BT501 – HEALTH BIOTECHNOLOGY

FINAL TERM IMPORTANT QUESTIONS

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Admin: **BS Biological Sciences** (One place to all your solutions)

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1. What are types of vaccines?

Types of vaccines

1. ***Live attenuated vaccines***
   Contain weakened forms of the pathogen that can replicate but do not cause disease in healthy individuals. ***Examples:*** BCG, oral polio vaccine (OPV), measles, mumps, rubella (MMR), varicella.
2. ***Inactivated (killed) vaccines***
   Prepared from pathogens that have been killed by heat or chemicals; they cannot replicate. ***Examples:*** Inactivated polio vaccine (IPV), rabies vaccine, hepatitis A vaccine.
3. ***Subunit vaccines***
   Contain only specific antigenic parts of the pathogen (proteins or polysaccharides). ***Examples:*** Hepatitis B vaccine, acellular pertussis vaccine.
4. ***Toxoid vaccines***
   Made from inactivated bacterial toxins (toxoids) that stimulate immunity against toxin-mediated sdisease. ***Examples:*** Diphtheria toxoid, tetanus toxoid.
5. ***Conjugate vaccines***
   Polysaccharide antigens are chemically linked to a protein carrier to enhance immunogenicity, especially in infants. ***Examples:*** Haemophilus influenzae type b (Hib) vaccine, pneumococcal conjugate vaccine.
6. ***Recombinant vaccines***
   Antigens are produced using recombinant DNA technology in host cells such as yeast or bacteria. ***Examples:*** Hepatitis B vaccine, human papillomavirus (HPV) vaccine.
7. ***mRNA vaccines***
   Contain messenger RNA encoding a specific antigen, leading to in-situ antigen production. ***Examples:*** COVID-19 mRNA vaccines.
8. ***Viral vector vaccines***
   Use a harmless virus to deliver genetic material coding for the antigen. ***Examples:*** Adenovirus-based COVID-19 vaccines.

2. What are strategies to produce animals?

Strategies to produce transgenic animals

1. **Pronuclear microinjection**
   Foreign DNA is directly injected into the pronucleus of a fertilized ovum. The embryo is implanted into a surrogate mother and screened for transgene integration.
2. **Embryonic stem (ES) cell–mediated gene transfer**
   Desired gene is introduced into cultured ES cells. Genetically modified ES cells are injected into blastocysts, which are then implanted to obtain chimeric animals.
3. **Retroviral / viral vector–mediated gene transfer**
   Recombinant viral vectors carrying the transgene infect early embryos, resulting in stable integration into the host genome.
4. **Sperm-mediated gene transfer (SMGT)**
   Sperm cells are incubated with foreign DNA and used for fertilization, allowing transfer of the gene into the embryo.
5. **Somatic cell nuclear transfer (SCNT)**
   A genetically modified somatic cell nucleus is transferred into an enucleated oocyte, followed by embryo development and implantation (cloning-based approach).
6. **Genome editing technologies**
   Site-specific modification of genes using tools such as CRISPR–Cas9**,** TALENs, or ZFN, enabling precise insertion, deletion, or replacement of genes.

3. What are disadvantages of artificial pancreas?

Disadvantages of an artificial pancreas

1. ***High cost:*** Devices and consumables (sensors, infusion sets) are expensive and may not be affordable for all patients.
2. ***Technical complexity:*** Requires calibration, troubleshooting, and regular maintenance; device malfunction can occur.
3. ***Risk of device failure:*** Sensor inaccuracies or pump failure may lead to hypoglycemia or hyperglycemia.
4. ***Delayed glucose sensing:*** Continuous glucose monitors measure interstitial glucose, which may lag behind blood glucose levels.
5. ***User dependency:*** Still requires user input for meals, exercise, and troubleshooting; not completely autonomous.
6. ***Skin-related problems:*** Prolonged use of sensors and infusion sets can cause skin irritation, infection, or discomfort.
7. ***Limited availability:*** Not widely accessible in all regions and healthcare settings.
8. ***Psychological burden:*** Continuous attachment to devices may cause stress or reduced quality of life for some patients.

4. Write a note on recombinant human insulin?

Recombinant human insulin

Recombinant human insulin is insulin produced by genetic engineering techniques using microorganisms, mainly *Escherichia coli* or yeast, to synthesize human insulin identical to the natural hormone.

Principle

The gene encoding human insulin (A and B chains or proinsulin) is inserted into a suitable plasmid vector and introduced into a host cell. The host expresses the insulin gene, producing insulin protein, which is then purified and processed to obtain biologically active insulin.

Method of production

1. Isolation and synthesis of the human insulin gene.
2. Insertion of the insulin gene into a plasmid vector using restriction enzymes and DNA ligase.
3. Transformation of the recombinant plasmid into *E. coli* or yeast cells.
4. Expression of insulin as separate A and B chains or as proinsulin.
5. Purification and enzymatic processing to form active insulin with correct disulfide bonds.

Advantages

- Identical to human insulin, reducing allergic reactions.
- High purity and consistent quality.
- Large-scale production is possible.
- Safe and free from animal-derived contaminants.

Applications

Used in the treatment of diabetes mellitus to control blood glucose levels and prevent long-term complications.

5. Explain Innate and Adaptive immunity.

Innate immunity

Innate immunity is the natural, inborn defense mechanism that is present from birth and provides immediate protection against pathogens. It is non-specific in nature, meaning it acts against a wide range of microorganisms without recognizing specific antigens. This system includes physical barriers such as skin and mucous membranes, chemical barriers like gastric acid and lysozyme, and cellular components including neutrophils, macrophages, dendritic cells, and natural killer cells. Innate immunity does not develop immunological memory, but it plays a crucial role in preventing infection and activating adaptive immune responses.

Adaptive immunity

Adaptive immunity is an acquired, specific defense system that develops after exposure to an antigen. It is characterized by high specificity and the ability to form immunological memory, resulting in a stronger and faster response upon subsequent exposure to the same antigen. Adaptive immunity is mediated by lymphocytes and is divided into humoral immunity, involving antibody production by B cells, and cell-mediated immunity, involving T lymphocytes that destroy infected or abnormal cells. This system provides long-term protection and specificity against pathogens.

6. What factors affect the bioavailability of the drugs?

Factors affecting bioavailability of drugs

i. **Drug-related factors**
These include physicochemical properties such as solubility, particle size, chemical stability, degree of ionization, and lipid solubility. Drugs with poor aqueous solubility or instability in gastric conditions show reduced bioavailability.

ii. **Dosage form and formulation factors**
Bioavailability is influenced by the type of dosage form (tablet, capsule, solution), nature of excipients, disintegration and dissolution rate, manufacturing process, and presence of coatings. Modified-release formulations can alter both the rate and extent of absorption.

iii. **Route of administration**
The route determines the amount of drug reaching systemic circulation. Oral administration often shows lower bioavailability compared to parenteral routes due to degradation and incomplete absorption.

iv. **First-pass metabolism**
Metabolism of drugs in the liver and intestinal wall before reaching systemic circulation can significantly reduce bioavailability.

v. **Physiological factors**
These include gastric emptying time, intestinal motility, gastrointestinal pH, blood flow at the absorption site, and presence of food, all of which can enhance or reduce drug absorption.

vi. **Drug interactions**
Concomitant use of other drugs may affect absorption, metabolism, or transport of a drug, thereby altering its bioavailability.

vii. **Patient-related factors**
Age, disease state, genetic variations, and patient compliance influence the extent of drug absorption and availability in systemic circulation.

7. Write disadvantages of autologous transplantation.

Disadvantages of autologous transplantation

Risk of disease relapse

Since the patient's own cells are used, there is a possibility that malignant or diseased cells may be reinfused, leading to recurrence of the original disease.

Lack of graft-versus-tumor effect

Autologous transplantation does not provide the beneficial graft-versus-leukemia or graft-versus-tumor effect seen in allogeneic transplants, reducing long-term disease control.

Limited stem cell availability

Patients with prior chemotherapy or poor bone marrow function may have insufficient stem cells for collection and transplantation.

Delayed immune recovery

Recovery of the immune system can be slower compared to allogeneic transplants, increasing susceptibility to infections.

High cost and complex procedure

The process involves stem cell harvesting, storage, conditioning therapy, and reinfusion, making it expensive and technically demanding.

8. Differentiate between pharmagenetics and pharmadynamics.

Difference between Pharmacogenetics and Pharmacodynamics

Pharmacogenetics

Pharmacogenetics is the study of how an individual's genetic makeup affects their response to drugs. It focuses on genetic variations that influence drug metabolism, efficacy, and risk of adverse effects. For example, variations in the CYP450 enzymes can alter the metabolism of drugs like warfarin or codeine.

Pharmacodynamics

Pharmacodynamics is the study of the biochemical and physiological effects of drugs on the body and the mechanisms of their action. It explains how drugs interact with receptors, enzymes, or ion channels to produce therapeutic or toxic effects. For example, beta-blockers reduce heart rate by blocking beta-adrenergic receptors.

9. Explain Factor VIII.

Factor VIII

Factor VIII is a clotting protein (also called anti-hemophilic factor A) that plays a critical role in the intrinsic pathway of the coagulation cascade. It is a glycoprotein produced mainly in the liver and circulates in plasma bound to von Willebrand factor (vWF)**,** which protects it from degradation.

Function:

- Acts as a cofactor for Factor IXa, helping convert Factor X to Xa, leading to thrombin generation and formation of a stable fibrin clot.
- Essential for proper blood clotting; deficiency or dysfunction causes hemophilia A, a genetic bleeding disorder.

Clinical significance:

- ***Hemophilia A*:** Caused by inherited or acquired deficiency of Factor VIII, resulting in prolonged bleeding.
- ***Therapeutic use*:** Recombinant or plasma-derived Factor VIII is used for replacement therapy in patients with hemophilia A.

Structure and forms:

- Exists as inactive factor VIII in plasma; activated by thrombin during coagulation.
- Can be administered intravenously in purified form for bleeding prevention or treatment.

10. Name six areas of applications of artificial organs.

Cardiac support – e.g., artificial heart, ventricular assist devices (VADs)
Renal replacement – e.g., dialysis machines, artificial kidneys
Hepatic support – e.g., bioartificial liver devices
Respiratory support – e.g., artificial lungs, ECMO (extracorporeal membrane oxygenation)
Pancreatic support – e.g., artificial pancreas for diabetes management
Ocular implants – e.g., artificial cornea, retinal prosthesis

11. Explain inactivated vaccine.

Inactivated vaccine

An inactivated vaccine is a type of vaccine in which the pathogen (virus or bacteria) has been killed or inactivated so that it cannot replicate or cause disease, but still retains its ability to stimulate an immune response**.** The immune system recognizes the inactivated pathogen as foreign and produces antibodies, preparing the body to fight the real pathogen if exposed in the future.

Method of preparation:

- Pathogen is grown in culture.
- It is then killed using heat, chemicals (like formaldehyde), or radiation.
- The inactivated pathogen is formulated with stabilizers or adjuvants to enhance immune response.

Examples are: Inactivated polio vaccine (IPV), Rabies vaccine, Hepatitis A vaccine, etc.

Advantages: Safe, as it cannot cause disease. Also, it is stable and easier to store compared to live vaccines.

Disadvantages: May require booster doses to maintain immunity. It produces a weaker immune response compared to live attenuated vaccines.

12. What is sperm mediated transgenesis?

Sperm-mediated transgenesis (SMT)

Sperm-mediated transgenesis is a technique used to produce transgenic animals by using sperm cells as carriers to deliver foreign DNA into an egg during fertilization. Sperm cells can bind or take up exogenous DNA and carry it into the oocyte at the time of fertilization, resulting in embryos that carry the foreign gene in their genome.

Method:

- Sperm cells are incubated with the desired foreign DNA.
- The treated sperm fertilizes an egg either in vitro or in vivo.
- Embryos are implanted into a surrogate mother.
- Offspring are screened for the presence of the transgene.

Advantages:

- Simple and less technically demanding than pronuclear microinjection.
- Can produce transgenic animals efficiently.

Limitations:

- Low efficiency of stable gene integration.
- Random insertion of DNA can lead to variable expression of the transgene.

13. Explain manufacturing of artificial organs.

Manufacturing of Artificial Organs

The manufacturing of artificial organs involves a combination of biomedical engineering, materials science, and medical knowledge to create devices that can replace or support the function of damaged organs**.** Steps involved:

1. Design and planning

- Identify the organ function to be replicated (e.g., heart, kidney, liver).
- Determine mechanical, biochemical, and physiological requirements.
- Create prototypes using computer-aided design (CAD) and simulations.

2. Selection of materials

- Materials must be biocompatible, durable, and non-toxic.
- Common materials include silicones, polymers, metals, ceramics, or bioengineered tissues.

3. Fabrication

- Mechanical parts are made using precision engineering, 3D printing, or molding.
- For bioartificial organs, cells or tissues may be cultured on scaffolds to replicate organ function.

4. Integration of sensors and electronics

- Many artificial organs include sensors, pumps**,** and microcontrollers to monitor and regulate function (e.g., in artificial pancreas or heart devices).

5. Testing and sterilization

- Devices are tested in vitro for mechanical and functional performance.
- Sterilization ensures they are safe for implantation or use.

6. Clinical trials and approval

- Before use in humans, devices undergo preclinical and clinical trials to ensure safety and efficacy.

14. What are liposome? Explain its structure.

Liposomes

Liposomes are spherical vesicles made of phospholipid bilayers that enclose an aqueous core. They are used as drug delivery systems because they can carry both water-soluble and fat-soluble drugs and target specific tissues.

Structure:

- The phospholipid bilayer is made of molecules with a hydrophilic head and hydrophobic tail. The tails face inward, forming the membrane, while the heads face the aqueous environment.
- The aqueous core inside the liposome can contain water-soluble drugs.
- Liposomes can be unilamellar (single bilayer) or multilamellar (multiple bilayers), and their size ranges from nanometers to micrometers.

15. Write a note on flow cytometry technique.

Flow Cytometry Technique

Flow cytometry is a laboratory technique used to analyze the physical and chemical characteristics of cells or particles as they flow in a fluid stream through a beam of light, usually a laser. It allows rapid measurement of multiple parameters of thousands of cells individually.

Principle:

Cells are labeled with fluorescent dyes or antibodies, and as they pass through the laser beam, they scatter light and emit fluorescence. Detectors measure:

- ***Forward scatter (FSC):*** indicates cell size
- ***Side scatter (SSC):*** indicates internal complexity or granularity
- ***Fluorescence intensity*:** indicates presence of specific markers

Components:

- ***Fluidics system:*** aligns cells in a single-file stream.
- ***Optical system:*** laser light, lenses, filters, and detectors capture signals.
- ***Electronics/data system:*** converts light signals into digital data for analysis.

Applications:

- Counting and sorting cells
- Immunophenotyping (e.g., identifying blood cell types)
- Measuring cell viability and apoptosis
- Detecting biomarkers in research and clinical diagnostics

16. Write disadvantages of retrovirus mediated transfer.

Disadvantages of Retrovirus-Mediated Gene Transfer

Limited gene size – Retroviral vectors can carry only relatively small genes, restricting their use for large genetic sequences.

Risk of insertional mutagenesis – Integration of viral DNA into the host genome may disrupt important genes, potentially causing cancer or other genetic disorders.

Replication competence risk – Although vectors are engineered to be replication-defective, there is a small risk that they may regain the ability to replicate.

Immune response – The host immune system may recognize and attack the viral vector, reducing efficiency and causing inflammation.

Limited target cell types – Some retroviruses can only infect dividing cells, limiting their application for non-dividing tissues.

Transient expression in some cases – In certain cells, expression of the transferred gene may decrease over time.

17. Write names of immune diseases.

Names of Immune Diseases

Autoimmune diseases – e.g., rheumatoid arthritis, systemic lupus erythematosus (SLE), type 1 diabetes, multiple sclerosis

Immunodeficiency diseases – e.g., severe combined immunodeficiency (SCID), acquired immunodeficiency syndrome (AIDS), common variable immunodeficiency (CVID)

Allergic diseases (hypersensitivity) – e.g., asthma, hay fever (allergic rhinitis), anaphylaxis, atopic dermatitis

Inflammatory diseases – e.g., Crohn's disease, ulcerative colitis, psoriasis

Transplant-related immune disorders – e.g., graft-versus-host disease (GVHD), organ transplant rejection

18. What is seamless cloning?

Seamless Cloning

Seamless cloning is a molecular biology technique used to join DNA fragments precisely without introducing any extra nucleotides or restriction sites at the junctions. In this method, DNA fragments are designed with overlapping or complementary ends, which allows them to be joined together using enzymes or recombination-based systems. Common approaches include Gibson Assembly, which uses exonuclease, DNA polymerase, and ligase; In-Fusion Cloning, which relies on recombinase-mediated integration; and SLiCE (Seamless Ligation Cloning Extract), which uses cell extracts to promote homologous recombination. This technique is highly useful in gene cloning, construction of recombinant DNA, protein engineering, and synthetic biology because it preserves the original DNA sequence exactly and allows the assembly of multiple DNA fragments in a single reaction. Seamless cloning is preferred when accuracy, efficiency, and a scar-free sequence are critical.

19. What are the products which do not come into the category of biopharmaceuticals?

Products Not Considered Biopharmaceuticals

Biopharmaceuticals are medicines produced using biological sources like proteins, nucleic acids, or living cells. Products that do not fall under biopharmaceuticals are those that are synthetic, chemically manufactured, or not derived from biological sources. Examples include:

➤ ***Small-molecule drugs*** – e.g., aspirin, paracetamol, ibuprofen
➤ ***Chemically synthesized antibiotics*** – e.g., penicillin derivatives produced synthetically
➤ ***Non-biological vitamins and minerals*** – e.g., synthetic vitamin C, calcium supplements
➤ ***Conventional vaccines made from inactivated pathogens*** (without recombinant technology)
➤ ***Hormones produced by chemical synthesis*** – e.g., synthetic thyroxine

20. Write a brief note on Neutrophils.

Neutrophils

Neutrophils are the most abundant type of white blood cells in human blood and play a key role in innate immunity. They are granulocytes containing enzymes and antimicrobial proteins that help destroy invading pathogens. Neutrophils are usually the first immune cells to arrive at the site of infection, where they perform phagocytosis to engulf bacteria, fungi, and cellular debris. They also release reactive oxygen species (ROS) and enzymes from granules to kill microbes.
Neutrophils have a short lifespan of about 6–8 hours in circulation but are constantly replenished from the bone marrow. They are essential for acute inflammation, defense against infections, and initiating signals that recruit other immune cells. Abnormal neutrophil counts can lead to increased susceptibility to infections (low count) or excessive inflammation (high count).

21. What are the risks of Gene Therapy?

Risks of Gene Therapy

Gene therapy involves introducing, altering, or silencing genes in a patient's cells to treat disease, but it carries several risks:

- ***Immune reactions*** – The patient's immune system may attack the viral vector or modified cells, causing inflammation or severe immune responses.
- ***Insertional mutagenesis*** – Integration of the therapeutic gene into the genome may disrupt normal genes, potentially leading to cancer.
- ***Off-target effects*** – In genome-editing approaches (like CRISPR), unintended genes may be altered, causing harmful effects.
- ***Short-lived effects*** – Some therapies may not provide permanent benefit, requiring repeated treatments.
- ***Infection risk*** – Using viral vectors may pose a small risk of **viral infection** or replication.
- ***Unpredictable long-term effects*** – The long-term safety and impact of gene therapy are still being studied, so delayed adverse effects are possible.

22. Differentiate between Addictive Drugs and Abusive Drugs.

Difference between Addictive Drugs and Abusive Drugs

Addictive Drugs – These are substances that cause physical or psychological dependence when used repeatedly. The body or mind develops a strong craving, and stopping the drug leads to withdrawal symptoms. ***Example***: heroin, nicotine, alcohol.

Abusive Drugs – These are substances that are used in a harmful or non-medical way, regardless of dependence. Abuse can cause health, social, or legal problems but does not always lead to addiction. ***Example***: inhalants, prescription painkillers taken without prescription.

23. Write steps of indirect ELISA.

Steps of Indirect ELISA

Indirect ELISA is used to detect the presence of antibodies in a sample. The main steps are:

- ***Coating*** – The antigen of interest is immobilized on the surface of a microtiter plate.
- ***Blocking*** – Remaining binding sites on the plate are blocked with a protein solution (e.g., BSA or milk) to prevent non-specific binding.
- ***Incubation with sample*** – The patient's serum or sample containing primary antibodies is added and allowed to bind to the immobilized antigen.
- ***Washing*** – Unbound antibodies are washed away to reduce background noise.
- ***Addition of secondary antibody*** – An enzyme-linked secondary antibody that recognizes the primary antibody is added.
- ***Washing*** – Excess secondary antibody is removed.
- ***Substrate addition*** – A chromogenic substrate is added, which reacts with the enzyme to produce a color change.
- ***Detection*** – The color intensity is measured, usually by a spectrophotometer, indicating the presence and amount of antibodies in the sample.

24. Write types of Trisomy.

Types of Trisomy

Trisomy is a genetic disorder in which a person has three copies of a chromosome instead of the normal two. Common types include:

Trisomy 21 (Down syndrome) – Extra copy of chromosome 21; causes intellectual disability, characteristic facial features, and heart defects.

Trisomy 18 (Edward syndrome) – Extra copy of chromosome 18; associated with severe developmental delays, growth retardation, and congenital malformations.

Trisomy 13 (Patau syndrome) – Extra copy of chromosome 13; leads to severe intellectual disability, cleft lip/palate, and multiple organ defects.

Sex chromosome trisomies – Extra sex chromosomes:

- ***47,XXX (Triple X syndrome)*** – Extra X in females; usually mild or asymptomatic.
- ***47,XXY (Klinefelter syndrome)*** – Extra X in males; causes infertility, tall stature, and some developmental issues.
- ***47,XYY syndrome*** – Extra Y in males; may cause tall stature and learning difficulties.

25. Explain genetics of Tay Sach Disease.

Genetics of Tay-Sachs Disease

Tay-Sachs disease is a genetic disorder caused by a deficiency of the enzyme β-hexosaminidase A, which is required to break down GM2 ganglioside, a fatty substance in nerve cells. Its accumulation in neurons leads to progressive neurodegeneration.

Inheritance pattern:

- Tay-Sachs disease is inherited in an autosomal recessive manner.
- Affected individuals inherit one defective gene from each parent.
- Carriers (with one normal and one defective gene) usually do not show symptoms but can pass the gene to offspring.

Genetic cause:

- Mutations occur in the HEXA gene on chromosome 15.
- These mutations lead to absent or nonfunctional β-hexosaminidase A enzyme, causing GM2 ganglioside to accumulate in lysosomes of nerve cells.

Clinical features:

- Symptoms appear in infancy: loss of motor skills, exaggerated startle response, seizures, vision and hearing loss.
- Progressive neurodegeneration leads to early death, usually by 4–5 years of age.

26. Explain Variant Structural Chromosomal Abnormality.

Variant Structural Chromosomal Abnormality

Variant structural chromosomal abnormalities are alterations in the structure of chromosomes that differ from the normal karyotype and may involve deletions, duplications, inversions, translocations, or ring formations. These abnormalities can affect gene dosage or disrupt gene function, potentially leading to genetic disorders or developmental abnormalities.

Types of structural chromosomal abnormalities:

- ***Deletion:*** A portion of a chromosome is missing, e.g., Cri-du-chat syndrome (deletion on chromosome 5p).
- ***Duplication:*** A segment of the chromosome is repeated, which may lead to gene overexpression.
- ***Inversion:*** A chromosome segment is reversed end to end; may be pericentric (includes centromere) or paracentric (does not include centromere).
- ***Translocation:*** A segment of one chromosome is transferred to another chromosome; can be reciprocal or Robertsonian.
- ***Ring chromosome:*** A chromosome forms a ring due to loss of terminal ends and joining of the ends.
- ***Isochromosome:*** One arm of a chromosome is duplicated while the other is lost, forming a mirror-image chromosome.

27. What is biotechnology? Differentiate classical and modern biotechnology.

Biotechnology

Biotechnology is the use of living organisms, cells, or biological systems to develop products, processes, or technologies for human benefit. It combines biology, genetics, microbiology, and engineering to create applications in medicine, agriculture, industry, and environmental management.

Difference between Classical and Modern Biotechnology

➤ ***Classical Biotechnology*** – Uses traditional methods involving whole organisms or natural processes. Examples include fermentation, brewing, baking, cheese making, and selective breeding of plants and animals. It relies on naturally occurring microbes and organisms without genetic modification.

➤ ***Modern Biotechnology*** – Involves advanced techniques such as genetic engineering, recombinant DNA technology, monoclonal antibody production, and CRISPR gene editing. It allows precise modification of genes or cells to produce specific products like insulin, vaccines, and transgenic crops.

28. Write a note on Erythropoietin.

Erythropoietin (EPO)

Erythropoietin is a glycoprotein hormone produced mainly by the kidneys that regulates the production of red blood cells in the bone marrow. It is released in response to low oxygen levels (hypoxia) in the blood. Erythropoietin binds to erythroid progenitor cells in the bone marrow, stimulating their growth and differentiation into mature red blood cells, which helps improve the blood's oxygen-carrying capacity.

Clinical use: Recombinant erythropoietin is widely used to treat anemia caused by chronic kidney disease, chemotherapy, or other conditions that reduce red blood cell production. It helps reduce the need for blood transfusions.

Important points: While it is very effective, excessive use of erythropoietin can lead to thickened blood, high blood pressure, and an increased risk of blood clots. It is one of the important biopharmaceuticals produced using biotechnology.

29. Explain in detail cardiovascular diseases.

Cardiovascular Diseases (CVDs)

Coronary Artery Disease (CAD):

Coronary artery disease occurs when the coronary arteries supplying blood to the heart become narrowed or blocked due to atherosclerosis, which is the buildup of fatty plaques. This reduces oxygen supply to the heart muscle, leading to chest pain (angina)**,** shortness of breath, and in severe cases, a heart attack (myocardial infarction)**.** Risk factors include high cholesterol, smoking, hypertension, diabetes, and sedentary lifestyle.

Hypertension (High Blood Pressure):

Hypertension is a condition in which the blood pressure in the arteries is persistently elevated, increasing the workload on the heart and blood vessels. If left untreated, it can lead to heart failure, stroke, kidney damage, or aneurysms**.** It is often called the "silent killer" because many people may not show symptoms until complications arise.

Heart Failure:

Heart failure occurs when the heart is unable to pump blood efficiently to meet the body's needs. This leads to fluid accumulation, causing swelling in the legs, lungs, and other tissues, along with fatigue and shortness of breath. Heart failure can result from coronary artery disease, hypertension, heart valve problems, or prior heart attacks.

Arrhythmias:

Arrhythmias are abnormal heart rhythms caused by electrical disturbances in the heart. They can include tachycardia (fast heartbeat), bradycardia (slow heartbeat), and atrial fibrillation. Arrhythmias may cause palpitations, dizziness, fainting, and in severe cases, sudden cardiac death. Treatment can involve medications, pacemakers, or electrical cardioversion.

Stroke (Cerebrovascular Disease):

Stroke occurs when blood flow to the brain is interrupted, either due to a blockage (ischemic stroke) or rupture of a blood vessel (hemorrhagic stroke). This causes brain cells to die, leading to paralysis, speech difficulties, cognitive impairment, or death. Risk factors include hypertension, diabetes, smoking, high cholesterol, and heart disease.

Peripheral Artery Disease (PAD):

Peripheral artery disease is caused by narrowing of arteries in the limbs, usually the legs, due to atherosclerosis. Reduced blood flow leads to pain, numbness, weakness, or ulcers in the affected limbs. Severe cases may result in tissue death (gangrene) and require surgical intervention.

30. What is Nanopore sequencing?

Nanopore Sequencing

Nanopore sequencing is a next-generation DNA sequencing technology that allows direct, real-time reading of DNA or RNA sequences without the need for amplification. It works by passing a single DNA or RNA molecule through a nanopore, which is a tiny protein or synthetic channel embedded in a membrane.

Principle:

As nucleic acids pass through the nanopore, they disrupt an ionic current across the membrane. Each nucleotide (A, T, G, C) causes a characteristic change in the current, which is detected and translated into the sequence of bases.

Advantages:

- Can read very long DNA or RNA fragments (long reads).
- Provides real-time sequencing.
- Requires minimal sample preparation.
- Portable devices are available, e.g., Oxford Nanopore MinION.

Applications:

- Whole-genome sequencing
- Pathogen detection and surveillance
- Transcriptome analysis
- Rapid genetic diagnostics in clinical and field settings

31. Write disadvantages of nucleic acid and cell therapy.

Disadvantages of Nucleic Acid and Cell Therapy

Nucleic Acid Therapy:

- ***Immune reactions:*** Introduction of foreign DNA or RNA can trigger immune responses or inflammation.
- ***Low efficiency:*** Delivery of nucleic acids into target cells can be inefficient.
- ***Off-target effects:*** Gene-editing techniques may alter unintended genes, causing harmful mutations.
- ***Short-lived expression:*** Some therapies may not provide long-term gene expression, requiring repeated treatments.
- ***Safety concerns:*** Risk of insertional mutagenesis when DNA integrates into the genome, potentially causing cancer.

Cell Therapy:

- ***Immune rejection:*** Transplanted cells may be recognized as foreign and attacked by the patient's immune system.
- ***Tumor formation:*** Stem or progenitor cells may proliferate uncontrollably, leading to tumors.
- ***Limited cell availability:*** Sourcing sufficient healthy cells for therapy can be challenging.
- ***High cost and complexity:*** Cell isolation, culture, and transplantation require specialized facilities and are expensive.
- ***Infection risk:*** Manipulation of cells outside the body can introduce contamination or pathogens.

32. What is effective way of rDNA through plasmid?

Effective Way of rDNA Through Plasmid

The most effective way to produce recombinant DNA (rDNA) using a plasmid vector involves the following steps:

1. ***Gene isolation and insertion*** – The desired gene (DNA fragment) is isolated and inserted into a plasmid vector using restriction enzymes and DNA ligase. The plasmid serves as a carrier to replicate the gene in a host.
2. ***Transformation into host cells*** – The recombinant plasmid is introduced into bacterial cells, usually *Escherichia coli*, through methods like heat shock or electroporation.
3. ***Selection of transformed cells*** – Host cells that have taken up the recombinant plasmid are selected using antibiotic resistance markers or other selectable traits present in the plasmid.
4. ***Replication and expression*** – The plasmid replicates independently inside the host, producing multiple copies of the inserted gene. In some cases, the gene is also expressed to produce the corresponding protein.
5. ***Isolation of rDNA or protein product*** – The plasmid or expressed protein is extracted and purified for further use in research, medicine, or biotechnology applications.

33. What is recombinant DNA? Write steps of cloning recombinant DNA.

Recombinant DNA (rDNA)

Recombinant DNA is a molecule of DNA formed by joining genetic material from two different sources, usually from different organisms. It is created using biotechnology techniques to combine a gene of interest with a vector (like a plasmid), allowing it to be replicated and expressed in a host organism. Recombinant DNA is widely used in medicine, agriculture, and research to produce proteins, vaccines, and genetically modified organisms.

Steps of Cloning Recombinant DNA

1. ***Isolation of Gene of Interest*** – The specific DNA fragment or gene to be cloned is extracted from the donor organism.
2. ***Selection of Vector*** – A suitable vector, such as a plasmid, is chosen to carry the gene into the host cell.
3. ***Insertion of Gene into Vector*** – The gene is inserted into the vector using restriction enzymes to cut DNA and DNA ligase to join the fragments, forming recombinant DNA.
4. ***Introduction into Host Cells (Transformation)*** – The recombinant plasmid is introduced into host cells, usually bacteria, using transformation techniques like heat shock or electroporation.
5. ***Selection of Transformed Cells*** – Cells that have successfully taken up the recombinant DNA are selected using antibiotic resistance markers or other selectable traits.
6. ***Replication and Expression*** – The host cells replicate, producing multiple copies of the recombinant DNA, and in some cases, express the gene to produce the desired protein or product.
7. ***Isolation and Purification*** – The recombinant DNA or the expressed protein is extracted and purified for further use in research, medicine, or industry.

34. Write functions of Helper T-cells and Killer T-cells.

Functions of Helper T-Cells and Killer T-Cells

Helper T-Cells ($CD4^+$ T-Cells):

- Activate B-cells to produce antibodies.
- Stimulate cytotoxic T-cells (killer T-cells) to destroy infected cells.
- Release cytokines that coordinate the immune response.
- Enhance activity of macrophages and other immune cells to fight infections.

Killer T-Cells (Cytotoxic T-Cells, $CD8^+$ T-Cells):

- Directly destroy virus-infected cells, tumor cells, and damaged cells.
- Release perforin and granzymes to induce apoptosis (cell death) in target cells.
- Recognize antigens presented by MHC class I molecules on infected cells.
- Play a key role in cell-mediated immunity against intracellular pathogens.

35. Write a short note on Insulin.

Insulin

Insulin is a hormone produced by the beta cells of the pancreas in the islets of Langerhans. It plays a key role in regulating blood glucose levels by helping cells absorb glucose from the blood for energy. Insulin also promotes the storage of excess glucose as glycogen in the liver and muscles, and supports the synthesis of fats and proteins while inhibiting glucose production in the liver.

In medicine, recombinant human insulin is produced using biotechnology and is used to treat diabetes mellitus**,** a condition where the body either does not produce enough insulin or cannot use it effectively. Proper insulin therapy helps maintain normal blood sugar levels, preventing complications like nerve, kidney, and eye damage.

Lack of insulin or resistance to its effects leads to hyperglycemia, which, if untreated, can cause serious health problems such as diabetic ketoacidosis or long-term organ damage. Insulin is therefore essential for energy metabolism and overall health.

36. Write advantages of the DNA vaccine.

Advantages of DNA Vaccines

Strong immune response – DNA vaccines can stimulate both humoral (antibody-mediated) and cellular (T-cell-mediated) immunity**.**

Safety – They do not contain live pathogens, so there is no risk of causing the disease**.**

Stability – DNA is more stable than protein-based vaccines, making storage and transport easier.

Rapid development – DNA vaccines can be designed and produced quickly, which is useful during outbreaks.

Flexibility – Multiple antigens can be encoded in a single DNA vaccine, allowing broad-spectrum protection**.**

Cost-effective – Production is generally simpler and less expensive than traditional vaccines.

No need for adjuvants – Often, DNA vaccines can stimulate immunity without additional chemicals.

37. How liposomes are effective in drug delivery?

Effectiveness of Liposomes in Drug Delivery

Liposomes are spherical vesicles with a phospholipid bilayer that can carry both water-soluble and fat-soluble drugs, making them highly effective as drug delivery systems.

- ***Targeted delivery:*** Liposomes can be modified with molecules that direct them to specific tissues or cells, reducing side effects on healthy tissues.
- ***Controlled release:*** Drugs encapsulated in liposomes can be released slowly over time, improving therapeutic effects.
- ***Enhanced stability:*** Encapsulation protects drugs from degradation by enzymes or the immune system, increasing their lifespan in the body.
- ***Reduced toxicity:*** By delivering drugs directly to target cells, liposomes minimize exposure to other organs, lowering adverse effects.
- ***Versatility:*** Both hydrophilic drugs (in the aqueous core) and hydrophobic drugs (within the lipid bilayer) can be carried efficiently.