International Journal of Pharma and Bio Sciences V1(2)2010

BIOTECHNOLOGY IN DRUG DELIVERY: EVOLUTION, OBSTACLES, AND APPLICATIONS

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ABSTRACTS

Biotechnology is the application of biology to the development of products and services that use naturally occurring molecules created to restore biologic processes. In simple language it uses or modifies the living things for human purposes. Biotechnology has evolved dramatically during the past 50 years, beginning with the discovery of deoxyribonucleic acid. Biotechnical methods are now used to produce many proteins for pharmaceutical and other specialized purposes Gene therapy – altering DNA within cells in an organism to treat or cure a disease – is one of the most promising areas of biotechnology research. New genetic therapies are being developed to treat diseases such as cystic fibrosis, AIDS and cancer. The biggest challenge in the preclinical assessment of biotech products has been coping with species specificity and the associated detection and implications of altered immune status and unpredicted pleiotropic activity.Over the next two decades, discoveries in biotechnology and advances in gene therapy will transform the practice of medicine. The idea of merging biological and nonbiological systems at the nanoscale level is not a new one. The broad field of bioconjugate chemistry is based on combining the functionalities of biomolecules and non-biologically derived molecular species for specialized use in applications Gene therapy and RNAi technologies are considered the medical treatments of the future.

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INTRODUCTION

Bio-technology is a technological application which is related to biology, plants, animals, human beings, agriculture, food science, and medicine. Biotechnology is the application of biology to the development of products and services that use naturally occurring molecules created to restore biologic processes.[1] People now also refer biotechnology as Genetic engineering or cell- and tissue- culture. In simple language it uses or modifies the living things for human purposes. Bio-technology is a diverse field which uses biological applications. It has various branches which include Genetics, microbiology, molecular biology, biochemistry, embryology, cell biology, anatomy, animal tissue culture, plant tissue culture, etc. The biological problems also use computational techniques and make the organization and analysis of biological data. This is called bio-informatics, also referred to as computational biology. The main areas of bio-informatics are genomics, structural genomics and proteomics.

Bio-technology has four major applications:-

Blue biotechnology- It is used to describe the marine and aquatic applications of biotechnology.

Green biotechnology- It is applied to agricultural processes. An example- the selection and domestication of plants via micropropagation. Another example is the designing of transgenic plants to grow under specific environmental in the presence or absence of chemicals.

Red biotechnology- It is applied to medical processes. An example is the designing of organisms to produce antibiotics, and the engineering of genetic cures through genomic manipulation.

White biotechnology- It is known as industrial biotechnology. An example is the designing of an organism to produce a useful chemical.

EVOLUTION OF BIOTECHNOLOGY TIMELINE

Biotechnology has evolved dramatically during the past 50 years, beginning with the discovery of deoxyribonucleic acid (DNA) and its structure in the 1950s, the identification of its genetic code in the mid- 1960s, and the cloning of the first human gene, somatostatin, in 1977 (Fig 1) [2] . In the early 1980s, the ability to manufacture DNA using recombinant technology opened up many commercial possibilities. In 1982, for example, recombinant human insulin was approved for use in refractory diabetics. [3] Since then, biotechnology has progressed exponentially.

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Figure 1

Evaluation of Biotechnology

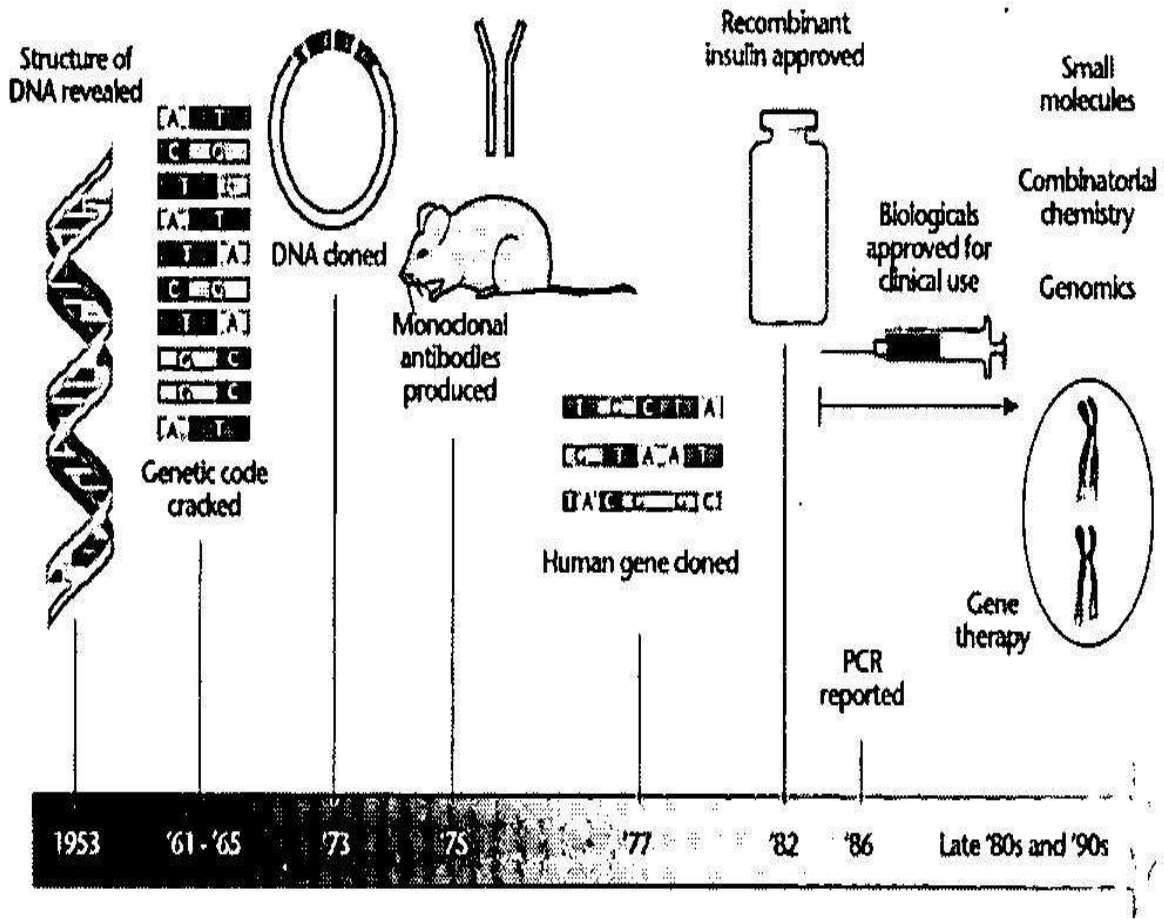

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Table: 1

Development in biotechnology [4-8]

Year	Development
Prior to 1750	Plants used for food. Animals used for food and to do work. Plants domesticated, selectively bred for desired characteristics. Microorganisms used to make cheese, beverages, and bread fermentation
1797 Edward Jenner	Used living microorganisms to protect people from disease.
1864 Louis Pasteur	Proved existence of microorganisms showed that all living things are produced by other living things.
1869 Johann Meischer	Isolated DNA from the nuclei of white blood cells.
1893 Koch and Pasteur	Fermentation process patented Diphtheria antitoxin isolated
1902 Walter Sutton	Coined the term "gene" Proposed that chromosomes carry genes (factors which Mendel said that could be passed from generation to generation)
1910 Thomas H. Morgan	Proved that genes are carried on chromosomes “Biotechnology" term coined
1938	Proteins and DNA studied by x-ray crystallography Term 'molecular biology" coined
Mid-1940	Penicillin produced Transition from animal power to mechanical power on farms
1950 Erwin Chargaff	Determined that there is always a ratio of 1:1 adenine to thymine in DNA of many different organisms Artificial insemination of livestock
1952 Alfred Hershey/ Margaret Chase	Used radioactive labeling to determine that it is the DNA not protein which carries the instructions for assembling new phages.
1953.James Watson/ Francis	Determined the double helix structure of DNA

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Crick	
1956 Dangr	Sequenced insulin (protein) from pork
1957 Francis Crick/ George Gamov	Explained how DNA functions to make protein
1958 Coenberg	Discovered DNA polymerase
1960	Isolation of m-RNA
1965	Classification of the plasmids
1966 Marshall Nirenberg/ Severo Ochoa	Determined that a sequence of three nucleotide bases determine each of 20 amino acids
1970	Isolation of reverse transcriptase
1971	Discovery of restriction enzymes
1972 Paul Berg	Cut sections of viral DNA and bacterial DNA with same restriction enzyme Spliced viral DNA to the bacterial DNA
1973 Stanley Cohen/ Herbert Boyer	Produced first recombinant DNA organism Beginning of genetic engineering
1975	Moratorium on recombinant DNA techniques
1976	National Institute of Health guidelines developed for study of recombinant DNA
1977	First practical application of genetic engineering human growth hormone produced by bacterial cells
1978 Genentech, Inc.	Genetic engineering techniques used to produce human insulin in E. coli. First biotech company on NY stock exchange. Stanford University First successful transplantation of mammalian gene. Discoverers of restriction enzymes receive Nobel Prize in medicine.
1979 Genentech, Inc.	Produced human growth hormone and two kinds of interferon DNA from malignant cells transformed a strain of cultured mouse cells new tool for analyzing cancer genes
1980	US. Supreme Court decided that manmade microbes could be

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	patented.
1983 Genentech, Inc.	Licensed Eli Lilly to make insulin, First transfer of foreign gene in plants
1986	First field trials of DNA recombinant plants resistant to insects, viruses, bacteria.

THERAPEUTIC APPLICATIONS OF BIOTECHNOLOGY

Monoclonal antibodies

Monoclonal antibodies (MAbs) are specialized forms of protein, designed to target other proteins, enzymes, or receptors that are elevated during disease. MAbs are used to facilitate delivery of drugs or toxins to pathologically altered cells and to carry enzymes to tumor surfaces to activate pro-drugs. Radiolabeled MAbs are used for site-directed delivery of radioisotopes. MAbs were first described in 1975 by Köhler and Milstein, and mass-scale production of MAbs for clinical investigation followed. [9] The development of MAbs as therapeutic agents was costly and time consuming, however, and generation of human MAbs was difficult.[10] These limitations led to the use of new technologies to redesign MAbs, which resulted in smaller, recombinant MAbs that closely resembled human immunoglobulins and retained the antigen-binding characteristics of the original murine MAbs.[10] These redesigned MAbs allowed for more adequate tumor penetration and elimination of immunogenicity problems. [10] Over time, the production of unlimited quantities of highly specific MAbs has facilitated their use for many diagnostic and treatment purposes. MAbs are prepared by various methods: (1) introducing a foreign antibody into an animal, which results in the formation of antibody-producing lymphocytes; (2) harvesting B lymphocytes from the spleen; (3) fusing antibody-producing B lymphocytes to cancer cells to impart the continuous reproductive characteristic of cancer cells; and (4) separating and cloning the hybrids to produce individual cell lines that secrete MAbs.[11]

The first MAb clinical trials began in the late 1970s, primarily in patients with hematologic malignancies. Therapeutic applications of MAbs have been particularly useful in oncology, with MAb use representing significant advancement compared with conventional methods of cancer therapy in the treatment of breast, gastrointestinal, and colorectal cancers; melanomas; and non-Hodgkin's lymphomas.[12] MAbs have also been used for imaging and in cancer therapy.

The remarkable efficacy of MAbs in the treatment of cancers in animals is the basis for continued research in human cancers.

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Signal-Transduction Inhibition

Recently, a better understanding of the molecular basis of cellular communication indicates that a number of diseases result from a malfunction of intracellular signaling. The activity of a particular signal-transduction pathway is often enhanced or inappropriately active in the diseased cell; study results suggest that blocking a signaling element that is overactive in a tumor cell but essential for normal cell function is a promising therapeutic approach.[13]. Signal-transduction malfunction results in proliferative diseases, such as cancers, atherosclerosis, and psoriasis, as well as inflammatory conditions, such as sepsis, rheumatoid arthritis, and multiple sclerosis[13].Signal transduction may be inhibited using reagents, such as small molecules, antibodies, DNA proteins, antisense RNA, and target-specific RNA ribozymes; in particular, this approach to therapy has been developed for protein tyrosine kinases (PTKs). PTKs is essential to normal cell growth, play a role in proliferative diseases when over expressed. They function in signaling, and their enhanced activity leads to a cellular abnormality, such as activation of genetic mutations or persistent stimulation of cell division by growth factors. [14]

Matrix Metalloproteinase Inhibition

Matrix metalloproteinases (MMPs) are a gene family of at least 15 structurally related enzymes responsible for the degradation of extracellular matrix components associated with angiogenic and metastatic processes. The proteolytic activity of MMPs is normally regulated by the tissue inhibitors of metalloproteinases (TIMPs); disturbance of the MMP and TIMP balance can result in pathologies such as rheumatoid arthritis, osteoarthritis, and atherosclerosis, as well as tumor growth and metastasis.[15] MMP over expression has been shown in prostate, lung, breast, and colon cancers and is five times higher in low-graded kidney tumor tissue than in normal tissue. [16]

Antisense Oligonucleotides

Antisense oligonucleotides are a novel class of therapeutic agents used in the prevention and treatment of gene-mediated disorders. This class of compounds was developed on the premise that inhibiting the process and translation of messenger RNA (mRNA) blocks the expression of target genes involved in pathologic processes. Gene expression is inhibited by hybridization of an oligonucleotide to sequences in the mRNA target by base-pairing rules. These base-pairing rules govern the interaction between the antisense oligonucleotide and the target, allowing the design of these compounds to target any gene of a known sequence.[17] Several types of antisense approaches have been developed: one uses antisense DNA and the other uses RNA. Different antisense compounds act at various stages in the synthesis of a biologically active target protein.[18]. Advantages of antisense approaches over conventional pharmacotherapy include extremely high specificity of antisense DNA and RNA for their target, ease of design, and requirement of information only on the nucleic acid sequence encoding a given protein." Antisense oligonucleotide therapy is being

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applied to oncology and hematology, and to cardiovascular, infectious, and viral diseases.[19]

Human Applications

Biotechnical methods are now used to produce many proteins for pharmaceutical and other specialized purposes. A harmless strain of Escherichia coli bacteria, given a copy of the gene for human insulin, can make insulin. As these genetically modified (GM) bacterial cells age, they produce human insulin, which can be purified and used to treat diabetes in humans. Microorganisms can also be modified to produce digestive enzymes. In the future, these microorganisms could be colonized in the intestinal tract of persons with digestive enzyme insufficiencies.[20]

Gene therapy – altering DNA within cells in an organism to treat or cure a disease – is one of the most promising areas of biotechnology research. New genetic therapies are being developed to treat diseases such as cystic fibrosis, AIDS and cancer.[21]

DNA fingerprinting is the process of cross matching two strands of DNA. In criminal investigations, DNA from samples of hair, bodily fluids or skin at a crime scene are compared with those obtained from the suspects. In practice, it has become one of the most powerful and widely known applications of biotechnology today. Another process, polymerase chain reaction (PCR), is also being used to more quickly and accurately identify the presence of infections such as AIDS, Lyme disease and Chlamydia.

Paternity determination is possible because a child's DNA pattern is inherited, half from the mother and half from the father. To establish paternity, DNA fingerprints of the mother, child and the alleged father are compared. The matching sequences of the mother and the child are eliminated from the child's DNA fingerprint; what remains comes from the biological father. These segments are then compared for a match with the DNA fingerprint of the alleged father.

DNA testing is also used on human fossils to determine how closely related fossil samples are from different geographic locations and geologic areas. The results shed light on the history of human evolution and the manner in which human ancestors settled different parts of the world.[22]

CURRENT OBSTACLES

The aim of any drug delivery is to deliver drug at the right time in a safe and reproducible manner to a specific target at the required level. For many drugs, however, these ideal requirements constitute hype rather than hope. The lack of suitable drug delivery systems not only has implications for conventional drug administration routes and dosage forms, but is becoming a drawback for the advance of novel therapeutic strategies like cell-therapy, RNA interference (rnai) Check E 2003and gene therapy. In gene therapy, a virus is used as a vector to deliver corrective and active genes into the cells of a patient.

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But, if the vector stitches itself into the genes of a cell it can cause uncontrolled mutations and induce cancer. this therapy is clearly not trivial, as evidenced by the tragic death in 1999 of a gene therapy trial volunteer Marshall E and more recently when two children administered gene therapy for severe combined immunodeficiency disease developed leukaemia Check E2002 similarly, if the promise of RNAi therapies is to be realized, scientists will need to develop ingenious systems to protect the small interfering RNAs (sirnas) in the bloodstream and to target them into the right cells.

SAFETY EVALUATION IN BIOTECHNOLOGY

The principles of preclinical safety evaluation are similar between conventional pharmaceuticals and biotechnology-derived pharmaceutical (biotech products). The difference lies in the way that these principles are put into practice. The biggest challenge in the preclinical assessment of biotech products has been coping with species specificity and the associated detection and implications of altered immune status and unpredicted pleiotropic activity. [23] The introduction of biotechnology-derived pharmaceuticals for clinical use has often required the application of unique approaches to assessing their safety in preclinical studies. There is much diversity among these products, which include the gene and cellular therapies, monoclonal antibodies, human-derived recombinant regulatory proteins, blood products, and vaccines. For many of the biological therapies, there will be unique product issues that may require specific modifications to protocol design and may raise additional safety concerns (e.g., immunogenicity). Guidance concerning the design of preclinical studies for such therapies is generally based on the clinical indication. Risk versus benefit decisions are made with an understanding of the nature of the patient population, the severity of disease, and the availability of alternative therapies. Key components of protocol design for preclinical studies addressing the risks of these agents include (a) a safe starting dose in humans, (b) identification of potential target organs, (c) identification of clinical parameters that should be monitored in humans, and (d) identification of at-risk populations. One of the distinct aspects of the safety evaluation of biotechnology-derived pharmaceuticals is the use of relevant and often nontraditional species and the use of animal models of disease in preclinical safety evaluation. Extensive contributions were made by the Center for Biologics Evaluation and Research to the ICH document on the safety of bio-therapeutics, which is intended to provide worldwide guidance for a framework approach to the design and review of preclinical programs. Rational, scientifically sound study design and early identification of the potential safety concerns that may be anticipated in the clinical trial can result in preclinical data that facilitate use of these novel therapies for use in humans without duplication of effort or the unnecessary use of animals. [24]

FUTURE TRENDS IN BIOTECHNOLOGY

Over the next two decades, discoveries in biotechnology and advances in gene therapy will transform the practice of medicine- the traditional treatment concepts of palliation, cure, and prevention

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will move toward human enhancement and capability The drug-discovery process will be even more accelerated, and new drugs will be developed based on the biologic cause or pathway of human disease. Development of gene chips containing the DNA representative of all the human genes will facilitate individual gene profiling and analysis of distinctive disease- specific gene patterns. These chips will allow clinicians to make more accurate diagnoses and recommend particular therapies with much greater certainty. Health care will evolve to a more sophisticated level of customization, enabling therapeutic selection precisely tailored to an individual's biochemistry. The advances expected to occur through biotechnology in the coming decade will have far-reaching effects on patients, clinicians, and payors, and will redefine the concept of medical practice in the new millennium.

The idea of merging biological and non biological systems

The idea of merging biological and non biological systems at the nano scale level is not a new one. The broad field of bio-conjugate chemistry is based on combining the functionalities of biomolecules and non-biologically derived molecular species for specialized use in applications ranging from markers for research in cell and molecular biology to biosensing, bioimaging and masking of immunogenic moieties to targeted drug delivery. Many current applications of nanostructures in biotechnology are a natural evolution of this approach. In fact, several of the 'breakthrough' applications recently demonstrated using nanostructure–biomolecular hybrids are in fact traditional applications originally addressed by standard molecular bio-conjugate techniques that have been revisited with these newly designed nanostructure hybrids.

EMERGING BIO TECH DELIVERY METHODS

The introduction of biotechnological methods for the production of drugs brought a revolution to the pharmaceutical field. Nowadays, drugs like insulin are produced by recombinant technology. This reduces not only the price for injectables but makes it simultaneously so relatively cheap that enough insulin is available at a sufficiently low price to go for other, more patient friendly administration routes, i.e. pulmonary delivery. After pulmonary delivery, the bioavailability of insulin is much lower compared to injection (only approximately 10%). [25, 26] Gene therapy and RNAi technologies are considered the medical treatments of the future. In fact, RNAi was announced as the scientific breakthrough of 2002. The hopes from these technologies have been partially dashed, however, following safety concerns and the lack of targeted gene and siRNA delivery.[3] To overcome this hurdle, researchers are studying a virus known as adenoassociated virus, which does not cause disease in animals or humans.[27] Furthermore, novel, harmless viral vectors [28] and non viral gene therapy systems such as the 'gene gun' [29] and liposomes are also under investigation. Finally, micro-fabricated systems combine the principles of micro technology and biology to provide sophisticated drug delivery systems that could provide advantages over existing technologies. Micromachining presents the opportunity to create multiple reservoirs of desired

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size to contain not just one, but many drugs or biomolecules of interest. The wide range of possibilities include implanted microchips for localized drug delivery[30] and nanoporous immuno-isolating devices for cell immobilization that are surrounded by microfabricated membranes with perfectly defined monodisperse pores in the nanometer scale. [31, 32]

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