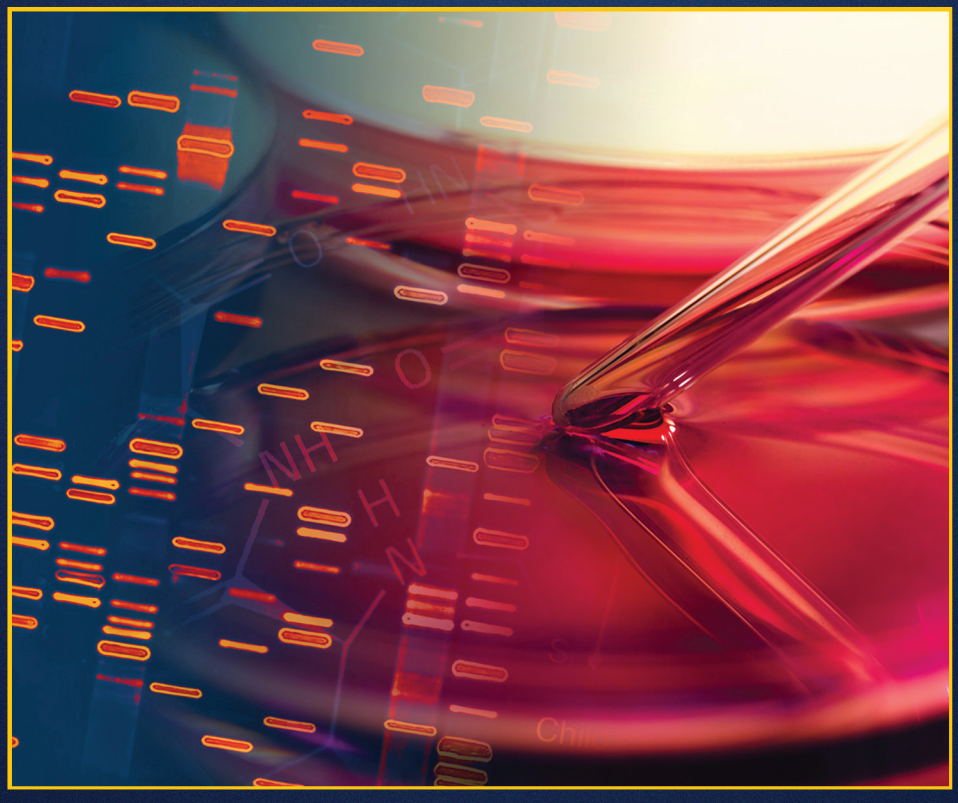
BIOTECHNOLOGY IN MEDICAL SCIENCES



Firdos Alam Khan

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Contents

Preface

Over the past few decades, we have seen an exponential growth in biotechnology products especially in the medical and health care sectors. Most prominently the discovery of penicillin (an antibiotic), recombinant insulin, and cell-based therapy have completely revolutionized the field of medical diagnosis and treatments. Biotechnology is a field of applied biology that involves the use of living organisms with engineering tools to develop useful therapeutic products. Biotechnology has applications in four major industrial areas: health care (medical), crop production and agriculture, nonfood (industrial) uses of crops and other products (e.g., biodegradable plastics, vegetable oil, biofuels), and environmental uses. Among these, the medical aspects of biotechnology have been extensively used in the development of health products and diagnostic tools. In medicine, modern biotechnology finds promising applications in such areas as drug production, pharmacogenomics, gene therapy, and genetic testing. The field of medical biotechnology keeps growing with new discoveries and products. It becomes necessary to have a book that addresses the basic to the advanced level and from laboratory to clinic levels of medical biotechnology in a lucid and concise way.

There are many books available in the market that discuss the applications and fundamentals of biotechnology, but there is no single book available that deals with all aspects of medical biotechnology. Many times, students and researchers need to refer to various books to get a holistic view of the recent progress in medical biotechnology. In this book, I collate and discuss topics that are associated with medicine. The book consists of 15 chapters. Each chapter begins with a brief introduction of the topic followed by significances and applications, including colorful illustrations to explain the significance of particular topics. I have also included the names and brief contributions of important scientists and a chapter-focused bibliography at the end of each chapter to help the reader to learn each topic with great ease and interest. Chapter 1 deals with an introduction to biotechnology in medical sciences. Chapter 2 discusses human diseases and epidemiology. Chapter 3 focusses on bacteriology and antibiotics. Chapter 4 furnishes details with regard to virology and vaccines. Chapter 5 deals with immunology and monoclonal antibodies. Chapter 6 provides details on recombinant DNA technology and therapeutic proteins. Chapter 7 discusses stem cell technology. Chapter 8 covers tissue engineering. Chapter 9 includes molecular diagnostics and forensic science. Chapter 10 contributes to gene therapy. Chapter 11 focusses on synthetic biology and nanomedicine. Chapter 12 contributes to pharmacogenomics. Chapter 13 discusses bioethics. Chapter 14 deals with biobusiness and intellectual property rights. And Chapter 15 discusses career opportunities.

Acknowledgments

First and foremost, I am grateful to Almighty Allah who gave me the strength to write a second book on topics in biotechnology. I am thankful to CRC Press and especially to Dr. Michael J. Slaughter, executive editor, for having complete faith in me and giving yet another opportunity to write this book. My first book titled *Biotechnology Fundamentals* was published in 2011 and has been adopted as a textbook in the United States, Canada, and Finland. I am thankful to many people who helped me in producing this book, especially to Stephanie Morkert, project coordinator, and Ed Curtis, project editor, Taylor & Francis Group, for their valuable help in completing the book in record time. I am also thankful to Syed Mohamad Shajahan for copyediting and to the entire team of CRC Press/Taylor & Francis Group for making this book a reality.

I would like to thank the entire management team, faculty, and staff of Manipal University, India and Dubai, for their constant support. I am thankful to all my teachers and mentors, especially to Professor Nishikant Subhedar and the late Professor Obaid Siddiqi, FRS, for their training.

I am thankful to all my friends, well-wishers, and colleagues for their support and cooperation. I am grateful to all my family members, especially my father, the late Nayeemuddin Khan, and mother, Sarwari Begum; my brothers Aftab Alam Khan, Javed Alam Khan, Intekhab Alam Khan, and Dr. Sarfaraz Alam Khan; my sisters Sayeeda Khanum, Kausar Khanum, Kahkashan Khanum, and Aysha Khanum; my wife Samina Khan; my sons Zuhayr Ahmad Khan, Zaid Ahmad Khan, and Zahid Ahmad Khan; and my daughter Azraa Khan; father-in-law Abdul Qayyum Siddiqui; and mother-in-law Uzma Siddiqui for their constant support and prayers.

I welcome your comments and suggestions to make this book error-free and more thought-provoking in the future. You may send your comments to the address below.
Enjoy reading!

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Firdos Alam Khan is a professor and chairperson in the School of Life Sciences, Manipal University Dubai Campus, Dubai, United Arab Emirates. He earned his doctorate from Nagpur University, India in 1997. He has more than 20 years of research and teaching experience.

Dr. Khan did his first postdoctoral research from the National Centre for Biological Sciences in Bangalore, India, where he worked on a World Health Organization sponsored research project titled Olfactory Learning in *Drosophila melanogaster*. In 1998, Dr. Khan moved to the United States and joined the Department of Brain and Cognitive Sciences, Massachusetts Institute of Technology, and worked on the research project titled Axonal Nerve Regeneration in Adult Syrian Hamster. In 2001, he returned to India and joined Reliance Life Sciences, a Mumbai-based biotechnology company, where he was associated with adult and embryonic stem cell research projects. Dr. Khan showed that both adult and embryonic stem cells have the ability to differentiate into neuronal cells. He also developed novel protocols to derive neuronal cells from both adult and embryonic stem cells.

Over the past 7 years, Dr. Khan has been associated with the Department of Biotechnology, Manipal University Dubai as chairperson. He teaches bioethics and biotechnology business courses to graduate and postgraduate students.

Dr. Khan's area of specialty in biotechnology includes stem cell technology, pharmacology, and neuroscience. He has written numerous articles in various national and international journals in the areas of neuroscience, neuropharmacology, and stem cell biology. He holds two US patents in the field of stem cell technology. He has been associated with various international scientific organizations such as the International Brain Research Organization, France, and Society for Neuroscience, USA. He has presented his research work at more than 23 different national and international conferences in India, Singapore, UAE, and the United States.

chapter one

Introduction to biotechnology in medical sciences

1.1 Introduction

The term medical biotechnology is the application of living organisms or cells or tissues to produce pharmaceutical and diagnostic products that help to treat and to prevent the progress of human diseases. One of the best-known examples of medical biotechnology is the production of antibiotics for treating various bacterial infections; these antibiotics are produced by using known microorganisms. Similarly, over the past few decades various other types of products have been produced, which include biopesticides, pest-resistant crops, and bioremediation techniques and many more. The most remarkable discovery made in recent times is the production of human proteins (insulin) outside of the human body, which has completely revolutionized the medical treatment modality. The synthesis of human insulin and growth hormones are considered to be the rewards of modern biotechnology. In both insulin and growth hormone production, scientists have used recombinant DNA technology to be administered in patients suffering from various incurable diseases such as diabetes and genetic disorders. It has been suggested that one of the major problems in treating patients with cancer and diabetes conditions is not being able to completely eradicate the disease. The only available option for any physician is to either go for drug therapy or surgical interventions to give symptomatic relief.

1.2 What is biotechnology all about?

The field of biotechnology has been in use for ages in various forms, which include the growing of better crops (agricultural biotechnology) and animal breeding (animal biotechnology). Similarly, the use of biotechnology has been around for thousands of years, especially the application of microorganisms in the production of cheese and yogurt (food biotechnology). In addition, the tools of biotechnology have been implied in animal husbandry, to develop pest-resistant crops, bioremediation (environmental biotechnology), as well as in bioethanol production. But the most promising application of biotechnology is found to be in the medical field by generations of biotherapeutics (insulin, growth hormones) and diagnostics tools (PCR, FISH, micro-array technique). Before we discuss various applications of medical biotechnology, let us briefly go through the historical aspects of biotechnology and this information will make you to understand the field better. It all began with the discovery made by Sir Alexander Fleming in the year 1918 where he observed that the mold *Penicillium* inhibited the growth of human skin disease-causing bacteria called *Staphylococcus aureus.* The discovery by Sir Alexander Fleming lead to the making of antibiotics that we use today. These antibiotics are highly recommended and extensively used medicinally for bacterial infections (Figures 1.1 and 1.2). These antibiotics are basically substances produced by microorganisms that normally inhibit the growth of

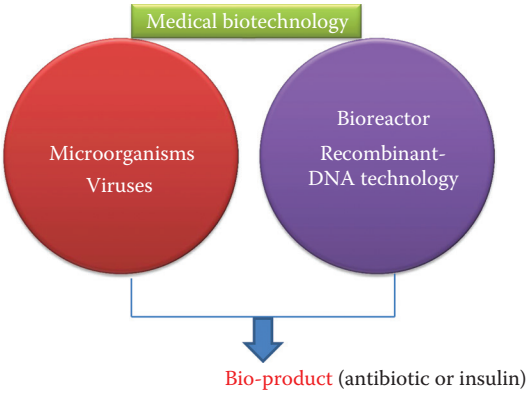

Figure 1.1 Application of microorganisms and viruses in making of medicine using bioreactor and recombinant DNA technology.

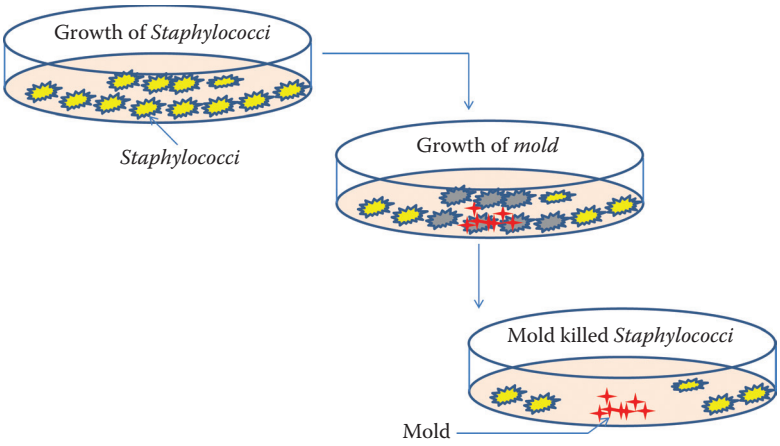

Figure 1.2 The pioneering experiment conducted by Dr. Alexander Fleming resulted in the discovery of the penicillin drug, an antibiotic drug.

other microorganisms. Later on, antibiotics became widely available as a drug for treating microbial infections in human beings, especially with the development of penicillin (Figure 1.3) as the most used antibiotic. Currently, a variety of microorganisms have been used to generate thousand liters of antibiotic drugs by using advanced biotechnology tools.

The field of biotechnology has taken a leap with the discovery of the double-helix structure of the deoxyribose nucleic acid (DNA) molecule and the credit goes to one research publication titled "This structure has novel features which are of considerable biological interest" authored by James Watson and Francis Crick in 1953, which claimed to discover the structure of the human DNA helix, the molecule that carries genetic information from one generation to the other. Nine years later, in the year 1962, they shared the Nobel Prize with Wilkins for cracking one of the most important of all biological puzzles. This discovery has led to the birth of genetic mapping, manipulation, and genetic engineering-type fields. Surprisingly, with the help of genetic engineering, the gene of interest can be cut and inserted into the genome of other living organisms (microorganisms and viruses) and

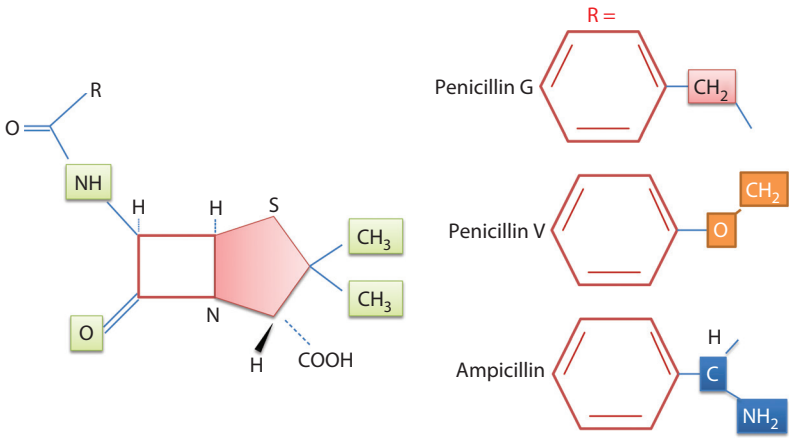

Figure 1.3 Structures of penicillin G, penicillin V, and ampicillin.

this process of gene insertion and manipulation is called recombinant DNA technology. Over the past few years recombinant DNA technology has been extensively employed to generate therapeutic products (insulin and growth hormones) for treating human diseases. With a rapid increase in the number of patient's worldwide, there has been a tremendous scope to identify and create medicine for various human diseases. Thankfully with the availability of biotechnology tools, now it is possible to develop therapy for various diseases. In Table 1.1, major biotechnology-related discoveries and milestones are listed to appreciate the contribution of biotechnology in human health care.

1.3 Medical products developed by using biotechnology tools

One of the salient features 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). We have briefly discussed below some of the major biotechnology-based products which have been widely used around the world.

1.3.1 Antibiotics

Penicillin is one of the earliest discovered antibiotics which is basically derived from molds such as *Penicillium*. Penicillin was discovered by Scottish scientist and Nobel laureate Sir Alexander Fleming in 1928. It all started with a basic experiment where Sir Fleming noticed a Petri dish containing *Staphylococcus* plate culture which he had mistakenly left open was contaminated by blue-green mold, which had formed a visible growth. But surprisingly he also found that there was a circle of inhibited bacterial growth around the mold. Later on, Sir Fleming hypothesized that the mold was releasing a substance that was preventing the growth and could contain a substance with antibiotic properties. In order to prove his hypothesis, he grew a pure culture and discovered the first antibiotic substance from the Penicillium mold, known as *Penicillium notatum*. Sir Alexander showed that *P. notatum* when grown in the appropriate substrate caused the release of chemical substances and these chemical substances were named as antibiotics. He later on named

Table 1.1 Milestones in Medical Biotechnology

Year	Nature of discovery/milestone
1882	Chromosomes discovered in salamander larva
1944	DNA is a hereditary material
1963	Genetic materials decoded
1971	The world's first biotech company is founded in California, US
1979	First biotech product human growth hormone, which is also becoming the first recombinant biotech drug manufactured and marketed by a biotechnology company
1980	Genentech becomes the first biotech company to go public, generating $35 million in its initial public offering
1983	Stanford School of Medicine becomes the first to screen blood to prevent AIDS transmission
1984	World's first DNA fingerprinting technique is developed
1984	Chiron Corporation announced the first cloning and sequencing of the entire HIV virus genome
1984	Genentech obtains USFDA approval to market human growth hormone, the first recombinant product to be sold by a biotechnology company
1986	The USFDA awards Chiron Corporation, a license for the production of first recombinant vaccine to battle the hepatitis B virus
1988	The "Harvard Mouse" becomes the first mammal patented in the United States
1991	Cancer patients are treated with a gene therapy that produces the tumor necrosis factor, a natural tumor-fighting protein
1995	The first full gene sequence of a living organism other than a virus is completed for the bacterium hemophilic influenza
1997	A sheep named "Dolly" becomes the first mammal cloned
2002	First vaccine against cervical cancer development

that chemical substance as penicillin. Soon after this discovery, penicillin was considered to be the most effective drug against bacteria (Gram-positive bacteria), and not at all effective against Gram-negative bacteria. The discovery of penicillin marks the beginning of the antibiotic production and so far more than 200 different types of antibiotics have been produced (Figure 1.3).

1.3.2 Recombinant insulin

Another discovery which was made in the medical field was the recombinant DNA technology, which has completely revolutionized disease treatments especially in the Type 1 diabetes mellitus, where insulin-producing cells become dysfunctional and do not produce sufficient amount of insulin to regulate blood-sugar level. In a healthy human being, insulin regulates glucose metabolism in the body. In diabetic conditions, the level of insulin decreases which causes the elevation of the blood sugar level and this clinical condition is known as diabetes mellitus. One of the best treatments of diabetes mellitus is insulin injections, where insulin is injected into the patient's body and the insulin regulates the blood-sugar level. The insulin is either synthesized chemically or produced by recombinant DNA technology. These synthesized insulins are used medically to treat patients with Type 1 diabetes mellitus, whereas patients with Type 2 diabetes mellitus are insulin resistant.

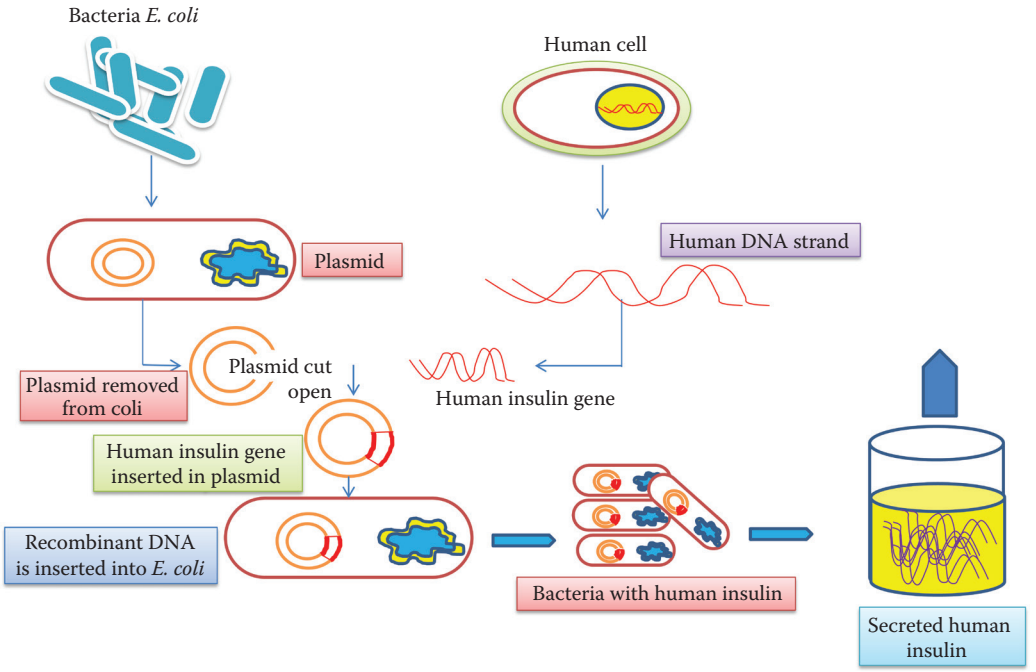

Figure 1.4 Making of human insulin by recombinant DNA technology.

However, some patients with Type 2 diabetes may eventually require insulin treatment if other medications fail to control blood glucose levels adequately, though this is somewhat uncommon. With the advent of recombinant DNA technology, it became possible to make insulin outside the human body and this biosynthetic insulin is now manufactured for widespread clinical use. More recently, scientists have succeeded in introducing human insulin gene into plants in order to produce human insulin in plants, and the safflower plant has been used for this purpose. In addition to recombinant DNA technology, there are other ways to synthesize human insulin outside the human body and they are usually referred to as insulin analogues and these analogues are chemically synthesized (Figure 1.4). One of the major hurdles in the diabetes treatment is the delivery of insulin, as insulin cannot be taken orally because it will not be absorbed properly and will lose its biological activity. In recent years, an attempt has been made to develop insulin which can be safely given through the oral route or sublingually. It has been reported that insulin can be taken as subcutaneous injections; few companies have also attempted to develop an oral form of insulin and trials are underway.

1.3.3 Vaccines

The vaccine is basically a biological preparation that improves the human immune defense system to fight diseases. The vaccines normally contain an agent that diligently resembles a disease-causing bacteria, and is frequently produced from debilitating or killed forms of the microorganisms. Upon injection into the human body, the vaccine can stimulate the body's immune system to recognize the agent as a foreign body, and recognize it, so that the immune system can recall and destroy or kill any of these microorganisms that later

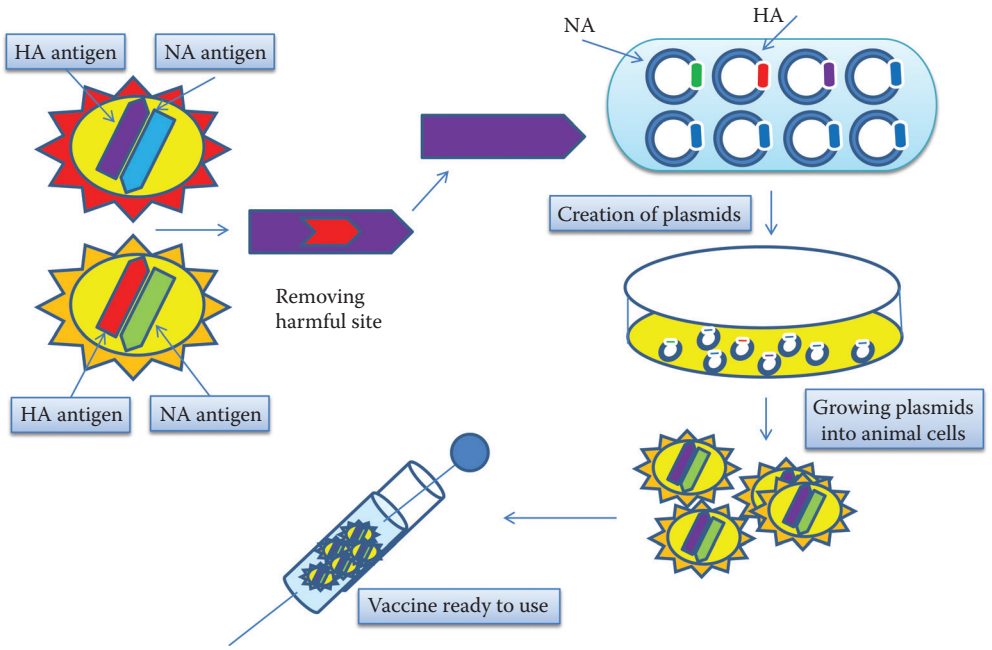

Figure 1.5 Making of vaccine.

infect the human body. In a simpler way, the vaccine basically trains the human body to defend or fight or kill the microorganisms. The vaccines are classified based on its application; the vaccines that are used to prevent the effects of an upcoming infection by any natural or wild pathogen are called prophylactic, and the vaccines which are used against cancer are called therapeutic vaccines (Figure 1.5).

The story of vaccine development dates back to the seventeenth century when Edward Jenner found a milkmaid infected with smallpox, and a few days later he took pus from the hand of the milkmaid with cowpox and inoculated an eight-year-old boy with it. After six weeks he found that the boy did not contract smallpox. After a few years, Sir Louis Pasteur adapted Jenner's idea of developing a rabies vaccine and, subsequently, vaccine development progress started and became a matter of national concern. After that obligatory vaccination laws were passed in various countries around the globe. During the twentieth century, we have seen an introduction of several successful vaccines against diseases such as diphtheria, measles, mumps, and rubella. Interestingly, the major achievement was done in the 1950s when the polio vaccine was made and clinically used; also vaccines were synthesized to eradicate smallpox.

1.3.4 Monoclonal antibodies

Another milestone achieved in the beginning of the twentieth century was the making of monoclonal antibodies, which was proposed by Paul Ehrlich. He suggested that drug compounds can be accurately, precisely, and specifically delivered along with monoclonal antibodies. Monoclonal antibodies are mono-specific antibodies as they are made by identical cells that are all clones of a distinctive parent cell (Figure 1.6). Currently, it is possible to produce monoclonal antibodies that specifically bind to that antigen and have a variety

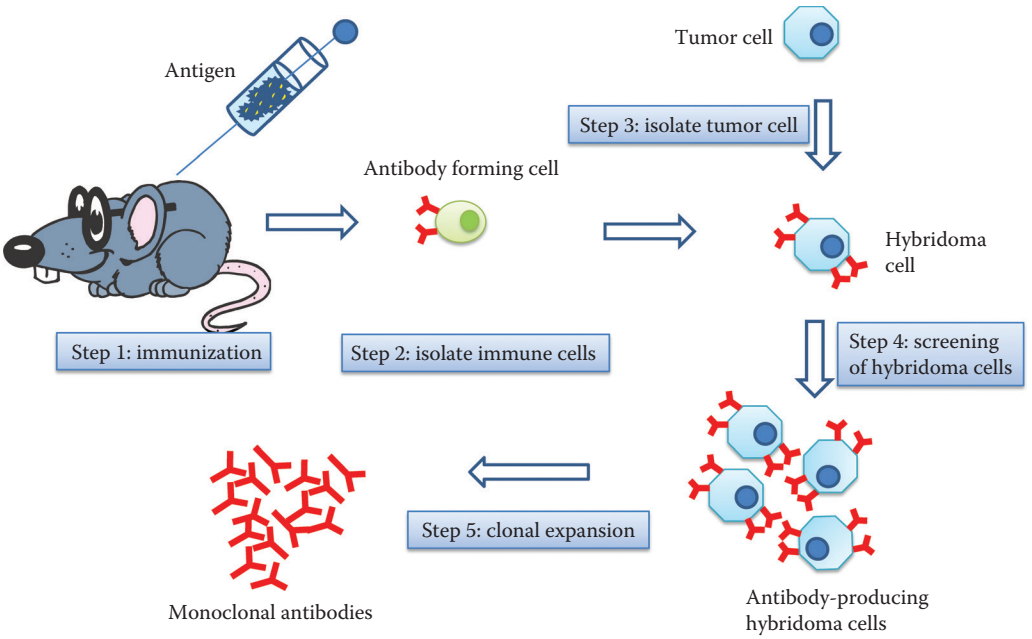

Figure 1.6 Making of monoclonal antibodies.

of applications, especially to detect or purify that substance (antigen), and has become an important tool in diagnostics and biomedical research.

In diagnostics and biomedical research, monoclonal antibodies are very useful tools. First, they are extremely specific; that is, each antibody binds to the specific site of the antigen. Second, some antibodies, once activated by the occurrence of a disease, continue to confer resistance against that disease; classic examples are the antibodies to the childhood diseases chickenpox and measles. Another important application of monoclonal antibodies is to develop vaccines against various diseases. As you are aware, a vaccine is made from bacteria or viruses either killed or inactivated. Upon introduction into the human body this vaccine stimulates the production of antibodies against the antigens to fight back the diseases. The production of monoclonal antibodies involves human and mouse hybrid cells and this technology is known as hybridoma technology. During monoclonal antibody production, tumor cells are merged with mammalian cells that produce an antibody against a particular antigen. The result of these merged tumors with mammalian cells is a called hybridoma, which can frequently produce antibodies. These antibodies are called monoclonal antibodies because they come from only one type of cell; whereas, the antibodies that are produced by conventional methods contain many kinds of cells and are called polyclonal antibodies.

1.3.5 Bioengineered tissues

Over the last decade, tissue engineering became the most fascinating medical field, especially in body-parts reconstruction or cosmetic surgery areas. The basic concept about tissue engineering is to make human tissues through in vitro methods under controlled laboratory conditions and the making of such tissues is called bioengineered tissues. The

Table 1.2 Research in Tissue Engineering

Bioengineered cells/tissues	Properties
Biomaterials	These include novel biomaterials that are designed to direct the organization, growth, and differentiation of cells in the process of forming functional tissue by providing both physical and chemical cues
Biomolecules	These include angiogenic factors, growth factors, differentiation factors, and bone morphogenic proteins
Engineering design aspects	These include two-dimension cell expansion, three-dimension tissue growth, bioreactors, vascularization, cell and tissue storage and shipping
Biomechanical aspects of design	These include properties of native tissues, identification of minimum properties required of engineered tissues, mechanical signals regulating engineered tissues, and efficacy and safety of engineered tissues
Informatics to support tissue engineering	These include gene and protein sequencing, gene-expression analysis, protein expression and interaction analysis, quantitative cellular image analysis, quantitative tissue analysis, in silicon tissue and cell modeling, digital tissue manufacturing, automated quality assurance systems, data mining tools, and clinical informatics interfaces

definition of tissue engineering covers a broad range of applications in the healthcare field, however, in practice the term tissue engineering closely relates to repair or replacing portions of, or whole, human body tissues which include neurons, cardiomyocytes, bone, and cartilage. The bioengineered tissues are constructed by integrating certain mechanical and structural properties for proper functioning in the human body, so one can say that bioengineering is basically the use of a combination of cells, engineering, and biocompatible materials, and is finally suitable to improve or replace biological functions (Table 1.2). The bioengineered field was once categorized as a sub-field of biomaterials, but having grown in scope and application it is considered as a field in its own right (Figure 1.7). Tissue engineering is an emerging multidisciplinary field involving biology, medicine, and engineering that is likely to revolutionize the ways we improve the health of millions of people worldwide by repairing, maintaining, or enhancing tissue and organ function. The tissue engineering can also have diagnostic applications where the human cells or tissues are used to test drug metabolism and drug uptake, toxicity, and pathogenicity.

1.3.6 Adult stem cell therapy

Over the last few years, there has been tremendous interest in adult stem cells for the autologous cell transplantation as these specialized cells have the potential ability to repair or restore the dysfunctional cells or tissues in the human body afflicted with various diseases that include blood cancer and neurological disorders. The stem cells are generally found in all multicellular organisms and they are characterized by the ability to renew themselves through cell division and differentiate into a varied range of specialized cell types. Moreover, mammalian stem cells may be broadly classified into two major types: adult stem cells and embryonic stem cells (ESCs) that are isolated from the inner cell mass of blastocysts.

One of the main roles of adult stem cells is that they remain in an undifferentiated state in the human body and multiply by cell division to replenish dying cells and restore damaged tissues and organs. The adult stem cells are also known as somatic stem cells;

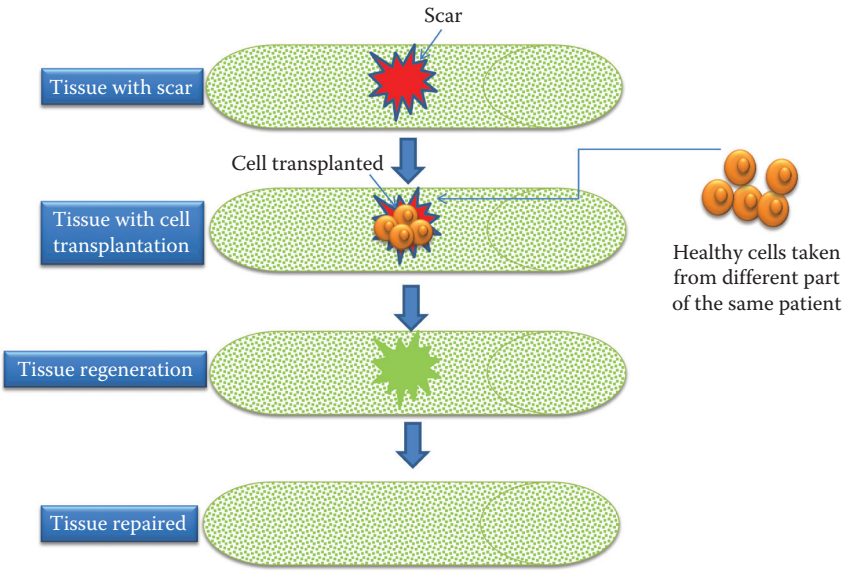

Figure 1.7 Tissue engineering.

these stem cells can be found in both juvenile and adult ages. Moreover, adult stem cells are specialized cells that have the capability to divide and generate all cell types of the organ from which they originate, and can also possibly regenerate the entire organ. Unlike ESCs, the use of adult stem cells is not contentious as they are derived from adult source or tissue rather than by killing human embryos. Moreover, 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) employing bone marrow transplants. Interestingly, the majority of the government funding especially in the United States is confined to adult stem cell-based research. Among adult stem cells, hematopoietic stem cells (HSCs) and mesenchymal stem cells (MSCs) are found to be the most successful stem cells because they can be clinically used in patients. These stem cells can be directly injected or placed at the site of repair or injected through vascular delivery (Figure 1.8).

1.4 Emerging trends

In addition to above-mentioned fields, there are other fields which are emerging and are in the initial phase of development. These emerging fields will provide improved technology or tools to treat or diagnose various diseases.

1.4.1 Embryonic stem cells

Over the last few years ESCs have been a highly discussed and debated scientific topic at all levels which include public life, scientific forums, ethical, legal, and political platforms. Before we discuss the ethical or legal issues associated with ESCs, we will first learn what ESCs are all about. The ESCs are basically pluripotent stem cells isolated from the inner cell mass of the blastocyst, which is an early-stage embryo. Moreover, human embryos reach the blastocyst stage 4 to 5 days post fertilization, at which time they consist of about

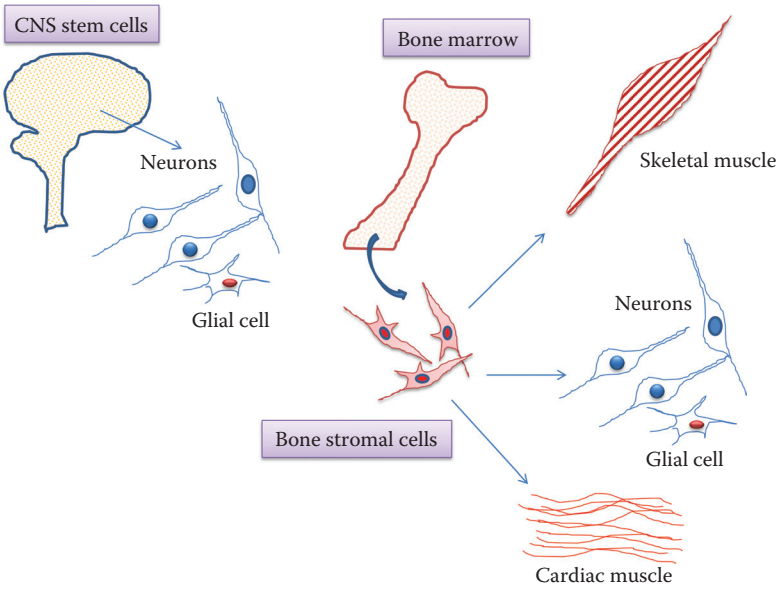

Figure 1.8 Adult stem cells.

50–150 cells. In addition to this, ESCs are adept at propagating themselves for an indefinite period under controlled culture situations. The culture and propagation of ESCs through in vitro methods is considered to be the best technology to culture human cells in large quantities and to be engaged as valuable tools for both research and cell-based therapy (Figure 1.9).

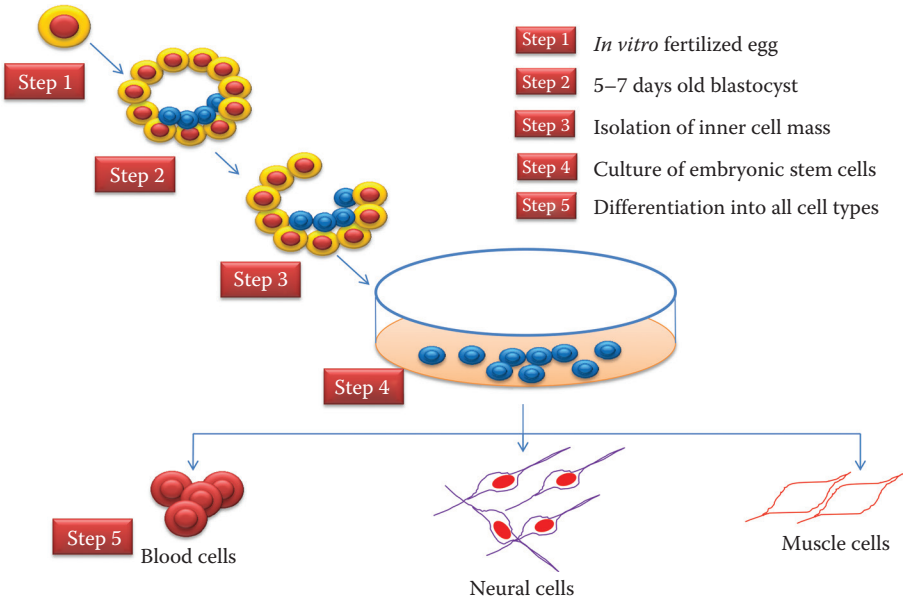

Figure 1.9 Embryonic stem cells.

One of the best applications of ESCs is to provide a large supply of required cells to be used as cell-based therapy and tissue replacement in patients who are suffering from degenerative diseases. Moreover, a number of diseases that could possibly be treated by ESCs are cancers, diabetes, Parkinson's disease, Alzheimer's disease, stroke, blindness, and spinal cord injuries. In contrast to its various therapeutic applications, the issues related to ESCs need to be resolved before making them useful for human use. These issues are the problem of immune rejection associated with allogeneic stem cell transplantation. These problems can be solved by using adult stem cells in autologous transplantation. Besides, cell-based therapy, the ESCs can be used as diagnostic tools to study an early human development and genetic disease, as well as in vitro drug testing. Other issues related to ESCs transplantation is that there is a possibility that transplanted stem cells could form tumors and have the possibility of becoming cancerous if cell division continues uncontrollably. Contrary to this, supporters of ESCs research argue that such research should be continued because the resultant research findings will have significant medical potential. The recent development of induced pluripotent stems cells (iPSc) has created tremendous interest among scientists, as adult stem cells can be converted into pluripotent stem cells and there is no need to kill the human embryos.

In spite of all controversies over ethical use of human ESCs, on January 23, 2009, Geron Corporation, USA, has received an approval from US Food and Drug Administration (FDA) to test ESCs in humans. The Phase 1 clinical trial for transplantation of a human-ES-derived cell population into spinal cord-injured individuals became the first human ES cell human trial. The Phase 1 clinical study was conducted on eight to ten paraplegic patients who have had their injuries no longer than two weeks before the trial began, and stem cells were injected before the formation of scar in the tissue. Nevertheless, the researchers are stressing that the injections are not expected to fully cure the patients and restore all mobility. The success of such clinical trials goes back to the experiments conducted by University of California, USA, and which was supported by Geron Corporation of Menlo Park (Biotechnology Company), California. The results of an animal study showed that there was functional improvement in locomotive recovery in spinal cord-injured rats after stem cell transplantation. Interestingly, the first trial is mainly for testing the safety of cell-based transplantation. However, the trial had been put on hold in August 2009 due to apprehensions made by the FDA regarding a small number of microscopic tumors found in several treated rat models, but the hold has been lifted since.

1.4.2 Human genome project

The Human Genome Project (HGP) was an international collaborative research project with a primary aim to decode the human DNA genome. The main objective of the HGP was to map about 20–25 k genes of the human genome from both a physical and functional perspective. The initial task of the project was to identify the full set of genetic instructions enclosed inside human DNA. The project began with the end of several years of work supported by the United States Department of Energy (DoE). It has been reported that the $3-billion project was founded in 1990 by the DoE and the US National Institutes of Health. In addition to the United States, other countries also took part in the HGP, including the United Kingdom, France, Germany, Japan, China, and India. The team of scientists worked hard over the years and drafted a proposal for the genome project and the final sequencing of the human genome was done in 2006. Although the objective of the HGP is to understand the genetic organization of the human genome, the project has worked with other nonmammalian organisms.

1.4.2.1 Significance of the genome project

The reason for starting the HGP was to understand the role of genes in the development of diseases. There are reports that suggest more than 3000 genetic disorders known to be caused by genetic mutations. With the current treatment modalities, these genetic disorders cannot be cured without knowing the real cause. And to know the cause we have to know the role of genes in the diseases. Recent efforts have been made to find out the causes of cancer with genetic tools, but not much success was achieved. It has been recommended that information gained from the HGP would help to understand the genetic cause of the devastating illnesses, which include Parkinson's disease, schizophrenia, or Alzheimer's disease.

The genetic mutations play a role in many genetic diseases that include heart disease, diabetes, immune system disorders, and congenital defects. These diseases are thought to be the consequence from complex collaborations between genes and environmental factors. When genes for diseases have been identified, researchers can study how environmental factors, food, drugs, or pollutants can influence those genes. The location of the gene is important in identifying the type of protein which is produced by a particular gene. It has been reported that understanding the mechanism of a genetic disease is an important step in curing genetic diseases. It has been suggested that one day it may be possible to treat genetic diseases by gene therapy. Besides therapeutic applications, the information gained from the HGP can help to know the reason for the pluripotency nature of ESCs and how these ESCs can be differentiated into many different specialized cells such as muscle cell, neural cells, or hepatocytes.

1.4.3 RNA interference technology

The RNA interference (RNAi) is a natural process that cells dictate to turn down or go to a silent state; such activity cell is regulated by a specific gene known as RNAi. The RNAi technology provides excellent tools to study the role specific gene in the development, causes, and progression of particular diseases, and has taken the biomedical community by storm. It has been reported that RNAi was first noticed in the plant *Petunia* when plant biologists tried to develop the purple color of the flower by introducing a pigment-producing gene. Surprisingly, the gene which was introduced to intensify color in the plants was found to suppress the color. In the end the resulting flowers developed white patches or became completely white. Surprisingly, a few years later another group of researchers observed a similar kind of gene-silencing effect in *Caenorhabditis elegans*. Later on, it has been reported that the gene-silencing effect is caused by the presence of double-stranded RNA, and this double-stranded RNA is normally not found in healthy cells.

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. Interestingly, bioengineered plants such as *FlavrSavr* tomatoes and two cultivars of *ring-spot*-resistant papaya were originally developed by employing antisense technology.

One of the pronounced applications of RNAi technology is its application in medicine. Over the past few years, efforts have been made to understand RNAi's role in normal and diseased cells, and also to use the RNAi technology for use in medical therapies. It

has been suggested that human disease progression can be blocked by using RNAi-based therapies to turn down the activity of genes. Cancer, for example, is frequently caused by over-excited genes in the cells, and retarding their activities could stop the disease progression. Over the last few years, several pharmaceutical companies are using RNAi-based therapies to treat for various forms of cancer. 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. Using RNAi technology, the activity of a particular gene can be knocked down which will help us to understand its role in the disease development and progression. For many years, researchers have been studying how proteins regulate gene activity. Now with the help of RNAi technology, it would be possible to discover the role of proteins in gene regulation.

1.4.4 Phage therapy

Phage therapy is generally to treat pathogenic infections caused by microorganisms and bacteriophages. Phages are basically viruses that enter bacterial cells and disturb bacterial metabolism causing the bacterium to lyse. 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. Unlike antibiotics, phage therapies have special advantages for localized use in humans because they 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. With the increasing incidence of antibiotic-resistant bacteria in humans, there is a need to apply phages in treating various kinds' of infections. In addition, phage therapy has many potential applications that include medicine, dentistry, veterinary science, and agricultural science. In addition, bacteriophages are found to be much more specific than antibiotics, and this bacteriophage do not cause any harmful effects on the host organism such as human, animal, or plant, but instead maintains a healthy relationship with beneficial bacteria in the body.

Over the last few years, it has been reported that phages are being used to treat bacterial infections in the patients and especially in those patients who do not respond to antibiotics. It has been found that these phage therapies tend to be more successful than antibiotics because there is a biofilm covered by a polysaccharide layer, which antibiotics typically cannot penetrate. In the West, no therapies are currently authorized for use on humans, although phages for killing food poisoning bacteria such as *Listeria* are now in use. 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.

1.4.5 Recombinant DNA technology

Recombinant DNA (rDNA) technology is one of the most sought-after technologies that has completely revolutionized the current treatment modalities, especially the use of humanized insulin for diabetes treatment. The rDNA technology soon to be developed can also be used in the treatment of diseases such as diabetes, genetic disorders, cystic

fibrosis, cancer, and sickle cell anemia. The organisms that have been developed by using rDNA technology will be used to produce new vaccines, monoclonal antibodies, enzymes, and hormones. It has been suggested that biotechnology be used to enhance breeding in plants and animals. In addition, rDNA technology can also be used to develop disease and herbicide-resistant plants, disease-resistant animals, seedless fruits, and quick-growing chickens.

1.4.6 Biochips

With the advent of information technology, it is possible to store biological information in a chip format which is called as a biochip. Biochip is a chip made of metal and designed to work inside the human body to treat and to diagnose the disease. There are various applications of biochips, which include biochips that can be implanted in the body and used for delivering precise amounts of drugs to the affected organs. Biochips can also be used as biosensors to monitor levels of enzymes, monoclonal antibodies, proteins in the human body, to detect hazardous substances, and to monitor blood components in the body.

1.4.7 Gene therapy

To treat genetic disorders, the default or dysfunctional gene can either be replaced with a new gene or repaired with gene therapy. In brief, the gene therapy involves correcting defects in genes and in this process a normal/healthy gene is introduced to replace a defective gene. Gene therapy is still under development stages and various clinical trials are underway around the world. Its success will be based on the successful cure of genetic diseases by gene therapy.

1.4.8 Liposome-based drug delivery

One of the major challenges of current drug therapy is to successfully transport the drugs to targeted sites in the human body. In recent times researchers have made efforts to develop a system to accurately and precisely release the drugs at the sites and one of the approaches is to transport the drug using liposomes as a vehicle. Liposomes are basically tiny vesicles made out of the same material as the human cell membrane. It has been suggested that liposomes can be packed with drug molecules and be used to deliver drugs in a precise and accurate manner to the affected regions of cells, especially in the treatment of cancer, without affecting the surrounding healthy cells.

1.4.9 Bionanotechnology

Bionanotechnology is a term that refers to the intersection of nanotechnology and biology. This discipline helps to indicate the merger of biological research in various fields of nanotechnology. The concepts that are enhanced through nanobiology include: nanodevices, nanoparticles, and nanoscale that occurs within the discipline of nanotechnology. Moreover, this technical method in biology allows scientists to imagine and create systems that can be used for biological research. Biologically inspired nanotechnology uses biological systems as the inspiration for technologies not yet created. The most important objectives that are frequently found in nanobiology involve applying nanotools to relevant medical or biological problems and refining these applications. Moreover, developing new tools for the medical and biological fields is another primary objective in nanotechnology.

Interestingly, 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.

Recently, the use of microorganisms to synthesize functional nanoparticles have been of great interest. Microorganisms can change the oxidation state of metals. Moreover, these microbial processes have opened up new opportunities for us to explore novel applications, for example, the biosynthesis of metal nanomaterials. In contrast to chemical and physical methods, microbial processes for synthesizing nanomaterials can be achieved in aqueous phases under gentle and environmentally benign conditions. This approach has become an attractive focus on current green bionanotechnology research toward sustainable development.

The applications of bionanotechnology are extremely widespread and nanobiotechnology is best described as helping modern medicine progress from treating symptoms to generating cures and regenerating biological tissues. Three American patients have received whole cultured bladders with the help of doctors who use nanobiology techniques in their practice. Also, it has been demonstrated in animal studies that a uterus can be grown outside the body and then placed in the body in order to produce a baby. Furthermore, there is also funding for research into allowing people to have new limbs without having to resort to prosthesis. Artificial proteins might also become available to manufacture without the need for harsh chemicals and expensive machines. It has even been surmised that by 2055, computers may be made out of biochemical and organic salts. Another example of current nanobiotechnological research involves nanospheres coated with fluorescent polymers. Moreover, investigators are seeking to design polymers whose fluorescence are quenched when they encounter specific molecules. Different polymers would detect different metabolites. Interestingly, the polymer-coated spheres could become part of new biological assays, and the technology might someday lead to the particles which could be introduced into the human body to track down metabolites associated with tumors and other health problems. Another example, from a different perspective, would be an evaluation and therapy at the nanoscopic level, that is, the treatment of nanobacteria (25–200 nm sized) as is done by NanoBiotech Pharma.

Despite the fact that biological systems are inherently nano in scale, nanoscience must merge with biology in order to deliver biomacromolecules and molecular machines that are similar to the human body or organ. In the twenty-first century, scientists have developed the technology to artificially tap into nanobiology. This process is best described as organic merging with synthetic. Interestingly, colonies of live neurons can live together on a biochip device. The self-assembling nanotubes have the ability to be used as a structural system as they would be composed together with rhodopsins, which would help the optical computing process and also help with the storage of biological materials. The most fascinating aspects would be of DNA as the software for all living things, which can be used as a structural proteomics system—a logical component of molecular computing.

1.5 Summary

The term biotechnology generally refers to the integration of technology or engineering knowledge in biological science. The fundamental applications of biotechnology are to produce arrays of products which are beneficial for human consumptions and also for making our environment healthy and pollution free. In particular, the human body is

very critical and requires proper care, diagnosis, and treatment. There are many diseases (malaria, typhoid, cold flu, fever, etc.) which are being treated by using synthetically designed medicines (tablets, capsules, syrups, etc.), but there are many diseases (diabetes, genetic disorders) that cannot be treated with synthetically designed medicines and require biotechnological interventions like insulin and gene therapy. Medical biotechnology primarily deals with providing solutions to major human diseases either in the form of diagnostic kits or in the form of vaccines, antibiotics, monoclonal antibodies, cell-based therapy, or gene therapy. One of the best examples of modern medical biotechnology is the development of antibiotics and vaccines, which in fact is experienced by almost every human being.

1.6 Scholar's achievement

Sir Alexander Fleming: Sir Alexander Fleming, FRSE, FRS, FRCS (Eng) (August 6, 1881 to March 11, 1955) was a Scottish biologist, pharmacologist, and botanist. He wrote many articles on bacteriology, immunology, and chemotherapy. His best-known discoveries are the enzyme lysozyme in 1923 and the antibiotic substance penicillin from the mold *Penicillium notatum* in 1928, for which he shared the Nobel Prize in Physiology or Medicine in 1945 with Howard Florey and Ernst Boris Chain.

James Watson and Francis Crick: Francis Crick and James D. Watson, the two scientists who discovered the structure of DNA in 1953. James Dewey Watson, KBE, ForMemRS (born April 6, 1928), is an American molecular biologist, geneticist, and zoologist, best known as a co-discoverer of the structure of DNA in 1953 by Francis Crick. Watson, Crick, and Maurice Wilkins were awarded the 1962 Nobel Prize in Physiology or Medicine "for their discoveries concerning the molecular structure of nucleic acids and its significance for information transfer in living material." Francis Harry Compton Crick, OM, FRS (June 8, 1916 to July 28, 2004) was an English molecular biologist, biophysicist, and a neuroscientist.

Edward Jenner: Edward Anthony Jenner, FRS (May 17, 1749 to January 26, 1823) was an English physician and scientist from Berkeley, Gloucestershire, who was the pioneer of smallpox vaccine. He is often called "the father of immunology," and his work is said to have "saved more lives than the work of any other man."

Sir Louis Pasteur: Louis Pasteur was a French chemist and microbiologist who was one of the most important founders of medical microbiology. He is remembered for his remarkable breakthroughs in the causes and prevention of diseases. His discoveries reduced mortality from puerperal fever, and he created the first vaccines for rabies and anthrax. His experiments supported the germ theory of disease. He was best known to the general public for inventing a method to treat milk and wine in order to prevent it from causing sickness, a process that came to be called pasteurization. He is regarded as one of the three main founders of microbiology, together with Ferdinand Cohn and Robert Koch. He worked chiefly in Paris.

Paul Ehrlich: Paul Ehrlich (March 14, 1854 to August 20, 1915) was a German physician and scientist who worked in the fields of hematology, immunology, and chemotherapy. He invented the precursor technique to Gram staining bacteria, and the methods he developed for staining tissue made it possible to distinguish between different types of blood cells, which led to the capability to diagnose numerous blood diseases. His laboratory discovered Arsphenamine (Salvarsan), the first effective medicinal treatment for syphilis, thereby initiating and also naming the concept of chemotherapy. Ehrlich popularized the concept of a "magic bullet." He also made a decisive contribution to the development of an antiserum to combat diphtheria and conceived a methodology for standardizing

therapeutic serums. In 1908 he received a Nobel Prize in Physiology or Medicine for his contributions to immunology.

1.7 Knowledge builder

PCR: Polymerase chain reaction (PCR) enables researchers to produce billions of copies of a specific DNA sequence as shown in the picture. This automated process bypasses the need to use bacteria for amplifying DNA. Developed in 1983 by Kary Mullis, PCR is now a common and often indispensable technique used in medical and biological research labs for a variety of applications. The PCR technique was patented by Kary Mullis and assigned to Cetus Corporation, where Mullis worked when he invented the technique in 1983. The PCR techniques have mainly used to study DNA cloning for sequencing, DNA-based phylogeny, or functional analysis of genes; the diagnosis of hereditary diseases; the identification of genetic fingerprints (used in forensic sciences and paternity testing); and the detection and diagnosis of infectious diseases. In 1993, Mullis was awarded the Nobel Prize in Chemistry along with Michael Smith for his work on PCR.

FISH: Fluorescence in situ hybridization (FISH is a cytogenetic technique to detect and localize the presence or absence of specific DNA sequences on chromosomes. FISH uses fluorescent probes that bind to only those parts of the chromosome with which they show a high degree of sequence complementarity. FISH is frequently used for finding specific topographies in DNA for use in genetic counseling, medicine, and species identification. FISH can also be used to detect and localize specific RNA targets in tumor cells, and pathological tissue samples.

Microarray: A microarray, also known as a DNA chip or biochip, is basically a collection of microscopic DNA spots attached to a solid surface (see picture). Scientists use DNA microarrays to measure the expression levels of large numbers of genes simultaneously or to genotype multiple regions of a genome. The gene expression can be used to measure the difference between the normal and diseased tissues or samples to be able to find out the role of different genes in the development of disease.

Gram-positive bacteria: Gram-positive bacteria are those that are stained dark blue or violet by Gram staining. Gram-positive organisms are able to retain the crystal violet stain because of the high amount of peptidoglycan in the cell wall. The examples of Gram-positive bacteria are *Streptococcus* and *Staphylococcus*.

Gram-negative bacteria: Gram-negative bacteria are bacteria that do not retain the crystal violet dye in the Gram staining procedure because these bacteria contain an outer membrane. The Proteobacteria are a major group of Gram-negative bacteria, including *Escherichia coli*, *Salmonella*, *Shigella*, and other Enterobacteriaceae, *Pseudomonas*, *Moraxella*, *Helicobacter*, *Stenotrophomonas*, *Bdellovibrio*, acetic acid bacteria, *Legionella* and numerous others.

Micro-array technique: A DNA microarray (also commonly known as a DNA chip or biochip) is a collection of microscopic DNA spots attached to a solid surface. Scientists use DNA microarrays to measure the expression levels of large numbers of genes simultaneously or to genotype multiple regions of a genome. Each DNA spot contains picomoles (10 – 12 moles) of a specific DNA sequence, known as probes (or reporters or oligos). These can be a short section of a gene or other DNA element that is used to hybridize a CDNA or CRNA (also called antisense RNA) sample (called target) under high-stringency conditions. Probe–target hybridization is usually detected and quantified by detection of fluorophore-, silver-, or chemiluminescence-labeled targets to determine relative abundance of nucleic acid sequences in the target.

Recombinant DNA technology: Recombinant DNA (rDNA) molecules are DNA sequences that result from the use of laboratory methods (molecular cloning) to bring together genetic material from multiple sources, creating sequences that would not otherwise be found in biological organisms. Recombinant DNA is possible because DNA molecules from all organisms share the same chemical structure; they differ only in the sequence of nucleotides within that identical overall structure. Consequently, when DNA from a foreign source links to host sequences that can drive DNA replication, and then is introduced into a host organism, the foreign DNA is replicated along with the host DNA.

Hybridoma technology: Hybridoma technology is a technology of forming hybrid cell lines (called hybridomas) by fusing a specific antibody-producing B cell with a myeloma (B cell cancer) cell that is selected for its ability to grow in tissue culture and for an absence of antibody chain synthesis. The antibodies produced by the hybridoma are all of a single specificity and are, therefore, monoclonal antibodies (in contrast to polyclonal antibodies). The production of monoclonal antibodies was invented by Cesar Milstein and Georges J. F. Köhler in 1975.

Biocompatible materials: A biocompatible material (sometimes shortened to biomaterial) is a synthetic or natural material used to replace part of a living system or to function in intimate contact with living tissue. Biocompatible materials are intended to interface with biological systems to evaluate, treat, augment, or replace any tissue, organ, or function of the body. Biomaterials are usually nonviable, but may also be viable.

Autologous cell transplantation: Autologous stem cell transplantation is a medical procedure in which stem cells (cells from which other cells of the same type develop) are removed, stored, and later given back to the same person. Though most frequently performed with hematopoietic stem cells (blood forming), cardiac cells have also been used successfully to repair damage caused by heart attacks.

FDA: The Food and Drug Administration (FDA or USFDA) is a regulatory agency of the United States Department of Health and Human Services, belonging to the US federal executive departments. The FDA is responsible for protecting and promoting public health through the regulation and supervision of food safety, tobacco products, dietary supplements, prescription and over-the-counter pharmaceutical drugs, vaccines, biopharmaceuticals, blood transfusions, medical devices, electromagnetic radiation-emitting devices, and veterinary products.

Geron Corporation: Geron Corporation is a biotechnology company located in Menlo Park, California, USA, that focuses on the development and commercialization of products in three specific areas: therapeutic products for cancer treatment; pharmaceuticals that activate telomerase in tissues impacted by cell aging, injury, or degenerative diseases; and cell-based therapies derived from human ESCs for treatment of various chronic diseases.

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