Targeted Drug Delivery Systems of Biopharmaceuticals

Presented by: Dr. Areen Alshweiat

TARGETED DRUG DELIVERY SYSTEMS AND BIOTECHNOLOGICAL PRODUCTS

INTRODUCTION

- Many diseases occur as a result of defects or errors in the genes involved in producing essential enzymes or proteins in the body.
- The aim of human Genome Project is to sequence the human genome. This lead to provide more information on the role of genetics in congenital defects, cancer, disorders involving the immune system, and other diseases that have a genetic link.
- So the genetic basis of the disease leads to development of many new drugs to treat these diseases, particularly in the field of biotechnology.

What is Biotechnology?

- Biotechnology is the manipulation of living organisms and organic material to serve human needs.
- Examples:
  - Yeast in bread making and alcohol production
  - Use of beneficial bacteria (penicillin) to kill harmful organisms
  - Cloning of plants and animals
  - Artificial insemination

➢ The large biopharmaceuticals have potential to treat disease in novel ways by using of biological materials to create a specific drug product.

➢ E.g. Nucleic acid, protein and peptide drugs, and diagnostics are the main drug products emerging from the biopharmaceutical industry.

Protein Drugs

- The human genome produces thousands of gene products that prevent disease and maintain health. Many may have therapeutic applications if supplemented to normal or supraphysiologic levels in the body.
- Most of the biologic molecules are normally present in the body in small concentrations but are used for certain therapeutic indications.
- For example, some diseases such as insulin-dependent diabetes result from insufficient production of a natural product, in this case insulin. For these patients, the treatment is to supplement the patient's own insulin production with recombinant human insulin (e.g, Humulin).
- Similarly, human recombinant growth hormone (Protropin, Nutropin) and glucocerebrocidase (Ceredase, Cerezyme) are used to treat growth hormone deficiency and Gaucher's disease, respectively.
- See table 18.1: A Sample of Approved Recombinant Drugs p664

Protein Drugs

- Interferons in our body are proteins produced by the immune system in response to viral infection and other biologic inducers.

يقوى قدرة الجهاز المناعي للجسم

- When infection or cancer surpasses the capacity of the body's immune system, recombinant interferons or other immune-enhancing molecules such as can be used to boost immunity and strengthen the immune system during infection, immunosuppression, cancer, and multiple sclerosis.

مثبط للمناعة

تصلب

Protein Drugs

- Erythropoetin and growth factors (GF) are also used to stimulate red and white cell production for anemia or immune suppression following chemotherapy. These molecules were originally available only by purification from human or animal sources.

- Thereby, using biotechnology, bioengineering, and cell banks have facilitated the large-scale and reproducible production of these naturally occurring biologically derived drugs.

Protein Drugs

- A biotechnology-derived drug (also referred to as a biologic drug or biopharmaceutical) must be designed such that:
  - structure is stable, reproducible, and accurate during manufacture, storage, and administration.
- The size of a protein or peptide drug can range from a few hundred to several hundred thousand daltons.
- The 3D structure of a protein or peptide drug is important for its **pharmacodynamic** activity.

Protein Drugs

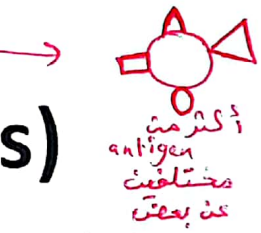
- Drug delivery of biologics can be a problem for therapeutic use because the protein drug must reach the site of action physically and structurally intact.
- Biologic drugs are notoriously unstable in plasma and the gastrointestinal tract, so modifications to improve drug delivery or stability are often required.
- Currently, most biologic drugs are generally too unstable for oral delivery and must usually be administered by parenteral routes. However, other, non-parenteral routes of administration, such as intranasal and inhalation, are being investigated for biologic drug delivery.

antigen يكون كلمة زيد بعضن

: Polyclonal/Monoclonal الفرق بين ال

one antigen ← Monoclonal ال يكون مرتبط

more than one antigen ← recognition يعمل Polyclonal ال



Monoclonal Antibodies (mAbs)

- Antibodies are produced by the body's immune system for specific recognition and removal of foreign bodies. الأجسام الغريبة
- The power of mAbs lies in their highly specific binding of only one antigenic determinant.
- The body responds immunologically to foreign substances containing antigenic sites by producing antibodies.
- These antigenic sites are usually on protein molecules, but non-protein material or haptens may be conjugated to a protein to form an antigen.

non protein molecule

بس لما يرتبط بالبروتين تزيد فعالية ال antibodies

Monoclonal Antibodies (mAbs)

- Periodic injections of an antigen into an animal result in production of antibodies that bind a site or sites on that antigen. The serum of the animal will also contain antibodies to antigens to which the animal has been previously exposed.
- Though these mixtures of antibodies in the serum (polyclonal antibodies) are too impure for therapeutic use, they can be used for diagnostic immunoassays.

Monoclonal Antibodies (mAbs)

- Unlike polyclonal antibodies, mAbs contain many copies of a single antibody that bind to and only detect one antigenic site.
- The techniques for the preparation of mAbs are quite complicated.
- In mAb production, normal antibody-producing cells, such as a mouse spleen cell, are fused with a myeloma cell and allow the hybrid cells (hybridoma) to grow in a test tube.
- The nonfused cells will die, the myeloma cells will be selectively destroyed with an antitumor drug such as aminopterin, whereas the hybridoma cells will continue to grow.
- Each hybridoma cell is then separated into a separate growth chamber or well in which they are allowed to multiply.
- Each cell and its clones in the respective growth chamber will make antibodies to only one antigen (mAb).

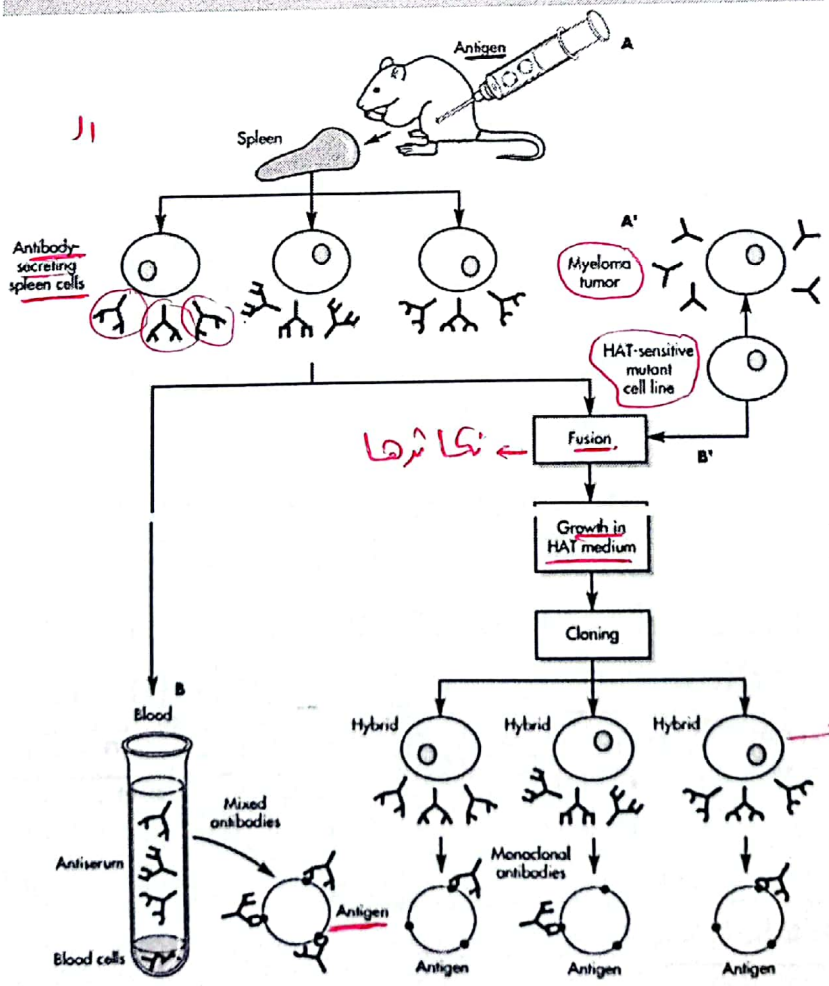
Monoclonal Antibodies (mAbs)

- The cells producing the desired antibody are selected by testing each well for mAb binding to the desired antigen.
- The desired cells (clones) are then expanded for mAb production.
- Since the resulting mAb is of murine origin, often genetic engineering is used to "humanize" the mAb, thus minimizing an immune response to the therapeutic mAb.

يعني جينات الـ mAb من animal source

و بعدين تعطيه لـ Human

Figure 18-1.Monoclonal antibody production.



الـ spleen بتنتج antibody

نكاثرها

Hybridoma

Monoclonal Antibodies (mAbs)

Table 18.2 Applications of Monoclonal Antibodies
Cancer treatment mAbs against leukemia and lymphomas have been used in treatment with variable results. Regression of tumor is produced in about 25%, although mostly transient. **Imaging diagnosis** mAbs may be used together with radioactive markers to locate and visualize the location and extent of the tumors. **Target-specific delivery**
mAbs may be conjugated to drugs or other delivery systems such as liposomes to allow specific delivery to target sites. For example, urokinase was conjugated to an antifibrin mAb to dissolve fibrin clots. The carrier system would seek fibrin sites and activate the conversion of plasmogen to plasmin to cause fibrin to degrade.
Transplant rejection suppression In kidney transplants, a mAb against CD3, a membrane protein of cytotoxic T cells that causes a rejection reaction, was very useful in suppressing rejection and allowing the transplant to function. The drug was called OKT3. mAbs are also used for kidney and bone marrow transplants.

Monoclonal Antibodies (mAbs)

- Monoclonal antibodies may be used therapeutically to neutralize unwanted cells or molecules.
- Several mAbs with proven indications are listed in page 670
- Monoclonal antibodies are used as antivenoms, for overdose of digoxin (DigiFab), or to neutralize endotoxin or viral antigen.
- Nebacumab is a human IgM mAb (HA-1A) with specificity for the lipid designed for septic shock treatment. Monoclonal antibodies (mAb) are named by a source identifier preceding "-mab," e.g., -umab (human), -omab (mouse), -zumab (humanized), and -ximab (chimeric).
- Other common indications for mAb drugs include imaging, cancer. rheumatoid arthritis, and transplant immunosuppression.

Gene Therapy

- Gene therapy refers to a pharmaceutical product that delivers a recombinant gene to somatic cells in vivo.
- In turn, the gene within the patients' cell produces a protein that has therapeutic benefit to the patient.
- The therapeutic approach in gene therapy is often the restoration of defective biologic function within cells, as is frequently seen in inherited disorders and cancer.
- Gene therapy has been applied to the inherited disorder cystic fibrosis

Gene Therapy

Two main approaches have been used for in-vivo delivery of rDNA.

1. **The first is a virus-based approach**

➢ Viral delivery systems (vectors)

➢ That involves replacing viral replicative genes with the transgene, then packaging the rDNA into the viral particle.

➢ The recombinant virus can then infect target cells, and the transgene is expressed, though the virus is not capable of replicating.

➢ Both retroviruses, RNA viruses that have the ability to permanently insert their genes into the chromosomes of the host cells, and DNA viruses (which remain outside host chromosomes) have been used successfully in viral gene delivery.

Gene Therapy

2. Non-viral approaches for in-vivo gene delivery.

- The transgene is engineered into a plasmid vector, which contains gene-expression control regions.
- These naked DNA molecules may enter cells and express product in some cell types, such as muscle cells.
- This naked DNA delivery technique is being tested as possible DNA vaccines, in which the muscle cells produce small amounts of antigen that stimulate immunity to the antigen.

Gene Therapy

- An alternative to direct in-vivo delivery is a cell-based approach that involves the administration of transgenes to cells that have been removed from a patient.
- For example, cells (usually bone marrow cells) are removed from the patient; genes encoding a therapeutic product are then introduced into these cells ex vivo using a viral or nonviral delivery system, and then the cells are returned into the patient.
- The advantage of ex-vivo (in vitro) approaches is that systemic toxicity of viral or nonviral delivery systems is avoided. (or minimized)

Gene Therapy

- **Effective gene therapy depends on several conditions:**

1. The vector must be able to enter the target cells efficiently.
2. Deliver the corrective gene without damaging the target cell.
3. The corrective gene should be stably expressed in the cells, to allow continuous production of the functional protein.
4. Neither the vector nor the functional protein produced from it should cause an immune reaction in the patient.

Gene Therapy

5. It is also difficult to control the amount of functional protein produced after gene therapy, and excess production of the protein could cause side effects, although insufficient production is more typically observed.
6. The physical and chemical properties of DNA and RNA molecules, such as size, shape, charge, surface characteristics, and the chemical stability of these molecules and delivery systems.
7. In-vivo problems may include bioavailability, distribution, and uptake of these macromolecules into cells.
8. Moreover, naked DNA molecules are rapidly degraded in the body.

Antisense Drugs

- Antisense drugs are drugs that seek to block DNA transcription or RNA translation in order to moderate many disease processes.
- Antisense drugs consist of nucleotides linked together in short DNA or RNA sequences known as oligonucleotides.
- Oligonucleotides are designed knowing the target DNA/RNA to bind to specific DNA or RNA sequences or regions (eg, messenger RNA) to block transcription or translation of that targeted protein.
- An oligonucleotide that binds complementary ("sense") mRNA sequences and blocks translation is referred to as antisense.

DRUG CARRIERS AND TARGETING

Targeted Drug Delivery

- Drug delivery systems (DDS) can selectively deliver drugs to the target cells with minimum side effects. The remarkable development of nanotechnology led to the development of efficient nanocarrier systems capable of delivering therapeutic agents to target tumour tissue.
- For biopharmaceuticals, selective and targeted drug therapy could result in a significant reduction in dose and cost.

Targeted Drug Delivery

- Site-specific drug delivery is classified into three broad categories or drug targeting:

1. First-order targeting, which refers to drug delivery systems that deliver the drug to the capillary bed of the active site
   arterial capillary → tissue → venous capillary
2. Second-order targeting, which refers to the specific delivery of drug to a special cell type such as the tumor cells and not to the normal cells
   targeting not for all cell
3. third-order targeting, which refers to drug delivery specifically to the internal (intracellular) site of cells. An example of third-order drug targeting is the receptor-mediated entry of a drug complex into the cell by endocytosis followed by lysosomal release of the lysosomally active drug.

General Considerations in Targeted Drug Delivery

1. The anatomic and physiologic characteristics of the target site, including capillary permeability to macromolecules and cellular uptake of the drug
2. The physicochemical characteristics of the therapeutically active drug
3. The physical and chemical characteristics of the carrier
4. The selectivity of the drug carrier complex
5. Any impurities introduced during the conjugation reaction linking the drug and the carrier that may be immunogenic, toxic, or produce other adverse reactions.

Targeted Drug Delivery

- **Site-Specific Carrier:** To target a drug to an active site, one must consider whether there is a unique property of the active site that makes the target site differ from other organs or tissue systems in the body.
- The next consideration is to take advantage of this unique difference so that the drug goes specifically to the site of action and not to other tissues in which adverse toxicity may occur.
- In many cases the drug is complexed with a carrier that targets the drug to the site of action.

Targeted Drug Delivery

- **Drugs:** Most of the drugs used for targeted drug delivery are highly reactive drugs that have potent pharmacodynamic activities with a narrow therapeutic range. These drugs are often used in cancer chemotherapy.
- Many of these drugs may be derived from biologic sources, made by a semisynthetic process using a biologic source as a precursor, or produced by recombinant DNA techniques.
- The drugs may be large macromolecules, such as proteins, and are prone to instability and inactivation problems during processing, chemical manipulation, and storage.

Formulation and Delivery of Protein Drugs

Advances in biotechnology have resulted in the commercial production of **naturally** produced active drug substances for drug therapy. These substances CHARACTERISED by:

- hold great potential for more specific drug action
- with fewer side effects
- complex molecules, such as large-molecular-weight proteins and peptides. →
- Conventional delivery of protein and peptide drugs is generally limited to injectables and implantable dosage forms. Because Formulating protein drugs for systemic use by oral, or even any extravascular, route of administration is extremely difficult due to **drug degradation** and **absorption from the site of administration**.

There are several requirements for effective oral drug delivery of protein and peptide drugs:

1. Protection of the drug from degradation while in the harsh environment of the digestive tract,
2. Consistent absorption of the drug in a manner that meets bioavailability requirements,
3. Consistent release of the drug so that it enters the bloodstream in a reproducible manner,
4. Nontoxicity,
5. Delivery of the drug through the GI tract and maintenance of pharmacologic effect similar to IV injection.

Absolute bioavailability
لازم تكون قريبة من 1

Designing, evaluating, and improving protein and peptide drug stability is considerably more complex than for small conventional drug molecules for the following reason:

1. **A change in quaternary structure, such as aggregation or deaggregation of the protein, may result in loss of activity.**
2. **Changes in primary structure of proteins frequently occur throughout the body**
3. **Because of protein drugs' complex structures, impurities are much harder to detect and quantify.**
4. **proteins may be recognized as foreign substances in the body and become actively phagocytized by the reticuloendothelial system (RES), resulting in the inability of these proteins to reach the intended target.**
5. **Proteins may also have a high allergenic or immunogenic potential, particularly when nonhuman genes or production cells are used.**

Humanization عشان نخفف من immunogenic property of the drug

The many stability and delivery problems associated with protein and nucleic acid drugs, provide a rationalism to generate new delivery systems to protect the drug from degradation, improve transport or delivery to cells, decrease clearance, or a combination of the above.

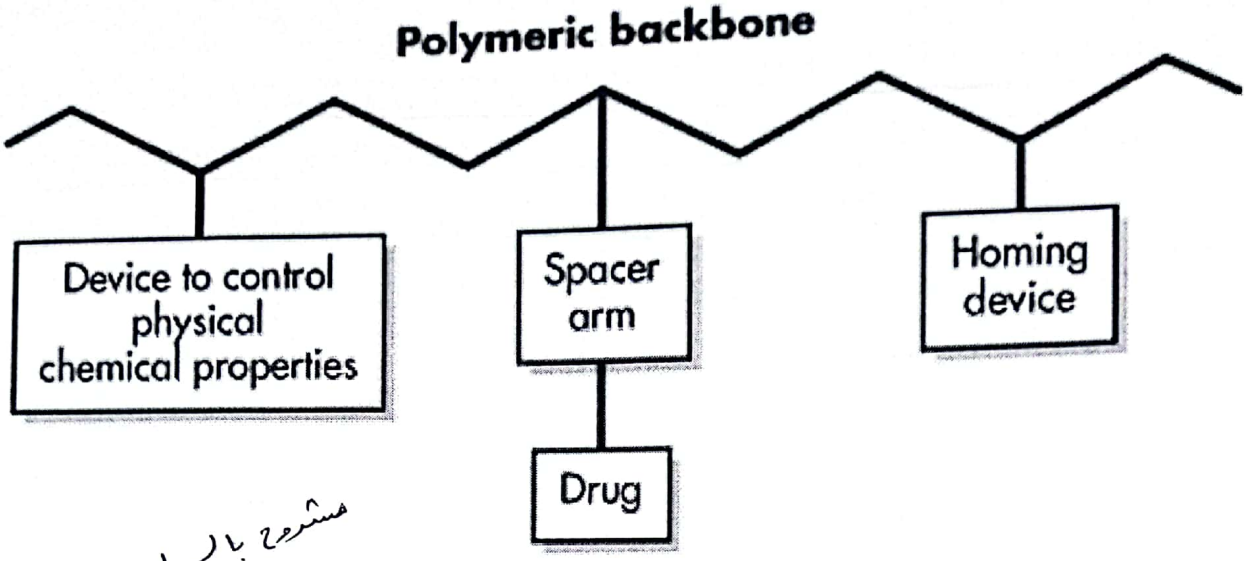
Carriers used for both small traditional drug and biopharmaceutical drug delivery, which may be covalently bound to the drug, where drug release is usually required for pharmacologic activity.
Noncovalent drug carriers such as liposomes typically require uncoating of the drug for biologic activity to occur.

Polymeric Carriers and Conjugates

- Polymers initially were used to prolong drug release in controlled-release dosage forms.
- The basic components of site-specific polymer carriers are

1. The polymeric backbone
2. a site-specific component (homing device) for recognizing the target
3. The drug covalently attached to the polymer chain, and
4. Functional chains to enhance the physical characteristics of the carrier system.

- In the case of polymeric prodrugs, a spacer group may be present, bridging the drug and the carrier. The spacer chain may influence the rate at which the drug will hydrolyze from the prodrug system.



مشروح بالسلايد الماضي

Site-specific polymeric carrier.

- Positively charged polymers such as polyethylenediamine (PEI), polylysine, and chitosan are used in noncovalent complexes for macromolecular drugs, such as gene or oligonucleotide therapy.

- Polymers may also be covalently conjugated to drugs to improve their solubility or pharmacokinetic properties. Polymers with molecular weights greater than 30 or 50 kDa bypass glomerular filtration, thereby extending the duration of drug circulation in the body.

لأنه اذا كان اقل من 20kDa يصير له glomerular filtration و drug عشان هيك نربط ال

- Polyethylene glycol (PEG) is used to improve the clearance of some drugs, such as adenosine deaminase, filgrastim, interferon, and asparaginase.

In addition to use as regular carriers, polymers may also be formulated into microparticles and nanoparticles.

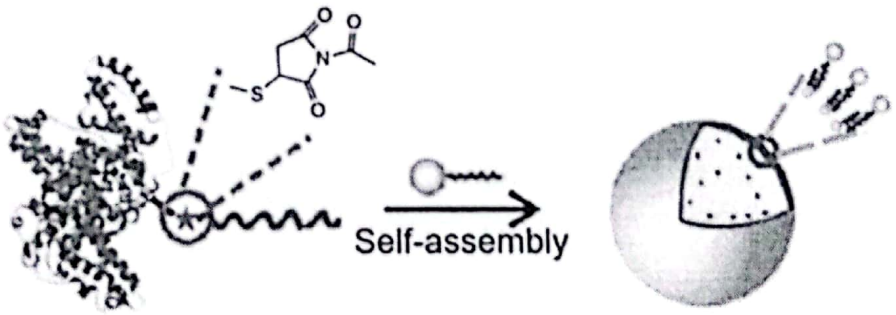
Micro- and nanosphere formulations are useful for:

- Solubilizing poorly soluble drugs,
- Improving oral bioavailability,
- Protecting against degradation,
- Or providing sustained drug delivery.

Lipophelic يكون منحبها عبارة عن suger ← يحب ال Lipophelic drug ويدخل لجوا

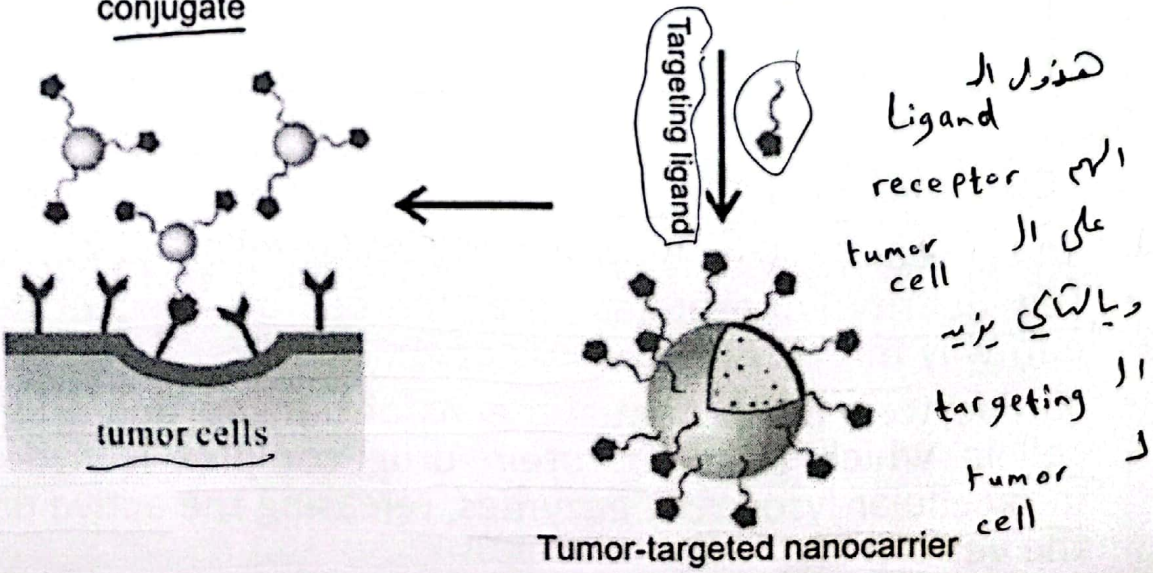
Cyclodextrins (CDs) are also used to improve stability, delivery, and water solubility of drugs. The lipophilic cavity of CDs typically contains the therapeutic agent, while the exterior of the CD molecule is hydrophilic and allows solubilization of the complex.

Hydro phelic



Biodegradable protein-polymer conjugate

Protein-based nanoparticle



هذول ال Ligand
الهم receptor على ال tumor cell
وبالتالي يزيد ال targeting لـ tumor cell

Ex:

Albumin

[redacted]

69KDa

- Albumin is a large protein (MW 69,000 Da) that is distributed in the plasma and extracellular water.
- Albumin has been experimentally conjugated with many drugs to improve site-specific drug delivery.

يصير لها concentration بال Liver

- As albumin concentrate in the liver, albumin complex has been used experimentally to deliver drug to the Kupffer cells in the liver for treatment of ectromelia virus.

Lipoproteins

- Lipoproteins are lipid protein complexes in the blood involved in the circulation and distribution of lipids in the body. The lipid components are polar phospholipids and cholesterol.
- Because of their various sizes, lipoproteins have been classified according to molecular weight based on centrifugation:

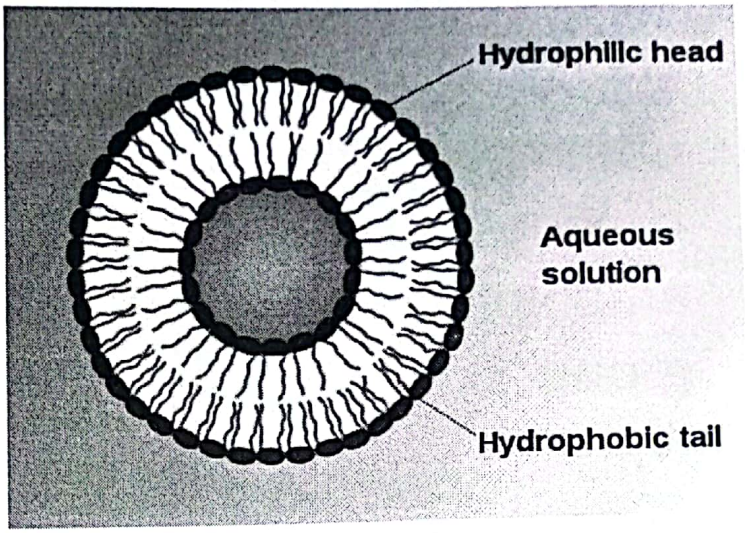
1. High-density lipoprotein (HDL, MW 300,000 to 600,000 Da),
2. Low-density lipoprotein (LDL, MW 2.3 x 10 Da),
3. very- low-density lipoprotein (VLDL, MW 10 x 10 Da),
4. Chylomicrons (MW 10 Da).

لأنه ال lipoprotein بال receptor ارتبط بال drug عشان يدخل لداخل الخلية من طريق endocytosis

- Low-density lipoproteins enter the cell by a receptor-mediated pathway through the process of endocytosis.
- Endocytosis is a potential means of transporting drugs into the cell in which the lipoprotein drug complex is hydrolysed by intracellular lysosomal enzymes, releasing the active drug within the vesicle.

Liposomes

- Liposomes have an aqueous, drug-containing interior surrounded by an exterior lipid bilayer.
- Typically range in size from 0.5 to 100 µm.
- Liposomes have been used successfully to reduce side effects of antitumor drugs and antibiotics.
- For example, doxorubicin liposomes have reduced cardiotoxicity and emetic side effects.



Liposomes

- There are three general ways of preparing conventional liposomes:

(1) Phase separation

(2) Spray or shear method through orifice

(3) Coacervation.

- The choice of method depends on the drug, the yield requirements, and the nature of the lipids.
- Formation of the liposome bilayer depends on the hydrophobic and hydrophilic orientation of the lipids.

surfactant تشبه الـ

Liposomes

- Liposomes have different electrical surface charges depending on the type of material used.
- Common anionic lipid materials are phosphatidyl choline and cholesterol. The phosphatidyl group is amphiphilic, with the choline being the polar group.
- This structure allows each molecule to attach to others through hydrophobic and hydrophilic interactions.

Liposomes

targeting نقدر نتحكم

- Liposomes can be engineered to be site specific. Generally, site specificity is conferred by the type of lipid or by inclusion of a targeting agent, such as a monoclonal antibody, into the liposome bilayer.
- Liposomes may be used to improve intracellular delivery, in which case the liposome must also be designed to fuse with the plasma or endosome membrane