Applications of Asymmetric Lipid Vesicles

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Liposomes have been widely studied for drug release applications, for which they are known to have the desired effect by releasing specific concentrations on site. In drug delivery, when a drug is supplied directly to the bloodstream, problems such as short circulation times, drug breakdown, and clearance are lessened. Liposomes are an alternative to avoid these problems because they can trap the drug, control the dosage need, and have an effective drug concentration to target the desired cells.

asymmetric vesicles drug delivery nucleic acid delivery cell models

1. Introduction

Numerous drug delivery methods have been developed for therapeutic applications. Conventional drug delivery systems, such as tablets, capsules, lozenges, syrups, and ointments [1], transport therapeutic molecules without any type of control into the human body by oral consumption, injection, or topical administration. The disadvantages of these conventional systems include the need for biocompatibility, poor distribution, burst or disrupted release, low specificity [2][3], and sometimes side effects [4]. Drug delivery can be improved using nanocarrier-based systems to deliver therapeutic molecules in a controlled, directed, and efficient manner, particularly when combined with local administration [5].

There is a need for highly effective and less toxic alternatives to treat existing and emerging diseases. Historically, researchers have studied nanocarrier-based drug delivery systems, such as solid lipid nanoparticles, liposomes, polymeric micelles, metallic nanoparticles, nanoemulsions, and nanoliposomes, to improve their therapeutic effects against specific diseases, protecting the active molecule against degradation and reducing size effects [3]. In addition, these novel systems could be the key to promoting the development of individualized and molecular medicine strategies [5].

2. Asymmetric Vesicles: Advantages and Applications

Liposomes have been widely studied for drug release applications, for which they are known to have the desired effect by releasing specific concentrations on site. In drug delivery, when a drug is supplied directly to the bloodstream, problems such as short circulation times, drug breakdown, and clearance are lessened [6]. Liposomes are an alternative to avoid these problems because they can trap the drug, control the dosage need, and have an effective drug concentration to target the desired cells [7].

In recent years, the applications of asymmetric liposomes have been gaining importance due to their benefits. Synthetic asymmetric bilayers mimic biological functions better than their symmetric counterparts because naturally occurring bilayers have an asymmetric behavior. Because of this, constructing these types of vesicles represents a step forward in understanding cell membranes and would allow for better delivery systems with cells as a target [8][9][10]. Asymmetric liposomes are also a helpful model system for the in vitro analysis of lipid–lipid and protein–lipid interactions and improve people's understanding of cellular processes [11]; innovative and novel models must be developed.

Asymmetric liposomes have properties that can optimize drug delivery due to their capacity to have different lipids in their layers and to control characteristics such as the charge. For example, it has been exhibited that a high charge density in the inner leaflet helps for an efficient condensation of biopharmaceuticals, such as nucleic acids, and simultaneously, a neutral or negative outer layer is more suitable for biocompatibility [12]. Some advantages of asymmetric lipid vesicles in different research applications are shown in **Table 1**.

Table 1. Advantages of asymmetric lipid vesicles in different research applications.

General Drug Delivery	Nucleic Acids Delivery	Cell Models
Capacity to enhance the properties of the inner and outer leaflets independently to optimize the composition depending on the drug to encapsulate.	Protects nucleic acids from degradation.	A helpful model system for the in vitro analysis of lipid–lipid and protein–lipid interactions.
Different lipids can be used in the outer leaflet to enhance drug delivery and vesicle stability.	A neutral or negative outer layer is an advantage for biocompatibility.	Crucial in developing strategies to understand how drugs could interact with cellular membranes.
Asymmetric liposomes can be engineered to target specific cell types.	Inner-layer engineering allows for better encapsulation capacity.	Better at biological mimetics than their symmetric counterparts.

2.1. Asymmetric Liposomes for Drug Delivery

Liposomes are representative of the development of new and better delivery systems regarding the enhancement of encapsulation, release, and efficiency. One of the objectives of this section is to describe the advantages and applications of asymmetric liposomes in drug delivery systems; a summary of studies related to this application is displayed in **Table 2**. London et al. developed asymmetric liposomes containing cationic or anionic outer leaflets and inner leaflets that had either the opposite charge or were uncharged, and the diameter of the asymmetric liposomes was around 120 nm. They found that anionic lipids in the inner leaflet maximized the amount and stability of doxorubicin entrapment within the vesicles, suggesting that it is possible to choose inner leaflet lipids to maximize the liposomal loading of charged drugs, and the outer leaflet should favor the bioavailability and biodistribution of the vesicles [6].

Table 2. Summary of asymmetric liposome studies with therapeutic applications. In some studies, the data is not presented, and it is indicated by a hyphen (-).

Synthesis Technique	Inner Leaflet	Outer Leaflet	Molecule	Size	Stability	Encapsulation Efficiency	Ref.
Modified reverse phase evaporation method	DODAP/DOPE	DSPC/DOPE/PEG-PE/cholesterol	siRNA	200 nm	siRNA encapsulated was protected from enzyme degradation for up to 24 h	90%	Mokhtarieh et al., 2012 [13]
Inverse emulsions	DMPC/DOTAP	DMPC/POPC/NBD-PC	siRNA	44, 188, and 489 nm	Modes remain relatively constant over a 160 h period	-	Whittenton et al., 2013 [14]
Modified reverse phase evaporation method	DODAP/DOPE	DSPC/DOPE/mPEG-PE/miPEG-PE/cholesterol	Calcein and indocyanine green (ICG).	-	-	90%	Lee., 2015 [15]
Cyclodextrin-catalyzed lipid exchange method	DOPE:POPS, phosphatidylserine (PS)-	bSM, DOPE	-	-	Up to 48 h	-	Petazzi et al., 2015 [16]
Inverse emulsions	DPPC/DOTAP	DPPC/DSPE-PEG2000	plasmid DNA	200 nm	-	10–15%.	De Matos et al., 2019 [6]
Cyclodextrin-catalyzed lipid exchange method	POPC, POePC, DOTAP, POPS, POPG, POPA	POePC, POPC, DOTAP, POPS, DOTAP, POPG	Doxorubicin	120 nm	Not changing significantly in the first 48 h	Up to 13 µM Dox/mM Lipid	London et al., 2020 [17]

Moreover, asymmetric liposomes can be helpful as alternative therapeutic strategies, as the research by Greco et al. shows, in which asymmetric liposomes were designed to act as apoptotic bodies that killed Mycobacterium tuberculosis bacteria without antibiotics to alleviate the incidence of antibiotic resistance in tuberculosis treatment. This therapy was successfully applied to mice in an inhalable route [18][19]. They used the inverted emulsion technique taken from Pautot et al. with phosphatidylserine in the outer membrane to resemble apoptotic bodies as well as phosphatidic acid in the inner layer to enhance the innate antimycobacterial activity in phagocytes while limiting the inflammatory response. They concluded that the possibility of distributing lipids in the liposome membrane asymmetrically is additionally of value in liposome-based therapeutic strategies because of the cargo of bioactive lipids, which can be used as unique immunomodulators, and can be preferentially delivered to specific target cells [19][20].

Jing et al. designed asymmetric lipid membranes in which the asymmetry was generated through the selective PEGylation of cationic lipids in the outer membrane leaflet, so an asymmetry between two membrane leaflets of liposomes was created while the charged surface function at the outer liposome surface of the symmetric liposomes was deactivated. This study mentions the importance of designing improved anticancer drugs and of using drug carriers in combination therapies [21].

Asymmetric-type liposomes continue to be studied for their application in the release of different drugs, so it is expected that in the future, the control over the layers of these nanosystems, as well as the benefits that these kinds of liposomes have, will lead to their application in drugs that require a more sustained release as well as better doses and routes of administration.

2.2. Asymmetric Liposomes for Nucleic Acid Delivery

In molecular medicine, nucleic acid therapeutics is one of the most significant advances encouraging the development of new technologies [12][17]. Using nucleic acids in therapy has disadvantages, including that molecules cannot enter or transfect the cell by themselves, mainly because of the negative charge of nucleic acids, which makes it difficult for them to pass through cell membranes. All nucleic acids exceed the size of conventional small drugs, and they are easily degraded by the nucleases present in physiological fluids, leading to limited biological stability [12][17]. For this reason, when delivering genetic material to the human body, it needs a carrier that protects and transports the nucleic acids safely [18][22].

Asymmetric lipid particles have proven to be an efficient tool for studying nucleic acid delivery due to their ability to control the inner and outer layer charge. It has been shown that a high positive charge density in the inner part is an advantage for the efficient encapsulation of nucleic acids. At the same time, a neutral or negative outer layer is an advantage for biocompatibility [23]. Cationic lipids are studied in the design of asymmetric liposomes, especially for the inner layer, where the cationic lipids form complexes with the nucleic acids, which are anionic [24]. Good encapsulation efficiencies and stability of the encapsulated nucleic acids in this type of vesicles have been observed (**Table 2**), but the encapsulation efficiency also depends on the synthesis technique.

De Matos et al. made improvements in and contributions to nucleic acid encapsulation using the inverse emulsion technique. They used positive phospholipids (DPPC/DOTAP) in the inner leaflet and (DPPC/DSPE-PEG2000) in the outer. The final liposomes had sizes below 200 nm and a bilayer asymmetry of 70%, making this an attractive methodology for encapsulating nucleic acid therapeutics because of the final size of the liposomes, genetic material integrity, and the successful production of the asymmetric bilayers. This study was the first report in which centrifugation technology was employed for the production of nanosized liposomes for pDNA encapsulation using the inverse emulsion technique [17]

Mokhtarieh et al. developed a method for making asymmetric liposomes with a high siRNA encapsulation efficiency (90%) and negatively charged surface that precludes nonspecific liposome uptake into cells. The inner layer was composed of ionizable cationic DODAP and DOPE, which entrap siRNA, and the outer layer was composed of

DSPC, DOPE, PEG-PE, and cholesterol. Moreover, these vesicles protect siRNA from ribonuclease A degradation, and the conjugation of the outer layer with different molecules induces mediated uptake into specific cells. These findings suggest that asymmetric lipid nanoparticles could be valuable cargo for delivering target-specific siRNA [13].

A suitable vehicle with a high capacity for nucleic acid loading and one that allows for an effective and timely release in the nucleus or cytoplasm of the cells is typically required for successful targeted delivery and efficient gene transfection outcomes. Inefficient delivery vectors can compromise therapeutic advances and, eventually, the potential of gene therapy [25]. Because of this, the development and study of asymmetric liposomes represent an opportunity for advances related to therapies in molecular medicine and pharmaceutical areas.

2.3. Cell Models

To study the biophysical characteristics of biological cell membranes, in vitro simplified systems are necessary. While artificial lipid bilayers are useful to model natural membranes, they are generally symmetric and lack at least some of the critical structural characteristics of natural cell membranes. Since cellular membranes have different lipid domains in different parts of the membrane that are required to control different cellular activities that are essential for differentiation, proliferation, protein interactions, and cell-to-cell communication [15][21][26], symmetric bilayers lack the capacity to emulate these behaviors, thus limiting the information obtained from working with this kind of model. Therefore, asymmetric systems could improve people's understanding of cellular processes due to their resemblance with the asymmetric behavior of eukaryotic or bacterial cellular membranes [15][27][28].

Lipid asymmetry has several roles in biological processes; for example, the display of phosphatidylserine in the outer leaflet of cell membranes signals the consumption of apoptotic cells by phagocytes [29]. Moreover, lipid charge asymmetry allows for determining the orientation of proteins in the membrane [30]. Another aspect likely affected by asymmetry is the physical lipid state (i.e., liquid-ordered and disordered states), which affects cellular functions such as amyloid formation, protein, lipid sorting, cell signal transduction, and pathogen invasion [31].

A study that pushed forward synthetic models resembling naturally occurring membranes was presented by Lin et al., which concluded that the cholesterol-containing asymmetric liposomes made from SM/POPC (inner leaflet) and POPE/POPS (outer leaflet) closely resemble mammalian plasma membranes because their vesicles emulate essential features, such as lipid composition and asymmetry [32].

Kamiya et al. demonstrated that the formation of asymmetric lipid vesicles with the inner leaflet of DOPS/DOPC (with a 1:1 molar ratio) and outer leaflet of DOPC emulated lipid flip-flop corresponding to the apoptotic cells' behavior and showed the promotion of the flop dynamics influenced by an antibiotic peptide. Additionally, these vesicles achieved at least seven days of long-term storage stability through microfluidics-based preparation methods [33].

Doktorova et al. measured lipid flip-flop using time-resolved small-angle neutron scattering (SANS) to study the asymmetric bilayers' stability. They concluded that asymmetric liposomes are better as biological mimetics than

their symmetric counterparts due to the alterations in lipid lateral diffusion, packing density, phase behavior, and the conformation, partitioning, and topology of transmembrane proteins. They produced asymmetric large unilamellar vesicles (aLUVs) through a lipid exchange methodology and experimented with different lipid combinations [11].

Asymmetric liposomes allow for the analysis of structural interactions resembling the ones naturally occurring in plasma membranes; therefore, the study and innovation of more realistic models are critical to developing strategies to understand how drugs could interact in vivo with cellular membranes.

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