techniques of gene cloning. Rabies Virus(RV) causes hydrophobia in animals and human in many countries like South America, Africa, Asia. Researches are being done to synthesise vaccines by including genetically engineered E.coli cells. However, the attempt has been made to isolate mRNA encoding viral glycoprotein coat has been successfully transferred to E.coli.

### **GENES FOR VACCINES AND IMMUNOGENIC SUBSTANCES**

Vaccines are chemical substances prepared from the proteins (antigens) of other animals, which confer immunity to a particular virus. Some of the vaccines are synthesized biologically through genetic engineering. Hepatitis B virus (HBV) is widespread in man and produces several chronic liver disorders such as chronic hepatitis, cirrhosis and primary liver cancer .HBV DNA is Double stranded circular molecule of about 3 kb size and has a large single stranded gap which must required with an endogenous polymerase before digestion with restriction enzymes for DNA .

### **MONOCLONAL ANTIBODIES**

Antibodies are proteins synthesized in blood against specific antigens just to combat and give immunity in blood. They can be collected from the blood serum of an animal. Such antibodies are heterogeneous and contain a mixture of antibodies (i.e., monoclonal antibodies). Therefore, they do not have characteristics of specificity. If a specific lymphocyte, after the isolation and culture in vitro, becomes capable of producing a single type of antibody which bears specificity against specific antigen. It is known as 'monoclonal antibodies'. Due to the presence of desired immunity, monoclonal antibodies are used in the diagnosis of diseases.

### **HYBRIDOMA**

Cesar Milstein of Argentina tried to culture myeloma cells. In 1973, in collaboration of Cotton, he succeeded to fuse rat and mouse myeloma cells with the result of production of hybrid cells. This hybrid cells secreted the immunoglobulins which consisted of several types of polypeptides. In 1974, George Kohler and Milstein successfully isolated clones of cells from the fusion of two parental cell line i.e., between lymphocyte from spleen of mice immunized with red blood cells from sheep and myeloma cells. The antibodies for specific antigens immunized the myeloma cells. These hybrid cell lines (cell clones) are known as hybridomas which are capable of producing unlimited supply of antibodies. For the discovery of monoclonal antibodies Kohler and Milstein along with Neils Jerns were awarded Nobel Prize in 1984, in physiology and medicine. Now the techniques are developed to obtain antibodies from hybridoma culture.

### **GENE THERAPY**

Gene therapy is the cure of genetic diseases through an insertion of an extra gene with the intention that the gene product will play a therapeutic role, whereas gene replacement therapy is the cure of similar diseases by replacing the abnormal genes with the normal ones. Through genetic engineering technique diagnosis, isolation and insertion of genes can be introduced into the germ cells e.g. blood, liver and skin cells. The somatic therapy is really an interesting area of research. Gene therapy is applied at two different levels.

### **PATIENT THERAPY**

A functional gene-preparation is introduced through different methods as described under ('methods of gene therapy')

### **EMBRYO THERAPY**

In this case after fertilization, the embryo is diagnosed for genetic diseases. If any such disease is present, the patient is advised for gene therapy at this stage or for abortion.

### **METHODS OF GENE THERAPY**

Following are some of the methods used for gene therapy.

i) Microinjection: It is a very tedious method. However, it is used in oocytes, eggs, and embryos. Jeffery S. Chamberlain et al, (1993) of Human Genome Centre, Michigan University U.S.A have cured mice that inherited a neuromuscular disease which is similar to muscular dystrophy of humans. The mutant mice lacking 417k gene in brain and muscle cells affected humans.

Chamberlain and coworkers microinjected the DNA containing 427k gene into the zygote of mutant mice. The transplanted genes worked properly and produced necessary protein and thus, prevented the diaphragm.

ii) Using detergent mixture.

Certain charged chemical compounds eg. calcium phosphate, dextran or lipids are mixed with functional cDNA of desired function. The mixture is introduced near the vicinity of recipient cells. Thereafter, it spread to the interior of the body organ. The chemical mixed with cDNA disturbs the cell membranes, widens pore size and allows the cDNA to pass into the cell. In this method the number of cells allowing the entry of cDNA is very small.

iii) Use of Viruses:

In recent years, use of harmless animal viruses or gene therapy has aroused the interest of the biotechnologies. The best example is retroviruses. They contain RNA as genetic material and do not kill the neighbouring cells after infection. Their genome can carry more foreign genetic material than the others. This genetically engineered viruses are produced by replacing the genes of the viral genome by functional genes for desired protein. The modified virus after entering into a cell will not replicate but express the certain desired proteins in the cells.

iv) Embryo therapy through IVF-technology.

In 1993, adapting IVF-technology, Dr. A. Handyside got success in producing a genetically engineered female baby, whose parents transmitted the genetically diseased (cystic fibrosis) in earlier four babies, who died later on. Even after

confirmation through prenatal diagnosis that the babies would be sufferer or carrier of this disease they decided to give birth to babies, which failed. This disease clogs the lungs of sufferer and makes them unable to digest the food properly.

**Figure 1**

Table 1: Microorganisms and their Cleavage Sites and Products.

| Microorganisms              | Restriction enzymes | Cleavage sites           | Cleavage products        |
|-----------------------------|---------------------|--------------------------|--------------------------|
| Bacillus amylo-liquefaciens | Bam HI              | 5-GGATCC-3<br>3-CCTAGG-5 | 5-GGATCC-3<br>3-CCTAGG-5 |
| E.coli RY 13                | Eco RI              | 5-GAATTC-3<br>3-CTTAAG-5 | 5-GAATTC-3<br>3-CTTAAG-5 |
| H.influenzae Rd             | Hin dIII            | 5-AAGCTT-3<br>3-TTCGAA-5 | 5-AAGCTT-3<br>3-TTCGAA-5 |
| H.parainfluenzae            | Hpa I               | 5-GTTAAC-3<br>3-CAATTG-5 | 5-GTTAAC-3<br>3-CAATTG-5 |
| K.pneumoniae OK 8           | Kpn I               | 5-GGTACC-3<br>3-CCATGG-5 | 5-GGTACC-3<br>3-CCATGG-5 |
| S.albus G                   | Sal I               | 5-GTCGAC-3<br>3-CAGCTG-5 | 5-GTCGAC-3<br>3-CAGCTG-5 |
| S.aureus 3AI                | Sau 3AI             | 5-GATC-3<br>3-CTAG-5     | 5-GATC-3<br>3-CTAG-5     |

## PRINCIPLES OF GENETIC ENGINEERING IN PHARMACEUTICAL INDUSTRY

The pharmaceutical industry was one of the first biotechnology industries to take advantage of the potentials of applied molecular genetics. This has been especially true in the development of efficient process for antibiotic production, in which applied genetics played a major role in improving the efficiency and productivity of these biological systems.

1. The genetic improvement of penicillin production is an example of long term efforts that lead to a dramatic improvement in strains of Penicillin fungus, *Penicillium chrysogenum*. The wild type strain was treated with toxic chemicals and UV radiations through successive stages generate mutant strains that yield many hundred-fold improvement in fermentation productivity.
2. Chemically induced mutations improved a valuable strain

of the bacterium, *Escherichia coli*, which produces an enzyme, L-asparaginase used to treat leukemia.

3. Genetic manipulation of the gentamicin bacterium, *Micromonospora purpurea*, resulted in sufficient yield improvement that its producer, Schering –Plough Corporation did not have to build a scheduled manufacturing plant, thereby resulting in a savings of \$50 million.

## CURRENT USES OF GENETIC ENGINEERING IN PHARMACEUTICAL INDUSTRY

The first areas of application of new biotechnology in the United States have been in the pharmaceutical field. As research using the new genetic techniques has progressed, the pharmaceutical industry has been the leader in industrial applications. Genetic Engineering and Biotechnology scientists can now identify genes that influence desirable physical features in one organism and transfer them into others. Such genetic engineering results in altered (or recombinant) organisms having a combination of desired traits. Using genetically modified living organisms or their products for commercial purposes is an emerging area in biotechnology (Table 2)

**Figure 2**

Table 2: Examples of Human biotechnology Products

| Sector       | Type                                                                  | Product name | Application     | Producer                 |
|--------------|-----------------------------------------------------------------------|--------------|-----------------|--------------------------|
| Vaccines     | Recombinant hepatitis B surface antigen                               | Shanvac B    | Hepatitis B     | Shantha Biotechnics      |
|              | Recombinant hepatitis B surface antigen                               | Revac B      | Hepatitis B     | Bharat Biotech           |
|              | Recombinant hepatitis B surface antigen                               | Genevac B    | Hepatitis B     | Serum Institute of India |
|              | Purified capsular polysaccharide Vi of <i>Salmonella typhi</i>        | Typhar Vi    | Typhoid         | Bharat Biotech           |
| Therapeutics | Recombinant human insulin                                             | Wosulin      | Diabetes        | Wockhardt (Mumbai)       |
|              |                                                                       | Indikinase   | Cardiovascular  | Bharat Biotech           |
| Diagnostics  | Immunoblot assays using recombinant HIV-1 antigens and HIV-2 antigens | HIV TRI-DOT  | HIV-1 and HIV-2 | J. Mitra (New Delhi)     |

The other areas of advances in genetic engineering are:

Production of pharmaceuticals by bacteria that produce human insulin for diabetics or human growth hormone for individuals with dwarfism. Scientists are perfecting ways to transfer human genes for important proteins into cows, sheep, and goats to obtain medically significant products from the milk of these animals.

1. The possibility of making products superior to those already marketed for a given purpose.
2. The technical feasibility of applying new methods (eg in recombinant DNA applications, The feasibility of cloning DNA that directs synthesis or desired substances)
3. The cost of conventional methods (eg. chemical synthesis, tissue extraction) and the potential to reduce costs with recombinant DNA technology or other new methods.
4. The nature of the market (i.e. whether it is of high enough value or volume to justify the substantial start up cost of newer production methodology and regulatory approval.

### **MAJOR AREAS OF GENETIC ENGINEERING IN PHARMACEUTICAL INDUSTRY**

#### **A) RECOMBINANT DNA TECHNOLOGY**

Gene coding for enzymes and other metabolic proteins can be cloned into antibiotic producing microorganisms to add steps to existing biosynthetic pathways that improve products or manufacturing processes. Research in progress includes the recombinant DNA mediated transfer of acyltransferase genes among species of bacteria to obtain solvent extractable cephalosporins, the combination of genes via recombinant DNA technology and transformation to obtain direct efficient synthesis of the antibiotic amikacin, utilization of recombinant DNA technology to improve the production of the antibiotic tyrosine etc.

The combination of new & traditional technology in the pharmaceutical industry holds tremendous potential for the improvement of microorganisms used in antibiotic production and in the isolation of new antibiotic products. With their extensive bioprocess resources are placing great emphasis on new antibiotic research. This emphasis may be due to the fact that antibiotic make up 25% of the ethical drug sales in Japan compared to about 8% in United States

and that at least 28% of the antibiotic sales in Japan now rise from antibiotics produced in the United States.

#### **B) MONOCLONAL ANTIBODIES**

Monoclonal Antibodies technology currently leads other forms of modern technology in commercial use as measured by numbers of products on the market. Its lead is largely because Mab invitro diagnostic products on the market. It leads largely because Mab invitro diagnostic products do not have to undergo some rigorous safety testing required for pharmaceuticals used within the bodies.

##### **1) IN VITRO DIAGNOSTIC PRODUCTS**

The roster of Mab -based invitro diagnostic products is growing rapidly. Mab technology is being to make novel diagnostic products. Although the competitive advantage of Mab products must ultimately be demonstrated in the market place, such products may prove superior to traditional methods used to identify infectious diseases, hormonal changes, detection of acquired immune deficiency syndrome or the presence of cancer.

##### **2) IN VIVO DIAGNOSTIC PRODUCTS**

Diagnosis of some diseases requires identification and localization of diseases within the body. Antibodies with detectable markers (eg radioactive chemicals) provide highly specific means for accomplishing these ends. Antibodies injected into the body are considered drugs, thus they require extensive testing prior to approval for marketing. Highly specific diagnostic tests are available for the detection of the AIDS retrovirus.

##### **3) PREVENTIVE AND THERAPEUTIC PRODUCTS**

Applications of Mabs to prevent or treat disease are being pursued on two fronts 1) administration of Mabs as passive vaccines to protect against specific diseases and 2) coupling cytotoxic agents (eg, diphtheria toxin, ricin, or cobra venom) to Mabs that direct the agents to diseased cells.

This is especially true in the case of viral diseases like hepatitis B, herpes and cytomegalovirus. Until recently no culture system for hepatitis B Virus has been available. However a human liver tumor has been adapted to cell culture and these tumor cells secrete the hepatitis B surface antigen. (HbsAg) The availability of this HbsAg may make Mab preparation possible, leading to Mabs that neutralize the virus and are effective as a passive virus. Mabs will undoubtedly play a major role in pharmaceutical research

and are proceeding rapidly in the United States.

### **3) REGULATORY PROTEINS**

The use of biotechnology to manufacture pharmaceutical products can be viewed in several ways. The successful cloning projects and microbial production of the proteins human insulin (Hi), interferon (IFNs) and human growth hormone (HGH) in recombinant DNA systems are valuable as paradigms for biotechnology's role in developing competitive pharmaceutical substitutes.

#### **I) HUMAN INSULIN**

The first therapeutic agent produced by recombinant DNA technology to achieve regulatory approval and market introduction was human insulin. The methodology was generated by Genentech, Inc and Eli Lilly and Company. Insulin derived from animals has long been the largest volume peptide hormone used in medicine. Chemically human insulin differs only slightly from that of pigs and cows and its incremental benefits have yet to be demonstrated.

#### **II) INTERFERON**

The IFNs represents an important class of immune regulators or lymphokines that regulate the response of cells to viral infections and cancer. IFNs inhibit tumor cell growth and may stimulate immune cells to destroy cancer cells; their effects on inhibiting tumor metastasis is better established than their ability to effect actual regression of primary tumors.

Alpha interferon is also showing promise in laboratory tests for treating in conjunction with chemotherapeutics, lung cancer. The hope is that by using the interferon, it might be possible to produce the amount of chemotherapeutic drug needed, thereby reducing the side effects of chemotherapy. Another promising use of alpha interferon is as the drug portion of immunotoxins.

Several biotechnology companies have reported significant progress using yeast for the manufacture of IFNs. Numerous genetic techniques are currently being used in yeast strains to increase IFN production.

#### **THERAPEUTIC PEPTIDES**

Therapeutic peptides offer perhaps the largest potential market for biotechnology-based products. However as pharmaceuticals they also face greater risks in terms of ultimate liability by the manufacture and raise the most challenging issues of protectability /patentability. A

competitive position will continue to depend on the effective use of research and development, regulatory financial and marketing and other resources (Table 1) shows selected therapeutic peptide market potential.

### **COMMERCIAL ASPECTS OF GENETIC ENGINEERING IN PHARMACEUTICAL INDUSTRY**

In the last 6 years, 65–70% of the 150 biotech drugs in today's market were approved, 11 of which reached blockbuster sales status in 2004. Such successes promise large medical and commercial advances for this area and 'hope' seems to be the major driving factor for investment (Table 2). However, despite revolutionary promises biotechnology remains a risky business, a fact demonstrated by previous unstable stock market performance. Big Pharma currently dominates the biotechnology industry. Several biotechnology-based products are still in the preclinical and clinical phase, raising the concerns associated with clinical based failures. Oncology, central nervous system diseases, cardiovascular autoimmune diseases, inflammatory diseases, diabetes, hormone/enzyme replacement respiratory and infectious diseases are the major therapeutic areas that are likely to see significant biotech product launches in the next 10 years.

### **SELECTED THERAPEUTIC MARKET POTENTIAL**

The innovation gap in the pharmaceutical industry has created demand for biotechnology-based product development with low productivity, rising R&D costs and generic competition hindering more traditional pharma business. The result is heavy investment in biotechnology-based products by pharmaceutical companies. Recombinant DNA-based products are one such group with high approval rates from regulatory authorities.<sup>10</sup> In future, advances in diagnostics coupled with biotechnology could lead to increased emphasis on personalized medicines. Moreover, advances in biotechnology are derived from developments in enabling technologies such as genomic, proteomics, cellomics, metabolomics, toxicogenomics and pharmacogenomics.

### **CONCLUSION**

#### **PRIORITIES FOR FUTURE GENETIC ENGINEERING RESEARCH**

Funding from government laboratories and agencies, small biotechnology companies and the pharmaceutical industry has been and will continue to be instrumental in developing

biotechnology for pharmaceutical use. The new biological techniques have dramatically increased our understanding of many disease mechanisms. Areas of research that would benefit from pharmaceutical innovations in biotechnology include the following

- 1 Clarification of the functions and mechanisms of action of immune regulators, such as IFN and interleukin-2.
- 2 Investigation into the clinical use of neuroactive peptides and thrombolytic and fibrynolytic peptides.
- 3 Development of improved drug delivery systems.
- 4 Clarification of the mechanisms of acquired immunity leading to better vaccine development procedures.
- 5 Development of the strategies and safe vector systems for human therapy<sup>11</sup>, somatic cell gene therapy, germ-line gene therapy<sup>12</sup> and eventually genetic engineering to enhance or alter human traits that influence our physical well being.

This brief study in genetic engineering concepts for biotechnology based drugs has certainly enabled us to understand the importance and made ourselves equipped well to face the biotechnology revolution towards the new

millennium.

### **CORRESPONDENCE TO**

Subin Mary Zachariah Faculty of Pharmaceutical Chemistry, Amrita School of Pharmacy, Amrita Vishwa Vidyapeetham University, AIMS Healthcare Campus, Elamakkara (P.O), Kochi, Kerala, India: 682026, E-mail: smz78@rediffmail.com  
subinmaryzachariah@aims.amrita.edu Fax: +91484-2802141, Phone: , 2802141, +91484-2801234-8275.

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**Author Information**

**Subin Mary Zachariah, MPharm, PDCR**

Amrita School of Pharmacy, Amrita Viswavidyapetham University, AIMS

**Leena K. Pappachen, MPharm**

PDCR, Amrita School of Pharmacy, Amrita Viswavidyapetham University, AIMS