Molecular biomedicine.

The use of genetic engineering in the production of vaccines and drugs. Genome Editing Technologies (CRISPR-Cas9). *Ex vivo* and *in vivo* gene therapy. Technologies for targeted delivery of drugs and gene therapy vectors: liposomes, dendrimers, aptamers, nanoparticles, genetically modified viruses, etc. Quantum dots. Prospects for the use of nanorobots in medicine. Pharmacogenomics. Pharmacogenetics.

1. Describe recombinant DNA technology.
2. Discuss about perspectives and dangers of creating the genetically modified organisms.
3. Describe the use of genetic engineering in the production of vaccines and drugs.
4. Explain the principles of CRISPR-Cas9 technology.
5. Explain what gene therapy is ex vivo and in vivo, analyze the problems and prospects of genomic technologies in medicine.
6. Give definitions of nanotechnology and bionanotechnology.
7. Describe and provide examples of various bionanotechnologies for targeted delivery of drugs and gene therapy vectors into the cells of the human body.
8. Analyze bionanotechnological methods for the diagnosis and treatment of cancer: quantum dots, magnetic and radioactive nanoparticles, etc.
9. Analyze the prospects for the use of nanorobots in biomedicine.
10. Give definitions and explain the difference between the terms "pharmacogenomics", "pharmacogenetics", "personalized medicine".
11. Explain how a hereditary predisposition can affect the individual reactions of the human body to drugs and dietary supplements, give specific examples.

Molecular Biomedicine is a branch of the Life Sciences that overlaps with Molecular Biology in many ways. It strives to elucidate molecular disease principles by studying the genome, transcriptome, proteome and metabolome, and their components, under healthy and disease conditions. It exploits our understanding of molecular biological and physiological mechanisms to devise new technologies for disease prediction, diagnosis and therapy, thereby improving healthcare and clinical practice and advancing the field of personalized medicine.

Genetic engineering, also called genetic modification, is the direct manipulation of an organism’s genome using biotechnology.Genetic engineering means the manipulation of organisms to make useful products and it has broad applications.

- Genetic engineering has applications in medicine, research, industry and agriculture and can be used on a wide range of plants, animals and microorganisms.
- In medicine, genetic engineering has been used to mass-produce insulin, human growth hormones, follistim (for treating infertility), human albumin, monoclonal antibodies, antihemophilic factors, vaccines, and many other drugs.
- In research, organisms are genetically engineered to discover the functions of certain genes.
- Industrial applications include transforming microorganisms such as bacteria or yeast, or insect mammalian cells with a gene coding for a useful protein. Mass quantities of the protein can be produced by growing the transformed organism in bioreactors using fermentation, then purifying the protein.
- Genetic engineering is also used in agriculture to create genetically-modified crops or genetically-modified organisms.

New DNA may be inserted in the host genome by first isolating and copying the genetic material of interest, using molecular-cloning methods to generate a DNA sequence; or by synthesizing the DNA, and then inserting this construct into the host organism. Genes may be removed, or “knocked out”, using a nuclease.

Gene targeting is a different technique that uses homologous recombination to change an endogenous gene, and can be used to delete a gene, remove exons, add a gene, or introduce point mutations. Genetic engineering has applications in medicine, research, industry and agriculture and can be used on a wide range of plants, animals and microorganisms.

Two fundamental kinds of cell are somatic cells and reproductive cells. Most of the cells in our bodies are somatic – cells that make up organs like skin, liver, heart, lungs, etc., and these cells vary from one another. Changing the genetic material in these cells is not passed along to a person's offspring. Reproductive cells are sperm cells, egg cells, and cells from very early embryos. Changes in the genetic make-up of reproductive cells would be passed along to the person's offspring. Those reproductive cell changes could result in different genetics in the offspring's somatic cells than otherwise would have occurred because the genetic makeup of somatic cells is directly linked to that of the germ cells from which they are derived.

Two problems must be confronted when changing genes. The first is what kind of change to make to the gene. The second is how to incorporate that change in all the other cells that are must be changed to achieve a desired effect.

There are several options for what kind of change to make to the gene. DNA in the gene could be replaced by other DNA from outside (called "homologous replacement). Or the gene could be forced to mutate (change structure – "selective reverse mutation.") Or a gene could just be added. Or one could use a chemical to simply turn off a gene and prevent it from acting.

There are also several options for how to spread the genetic change to all the cells that need to be changed. If the altered cell is a reproductive cell, then a few such cells could be changed and the change would reach the other somatic cells as those somatic cells were created as the organism develops. But if the change were made to a somatic cell, changing all the other relevant somatic cells individually like the first would be impractical due to the sheer number of such cells. The cells of a major organ such as the heart or liver are too numerous to change one-by-one. Instead, to reach such somatic cells a common approach is to use a carrier, or vector, which is a molecule or organism. A virus, for example, could be used as a vector. The virus would be an innocuous one or changed so as not to cause disease. It would be injected with the genetic material and then as it reproduces and "infects" the target cells it would introduce the new genetic material. It would need to be a very specific virus that would infect heart cells, for instance, without infecting and changing all the other cells of the body. Fat particles and chemicals have also been used as vectors because they can penetrate the cell membrane and move into the cell nucleus with the new genetic material.

Genetic engineering has produced a variety of drugs and hormones for medical use. The first successful products of genetic engineering were protein drugs like insulin, which is used to treat diabetes, and growth hormone somatotropin. These proteins are made in large quantities by genetically engineered bacteria or yeast in large "bioreactors. " Some drugs are also made in transgenic plants, such as tobacco. Other human proteins that are used as drugs require biological modifications that only the cells of mammals, such as cows, goats, and sheep, can provide. For these drugs, production in transgenic animals is a good option. Using farm animals for drug production has many advantages because they are reproducible, have flexible production, are easily maintained, and have a great delivery method

Interferon, which is used to eliminate certain viruses and kill cancer cells, also is a product of genetic engineering, as are tissue plasminogen activator and urokinase, which are used to dissolve blood clots.

Recombinant DNA technology not only allows therapeutic proteins to be produced on a large scale but using the same methodology protein molecules may be purposefully engineered. Genetic modifications introduced to a protein have many advantages over chemical modifications. Genetically engineered entities are biocompatible and biodegradable. The changes are introduced in 100% of the molecules with the exclusion of rare errors in gene transcription or translation. The preparations do not contain residual amounts of harsh chemicals used in the

conjugation process. Bacterial expression systems, due to their simplicity, are often not able to produce a recombinant human protein identical to the naturally occurring wild type.

The evolving field of gene therapy involves manipulating human genes to treat or cure genetic diseases and disorders. Modified plasmids or viruses often are the messengers to deliver genetic material to the body's cells, resulting in the production of substances that should correct the illness. Sometimes cells are genetically altered inside the body; other times scientists modify them in the laboratory and return them to the patient's body.

Since the 1990s, gene therapy has been used in clinical trials to treat diseases and conditions such as AIDS, cystic fibrosis, cancer, and high cholesterol. Drawbacks of gene therapy are that sometimes the person's immune system destroys the cells that have been genetically altered, and also that it is hard to get the genetic material into enough cells to have the desired effect.

Gene therapy and genetic engineering are two closely related technologies that involve altering the genetic material of organisms. The distinction between the two is based on purpose. Gene therapy seeks to alter genes to correct genetic defects and thus prevent or cure genetic diseases. Genetic engineering aims to modify the genes to enhance the capabilities of the organism beyond what is normal.

Gene therapy is an experimental technique that uses genes to treat or prevent disease. In the future, this technique may allow doctors to treat a disorder by inserting a gene into a patient's cells instead of using drugs or surgery. Researchers are testing several approaches to gene therapy, including:

- Replacing a mutated gene that causes disease with a healthy copy of the gene.
- Inactivating, or "knocking out," a mutated gene that is functioning improperly.
- Introducing a new gene into the body to help fight a disease.

Genome editing (also called gene editing) is a group of technologies that give scientists the ability to change an organism's DNA. These technologies allow genetic material to be added, removed, or altered at particular locations in the genome.

Several approaches to genome editing have been developed. A recent one is known as CRISPR-Cas9, which is short for clustered regularly interspaced short palindromic repeats and CRISPR-associated protein 9. The CRISPR-Cas9 system has generated a lot of excitement in the scientific community because it is faster, cheaper, more accurate, and more efficient than other existing genome editing methods.

CRISPR-Cas9 was adapted from a naturally occurring genome editing system in bacteria. The bacteria capture snippets of DNA from invading viruses and use them to create DNA segments known as CRISPR arrays. The CRISPR arrays allow the bacteria to "remember" the viruses (or closely related ones). If the viruses attack again, the bacteria produce RNA segments from the CRISPR arrays to target the viruses' DNA. The bacteria then use Cas9 or a similar enzyme to cut the DNA apart, which disables the virus.

The CRISPR-Cas9 system works similarly in the lab. Researchers create a small piece of RNA with a short "guide" sequence that attaches (binds) to a specific target sequence of DNA in a genome. The RNA also binds to the Cas9 enzyme. As in bacteria, the modified RNA is used to recognize the DNA sequence, and the Cas9 enzyme cuts the DNA at the targeted location. Although Cas9 is the enzyme that is used most often, other enzymes (for example Cpf1) can also be used. Once the DNA is cut, researchers use the cell's own DNA repair machinery to add or delete pieces of genetic material, or to make changes to the DNA by replacing an existing segment with a customized DNA sequence.

Genome editing is of great interest in the prevention and treatment of human diseases. Currently, most research on genome editing is done to understand diseases using cells and animal models. Scientists are still working to determine whether this approach is safe and effective for use in people. It is being explored in research on a wide variety of diseases,

including single-gene disorders such as cystic fibrosis, hemophilia, and sickle cell disease. It also holds promise for the treatment and prevention of more complex diseases, such as cancer, heart disease, mental illness, and human immunodeficiency virus (HIV) infection.

Ethical concerns arise when genome editing, using technologies such as CRISPR-Cas9, is used to alter human genomes. Most of the changes introduced with genome editing are limited to somatic cells, which are cells other than egg and sperm cells. These changes affect only certain tissues and are not passed from one generation to the next. However, changes made to genes in egg or sperm cells (germline cells) or in the genes of an embryo could be passed to future generations. Germline cell and embryo genome editing bring up a number of ethical challenges, including whether it would be permissible to use this technology to enhance normal human traits (such as height or intelligence). Based on concerns about ethics and safety, germline cell and embryo genome editing are currently illegal in many countries.

Nanotechnology, the manipulation of matter at the atomic and molecular scale to create materials with remarkably varied and new properties, is a rapidly expanding area of research with huge potential in many sectors, ranging from healthcare to construction and electronics. In medicine, it promises to revolutionize drug delivery, gene therapy, diagnostics, and many areas of research, development and clinical application.

The use of nanotechnology in medicine offers some exciting possibilities. The aim of nanomedicine may be broadly defined as the comprehensive monitoring, control, construction, repair, defence and improvement of all human biological systems, working from the molecular level using engineered devices and nanostructures, ultimately to achieve medical benefits. In this context, nanoscale should be taken to include active components or objects in the size range from one nanometre to hundreds of nanometres. These may be included in a micro-device (that have a macro-interface) or in a biological environment. The focus, however, is always on nano-interactions within the framework of a larger device or directly within a sub-cellular (or cellular) system.

Nanotechnology in medicine involves applications of nanoparticles currently under development, as well as longer range research that involves the use of manufactured nano-robots to make repairs at the cellular level.

Nanoparticulate drug delivery systems In the short and medium term, the main use of nanoparticle medicinal products (NMP) is vectorisation of active principles, corresponding to several products already marketed like Doxil™ or more recently Abraxane™
Generally three vector generations are considered:

• First generation vectors: nanospheres and nanocapsules (the best known and most accessible);
• Second generation vectors: nanoparticles coated with hydrophilic polymers such as polyethylene glycol (PEG), PEGylated nanoparticles;
• Third generation vectors, still under development, combining a biodegradable core and a polymer envelope (PEG) with a membrane recognition ligand.

Today, most current research projects in nano delivery systems are focused on the third type. Conventional chemotherapy employs drugs that are known to kill cancer cells effectively. But these cytotoxic drugs kill healthy cells in addition to tumor cells, leading to adverse side effects such as nausea, neuropathy, hair-loss, fatigue, and compromised immune function. Nanoparticles can be used as drug carriers for chemotherapeutics to deliver medication directly to the tumor while sparing healthy tissue [9] (Fig. 7).

Nanocarriers present several advantages over conventional chemotherapy. They can:

• Protect drugs from being degraded in the body before they reach their target;
• Enhance drug absorption into tumors and the cancerous cells themselves; • Allow for better control over the timing and distribution of drugs to the tissue, making it easier for oncologists to assess how well they work;
• Prevent drugs from interacting with normal cells, thus avoiding side effects.

Passive targeting

There are now several nanocarrier-based drugs on the market, which rely on passive targeting through a process known as “enhanced permeability and retention”. Because of their size and surface properties, certain nanoparticles can escape through blood vessel walls into tissues. In addition, tumors tend to have leaky blood vessels and defective lymphatic drainage, causing nanoparticles to accumulate in them, thereby concentrating the attached cytotoxic drug where it’s needed, protecting healthy tissue and greatly reducing adverse side effects. Another strategy for passive targeting consists in using myeloid cells like macrophages which absorb nanoparticles and concentrate them in the site to be treated, like a Trojan horse.

Active targeting On the horizon are nanoparticles that will actively target drugs to cancerous cells, based on the molecules that they express on their surface. Molecules that bind particular cellular receptors can be attached to a nanoparticle so that it specifically targets cells expressing this receptor. Active targeting can even be used to bring drugs into the cancerous cell, by inducing the cell to absorb the nanocarrier. Active targeting can be combined with passive targeting to further reduce interaction of carried drugs with healthy tissue. Nanotechnology-enabled active and passive targeting can also increase the efficiency of a chemotherapeutic, achieving more significant tumor reduction with lower drug doses.

. Destruction from within Moving away from conventional chemotherapeutic agents that activate normal molecular mechanisms to induce cell death, researchers are exploring ways to physically destroy cancerous cells from within. One such technology – nanoshells – is being used in the laboratory to thermally destroy tumors from the inside. Nanoshells can be designed to absorb light at different wavelengths, generating heat (hyperthermia). Once the cancer cells take up the nanoshells (via active targeting), scientists apply near-infrared light that is absorbed by the nanoshells, creating an intense heat inside the tumor that selectively kills tumor cells without disturbing neighbouring healthy cells. Similarly, new targeted magnetic nanoparticles are in development that will both be visible through Magnetic Resonance Imaging (MRI) and can also destroy cells by hyperthermia.

Drug delivery (mechanical) devices Implanted drug delivery devices – DDD – can take benefit of nanotechnology. Conventional water-soluble drugs can create difficulties in treatment, such as failed absorption in the diseased areas. However, nanomedicine applications such as diagnostic nanomachines provide the ability to monitor the internal chemistry of the body’s organs, providing direct access to diseased areas. Moreover, technology such as nanobots can be equipped with wireless transmitters, and this offers doctors opportunities to change the treatment method if a patient’s medical condition gets worse. Nanobots in medicine could also be planted into a patient’s nervous system to monitor pulse and brainwave activities.

According to scientists, nanobots can completely replace pacemakers by treating the heart’s cell directly. Research regarding nanobots in medicine offer several opportunities such as artificial antibodies, artificial white blood cells (WBCs) and red blood cells (RBCs), and antiviral nanobots. The major advantage that nanobots provide is that they are extremely durable. Theoretically, they can operate for years without any damage owing to their miniature size, which reduces mechanical damage.

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