Altogen Labs

Provider of Preclinical Research Services (GLP/non-GLP) for Drug Discovery
Efficacy and Pharm/Tox IND contract research studies (clients worldwide)
100+ Xenograft Models (validated in-house) and IND-enabling Toxicology studies
100% IP belongs to client, experienced IACUC-regulated barrier facility

Liposome Encapsulation

Contact us: **info@altogenlabs.com** | Read more at **AltogenLabs.com**
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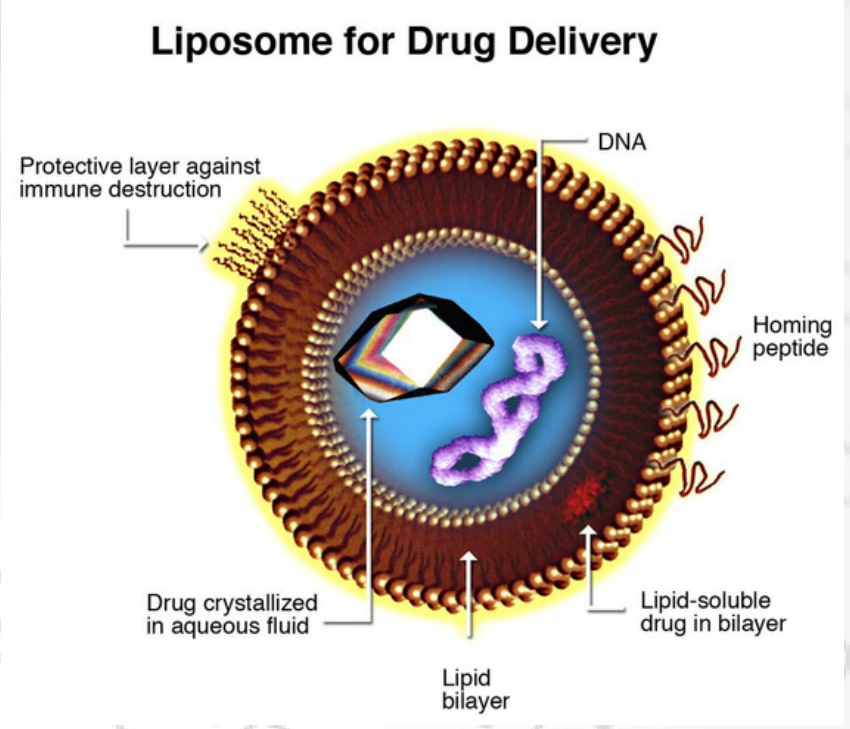
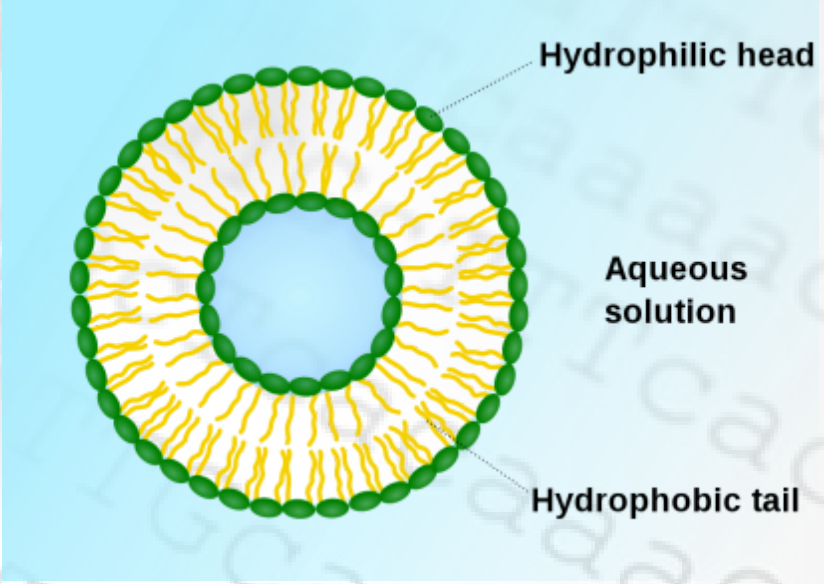
What are liposomes?

- **Liposomes** are phospholipid bilayers containing aqueous cores.
- Over 50 years ago, researchers discovered that these spheres could be filled with therapeutic agents and used to protect and deliver these agents into the body and even into specific cells of the body.
- Liposomes have been demonstrated to improve delivery of encapsulated cargo.



Structure

- Structurally, a liposome is a spherical vesicle that has at least one lipid bilayer and an aqueous core. The liposome can be used as a vehicle for administration of nutrients and pharmaceutical drugs.

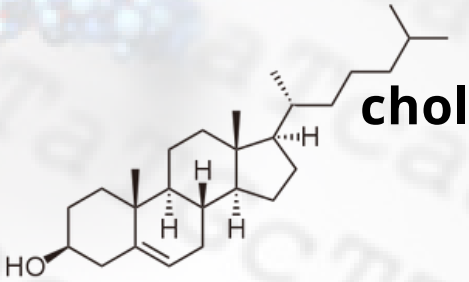


Liposomal Formulations

- Since the first liposomal pharmaceutical product, Doxil, was approved in 1995 there are now several liposomal-drug formulations on the market.
- Most of them have to be administrated intravenously due to the degradation of lipids in the gastrointestinal tract. However, some recent formulations such as Arikace can be subcutaneously injected or inhaled as aerosols.
- Apart from a broadened range of drugs being investigated for liposomal formulations, new strategies such as environmental sensitivity and combination therapy have been applied to the development process to achieve better efficacy.

Lipids, Phospholipids, and Liposomes

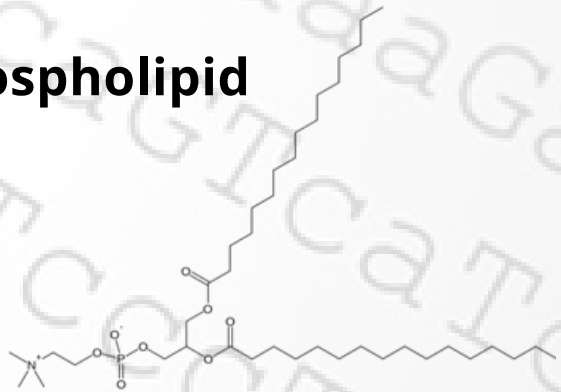
- **Lipids** are a group of naturally occurring molecules that include fats, waxes, sterols, and fat-soluble vitamins. The main biological functions of lipids include: storing energy, signaling, and acting as structural components of cell membranes.



cholesterol

- **Phospholipids** are a class of lipids that are a major component of all cell membranes as they can form lipid bilayers. The structure of the phospholipid molecule generally consists of hydrophobic tails and a hydrophilic head.

phospholipid



Clinically Approved Liposomal Drugs

Name	Trade name	Indication
Liposomal amphotericin B	Abelcet	Fungal infections
Liposomal amphotericin B	Ambisome	Fungal and protozoal infections
Liposomal cytarabine	Depocyt	Malignant lymphomatous meningitis
Liposomal daunorubicin	DaunoXome	HIV-related Kaposi's sarcoma
Liposomal doxorubicin	Myocet	Combination therapy with cyclophosphamide in metastatic breast cancer
Liposomal IRIV vaccine	Epaxal	Hepatitis A
Liposomal IRIV vaccine	Inflexal V	Influenza
Liposomal morphine	DepoDur	Postsurgical analgesia
Liposomal verteporfin	Visudyne	Age-related macular degeneration, pathologic myopia, ocular histoplasmosis
Liposome-proteins SP-B and SP-C	Curosurf	Pulmonary surfactant for Respiratory Distress Syndrome (RDS)
Liposome-PEG doxorubicin	Doxil	HIV-related Kaposi's sarcoma, metastatic breast cancer, metastatic ovarian cancer
Micellular estradiol	Estrasorb	Menopausal therapy
Liposomal vincristine	Marqibo	Acute Lymphoblastic Leukemia (ALL) and Melanoma
Liposome-PEG doxorubicin	Lipo-Dox	HIV-related Kaposi's sarcoma, metastatic breast cancer, metastatic ovarian cancer, Multiple Myeloma

Altogen Labs provides following standard liposomal formulations:

Standard Liposome #1: DSPC : Cholesterol = 2 : 1
Standard Liposome #2: DMPC : DMPG : Cholesterol = 1 : 1 : 1
Standard Liposome #3: DOPC : Cholesterol : DPPG : Triolein = 7 : 7 : 1 : 1
Standard Liposome #4: PC : DOTAP : PEG : Cholesterol = 10 : 1 : 1 : 3
Standard Liposome #5: EPC : Cholesterol = 55 : 45
Standard Liposome #6: HSPC : Cholesterol : PEG2000-DSPE = 12 : 8 : 1
Standard Liposome #7: HSPC : Cholesterol : DSPG = 2 : 1 : 0.8
Standard Liposome #8: DOPC : Cholesterol : Cardiolipin = 5 : 4 : 1

* Please note that the cost of encapsulation will depend on type of liposome, number of samples, and total amount (mgs) of compound to be encapsulated.

Benefits and Applications

- Due to their unique properties, including **low cytotoxicity**, **good biocompatibility,** and **biodegradability**, liposomes have wide-ranging applications in different fields including gene and drug delivery, food/nutrition industries, and cosmetic industries.

- A number of new liposome modification methods have emerged to improve stability and attain higher concentrations of bioactives in the target cells and cellular compartments for maximum therapeutic efficiency.

Methods

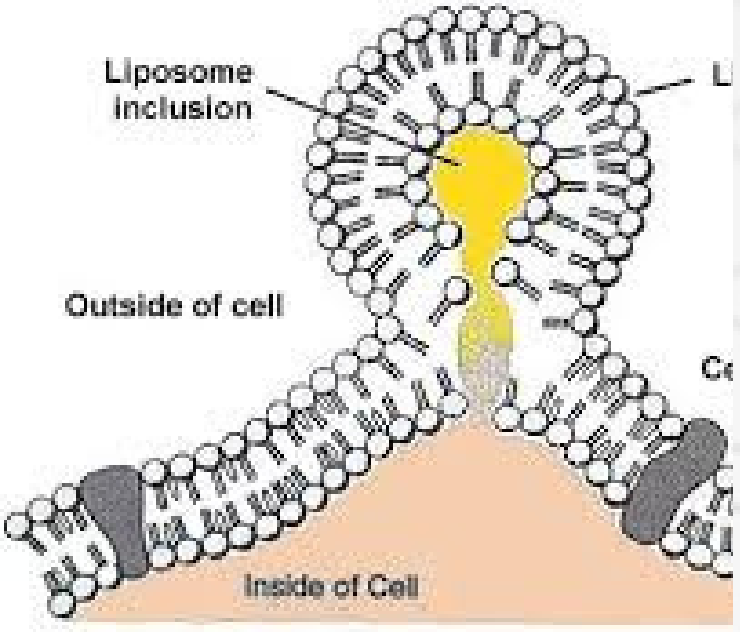
- **Active targeting** can be achieved via appropriately engineered modifications to the liposomal structure. For active targeting, thermo-labile, pH-sensitive, photo-sensitive and antibody coated vesicles, have been designed.

- **Passive targeting** is the mechanism by which the bioactive-carrier complex reaches its destination based on the physicochemical properties of bioactive carrier complexes and does not utilize any targeting strategy.

Mechanism

- Hydrophilic solutes dissolved in the core cannot readily pass through the bilayer. Hydrophobic chemicals associate with the bilayer via Van der Waals forces.

- This makes liposomes versatile and advantageous delivery vehicles as they can be formulated with hydrophobic and/or and hydrophilic cargo. To deliver the molecules to a site of action, the lipid bilayer can fuse with other bilayers such as the cell membrane, thus delivering the liposome contents.



A liposome fusing with a cell membrane.

Formation

- Useful liposomes rarely form spontaneously. They typically develop after supplying enough energy, in the form of sonication, to a dispersion of phospholipids in a polar solvent, such as water, to break down multilamellar aggregates into oligo- or unilamellar bilayer vesicles.

Mechanism of Delivery

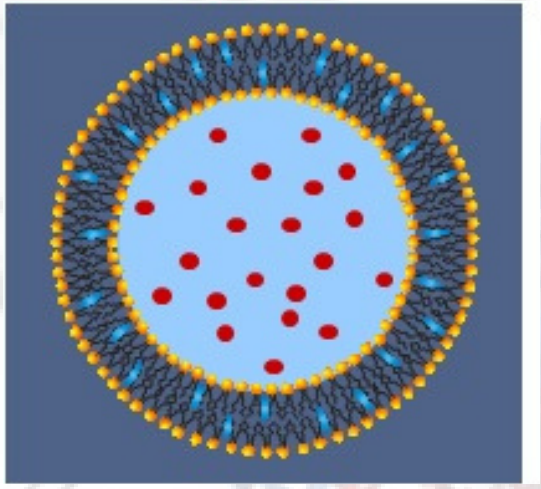
- By preparing liposomes in a solution of DNA or drugs (which would normally be unable to diffuse through the membrane) they can be (indiscriminately) delivered past the lipid bilayer, but are then typically distributed non-homogeneously.

- For drug delivery, liposomes that contain low (or high) pH can be constructed such that dissolved aqueous drugs will be charged in solution (i.e., the pH is outside the drug's pH range).

Membrane penetration

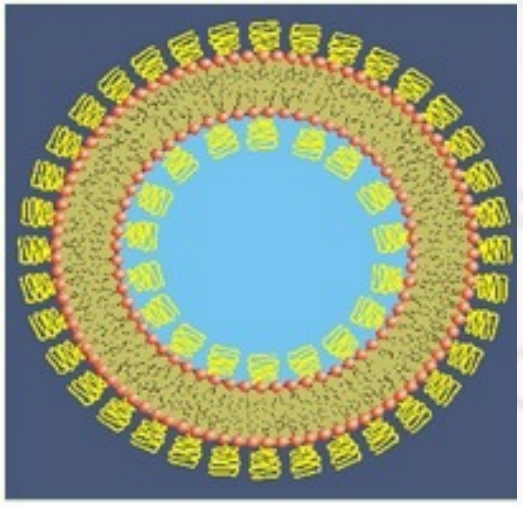
- As the pH naturally neutralizes within the liposome (protons can pass through some membranes), the drug will also be neutralized, allowing it to freely pass through a membrane. These liposomes work to deliver drug by diffusion rather than by direct cell fusion.

Evolution of Liposomes



First generation: Conventional Liposomes

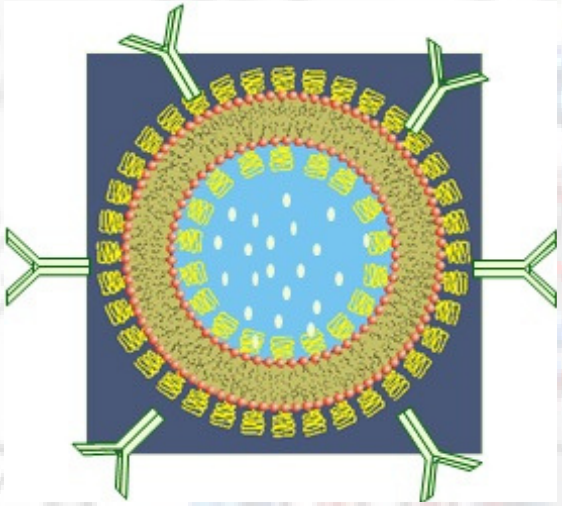
Various types of drugs can be loaded into the interior, in the bilayer or at the interface depending on the nature of the compounds.



Second generation: PEGylated Liposome

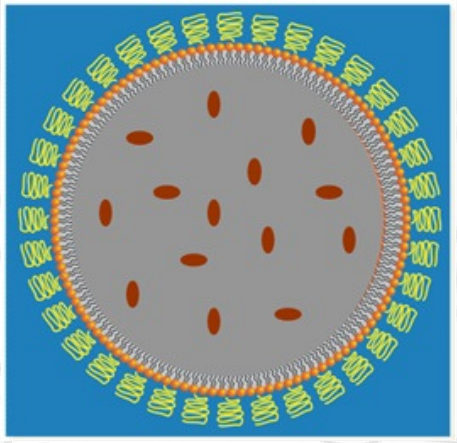
It offers significant advantages over conventional liposomes such as an extended half life. Other potential benefits include reduced toxicity, reduced dosing, frequency, etc.

Evolution of Liposomes



Third generation: Ligand-Targeted Liposome

PEGylated liposomes with targeting ligands (antibodies, antibody fragments, peptides and small molecules) have the potential of specific targeted delivery to the disease site through ligand-receptor binding.

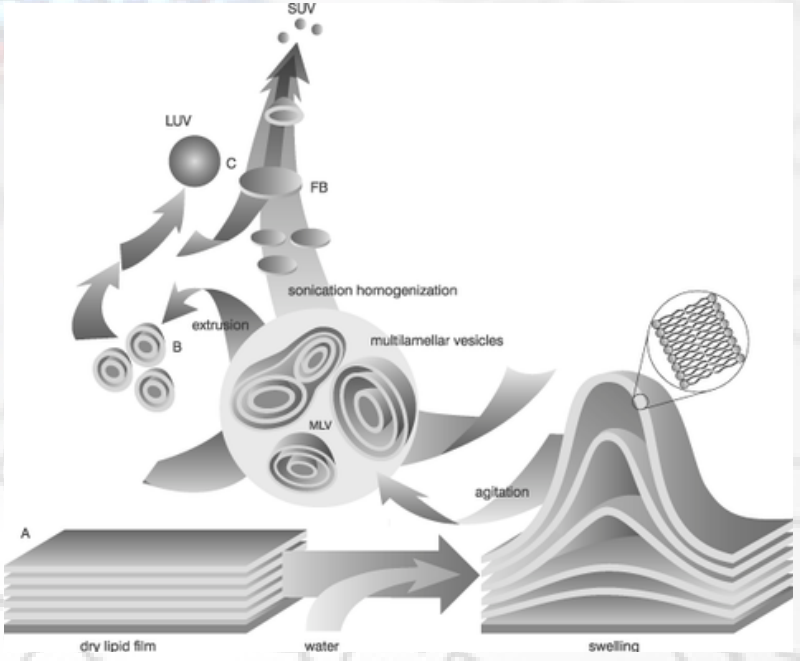


Lipid-Nanoparticles (Liposphere)

A liposphere is a nanoparticle with a lipophilic core (such as triglycerides) coated with a monolayer of lipids. Lipophilic drugs are contained in the oil core.

Methods of Liposome Preparation

- Ether/alcohol injection
- Freeze dry evaporation method
- Extrusion method
- Reverse phase evaporation
- Microfluidization (Microfluidics)
- Detergent depletion
- Supercritical fluid injection and decompression
- Dense gas techniques
- Dual asymmetric centrifugation
- High-pressure homogenization



Liposome formation requires an input of energy, usually in the form of sonication.

Protocols: Freeze Drying

Requirement	Measures
Preservation of liposome size	Add amorphous lyoprotective matrix inside and outside the liposomes.
	Small, unilamellar liposomes
	Freezing after undercooling
Retention of encapsulated, water-soluble compound after reconstitution	Rigid bilayers
	Prevent bilayer rupture by crystal growth (ice, salts, other components), provide substitute for water.
	Temperature at sublimation front and of dried cake must remain below T_g of the amorphous matrix throughout the freeze-drying process
Easy reconstitution; cake with attractive appearance (no collapse)	Formulate towards a high T_g of the amorphous matrix. Add a bulking agent.
Short freeze-dry cycle	Formation of large ice crystals during freezing phase
	Decrease the filling height of the sample.

Freeze-drying of liposomes can prevent hydrolysis of phospholipids and also help to stabilize encapsulated material.

Preservation of labile macromolecules (proteins)	Prevent pH changes during freezing.
Chemical and physical stability of dried product	Storage at a temperature well below T_g of the dried cake.
	Lower residual water content of (amorphous matrix of) the dried cake
	Crystalline water does not necessarily contribute to degradation processes.
	Minimize the oxygen content in the freeze-dried cake.

Protocols: Extrusion Method

- Liposome extrusion is a widely used method in which liposomes are forced under pressure through filters with defined pore sizes in order to generate liposomes of a uniform size.
- Prior to extrusion through the final pore size, multilamellar liposome (LMV) suspensions are disrupted either by several freeze-thaw cycles or by prefiltering the suspension through a larger pore size (typically 0.2μm-1.0μm). This method helps prevent the membranes from fouling and improves the homogeneity of the size distribution of the final suspension.
- Extrusion through filters with 100nm pores typically yields large, unilamellar vesicles (LUV) with a mean diameter of 120-140nm. Mean particle size also depends on lipid composition and is quite reproducible from batch to batch.

Protocols: Microfluidization

- A major challenge in the development of liposomes for drug delivery is the control of size and size distribution. **Microfluidics** is an emerging technology for liposome synthesis, because it enables precise control of the lipid hydration process and allows for the production of liposomes ranging from tens of nanometers to tens of micrometers in diameter.

IPA

- Isopropyl alcohol (IPA) containing the dissolved lipids flow through the center inlet channel, and an aqueous solution flows through the two side inlet channels. The stream of lipids in IPA is hydrodynamically focused by two aqueous streams at the cross junction of the microfluidic chip. The liposome formation is based on a diffusion-driven process in which the dissolved lipids self-assemble into liposomes as IPA quickly diffuses and dilutes into two aqueous streams at the interfacial region.

Injection

- The lipid IPA solution is injected into the center channel of the microfluidics network, while phosphate-buffered saline (PBS) is injected into two side channels intersecting with the center channel. Relatively high liposome concentrations can be produced at the center point in the channel once the focused IPA stream is diluted to the critical concentration for formation of the more stable liposomes along the interfacial region.

Research Development

- Custom formulation and composition can be produced to achieve specific goals for specific projects.

- These include multivesicular liposomes, thermally sensitive liposomes, etc. We are happy to work with our clients to produce ideal liposome delivery.

- A variety of preparation methods are available, such as extrustion and ultrasonication.

Contact Us

- Altogen Labs can encapsulate any charged oligonucleotide (siRNA, miRNA, plasmid DNA) into a standard (PC : Cholesterol, DSPC : Cationic Lipid, PEGylated Lipoid : Cholesterol) or custom lipid formulation.
- When provided with at least 0.1mg of a sample, Altogen Labs can generate liposomes of uniform size from 50 – 400nM particles.

Contact us to discuss details, timeline estimates, and price!

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