Liposomes as drug delivery: A review of innovations in disease treatment and tumor therapy

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ABSTRACT

Liposomes are a drug delivery model that are studied for the treatment of various pathologies. These nanoparticles are manufactured by redirecting phospholipids with the hydrophilic inner medium surrounded by the lipophilic bilayer. As an additional advantage of this model, several changes can be made to the bilayer to implement the transport of drugs in the biological media, such as the insertion of polyethylene glycol, peptides and carbohydrates, giving more specificity to the drug delivery model, such as multifunctional liposomes and ligand-directed liposomes. These modifications help in the different mechanisms of active and passive vectorization and make the liposome system cover several areas of action, such as pain control, antibacterial action, and vaccines. In addition, these nanoparticles are also used in new strategies in tumor therapy, which use cancer symptoms to target nanoparticles more effectively, such as double-ligand liposomes, co-delivery liposomes, and sensitive to stimuli that are still under development or already used in the clinic.

Keywords: Nanoparticles, Liposome, Drug delivery, Cancer.

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INTRODUCTION

With the advances in nanotechnology, the development of new therapeutic alternatives to overcome the conventional limitations of drug transport in biological media has become more specific. Thus, nanoparticles have the potential to improve the stability and solubility of encapsulated fillers, promote transport across membranes, and extend circulation times to increase safety and efficacy (Mitchell *et al.*, 2020). Among the various nanotechnological models, liposomes are widely studied and developed for the treatment of various pathologies. These nanoparticles are manufactured from the self-assembly of phospholipids, which consist of a group of phosphate polar head and hydrophobic lipid tails, typically 100-500nm in diameter. In aqueous environments, hydrophobic tails reorient themselves, resulting in a spherical structure composed of an aqueous core surrounded by a lipophilic double-layered membrane (Almeida *et al.*, 2020).

When compared to other colloidal drug delivery systems, liposomes have the advantage of enabling modifications in the structural and physicochemical characteristics of their envelope, which directs the nanoparticle to a specific target *in vivo*. Therefore, liposomes can be classified according to their composition and functionalization. In addition, other more recent changes in the literature can also be observed, such as the improvement of the design by inserting units sensitive to environmental stimuli and other functionalities (Nisini *et al*., 2018).

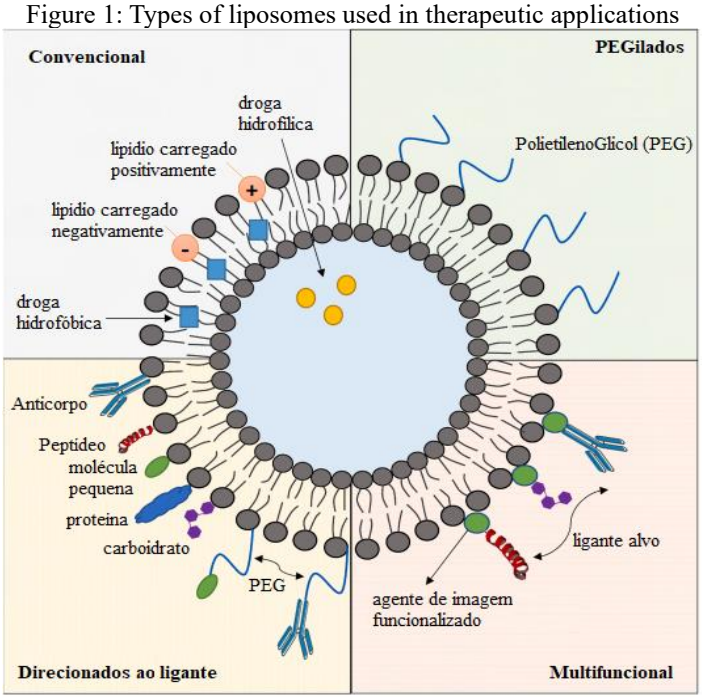
COMPOSITION AND FUNCTIONALIZATION

- **Conventional liposomes:** can be composed of neutral, cationic or anionic phospholipids, usually combined with CH to stabilize the liposomal bilayer (Figure 1). However, this type of liposome is unstable in plasma, which results in a reduced half-life, being quickly captured by the reticuloendothelial system and removed from the bloodstream. This is due to the fact that they are attacked by opsonins, serum proteins that cause macrophages to recognize the nanoparticle as a foreign body susceptible to phagocytosis by the mononuclear phagocytic system (Riaz *et al.* 2018).
- **PEGylated liposomes:** Also called stealth or long-circulating liposomes, they are the second generation of liposomes. To increase the half-life of these nanoparticles, they were coated with a layer of a biocompatible hydrophilic polymer such as polyethylene glycol (PEG) or chitosan (Figure 1) to increase the repulsive forces between the liposomes and the serum proteins, and they could remain stable for up to 12 days in the body (Guimarães; Cavaco-Paulo; Nogueira, 2021). However, this exacerbated residence time can cause some adverse effects such as the cell uptake blockade phenomenon, in which the hydrophilic barrier that increases the half-life makes it difficult to interact with target cells; and the ABC phenomenon, in which repeated doses by the parenteral route

induces accelerated blood clearance (ABC) by inducing the generation of an anti-PEG IgM antibody, increasing systemic elimination from the body (Saraf *et al.*, 2020; Wang *et al*., 2021).

- **Ligand-targeted liposomes:** To address the limitations of the previous generation, ligand-targeted liposomes have been developed for targeted distribution of compounds to target tissues, promoting greater and more selective therapeutic activity. Thus, in addition to the modification of liposomes with PEG, glycoproteins, polysaccharides, or ligands specific to receptors such as antibodies, small molecules, or peptides were inserted (Figure 1). Thus, new formulations have been developed, inserting different fragments to the liposomal surface and further increasing the specificity of the systems that respond to stimuli in the body (Nisini *et al*., 2018; Guimarães; Cavaco-Paulo; Nogueira, 2021).
- **Multifunctional liposomes:** This class has been studied for its potential to perform a combination of multiple functions through surface modification techniques, resulting in liposomes with a wide range of functionalities (Figure 1). In the literature, several examples of multifunctional liposomes such as theranostic liposomes have been reported, in which the same agent can have a target for diagnostic imaging and therapeutic assets (Xing, Hwang, Lu, 2016).

Figure 1: Types of liposomes used in therapeutic applications



Source: Guimarães, Cavaco-Paulo and Nogueira, 2021 (adapted)

VECTORIZATION STRATEGIES

PASSIVE VECTORIZATION

This approach has been applied mostly in the field of oncology due to its pathophysiological characteristics of cancer and the environment in which it is inserted. An example of this passive targeting occurs through distribution through the leaky tumor vasculature through fluid movement. As the endothelial space of tumor cells is larger and widely irrigated, and often without lymphatic return due to obstruction, 10-500nm liposomes are able to passively reach the site and remain in the tumor due to this effect called the enhanced retention and permeability (RPE) effect (Guimarães; Cavaco-Paulo; Nogueira, 2021).

Another example is through the stealthy liposomes with PEG and their system of not adhering to serum opsonins, increasing the circulation time, as seen previously (Saraf *et al.*, 2020; Wang *et al.*, 2021). Finally, the use of electrostatic interactions can also be performed by inserting charge properties to induce targeting in the tumor. There are certain phospholipids, proteoglycans, and other negatively charged molecules in tumor neovascular endothelial cells that can serve to guide cationic liposomes, which will accumulate in the endothelium through electrostatic interactions (Wang *et al.*, 2021).

ACTIVE VECTORIZATION

It is the active targeting of the liposome by inserting one or multiple ligands on its surface to increase the distribution of liposomal systems in the target, thus forming multi-functionalized liposomes. This chemical binding of liposomes to ligands occurs primarily through covalent and non-covalent bonds between the active groups on the surface of the liposomes and specific groups present in the ligand (Wang *et al.*, 2021).

There are several aspects that must be considered in the selection of ligands that will direct the liposome, which include: degree of relative overexposure or selective expression in the target; the capture of the target cells of the targeted formulation; and the degree of coverage of the target molecule. Furthermore, the main focus of ligand selection is to allow binding to the target while minimizing binding to healthy cells as much as possible (Guimarães; Cavaco-Paulo; Nogueira, 2021). Some examples of active vectorization are:

- **Vectorization mediated by polypeptide and protein:** in this type an example is transferrin, a protein that is normally used in the body to transport iron absorbed by the digestive tract and by erythrocytic degradation, in addition to being the largest carrier of iron ions. Because tumor tissue with rapid cell proliferation mainly requires iron as a nutrient, the transferrin receptor is overexpressed in tumor cells when compared to normal cells, which facilitates receptor-mediated endocytosis of a liposome with

transferrin (Jhaveri *et al*., 2018). Another example is BR2, a polypeptide with 17 amino acids, is a derivative of an anticancer peptide of nonspecific cell penetration called buforin IIb. BR2 penetrates cancer cells four times faster than normal cells (Zhang *et al*., 2017).

- **Polysaccharide-mediated vectorization:** hyaluronic acid is a mucopolysaccharide widely used in tumor treatment because of its special structural characteristics. CD44 is largely overexpressed in tissues where inflammation and tumorigenesis occur. hyaluronic acid binds to CD44 molecules, allowing the concentration of drugs in the tumor region (Wang *et al*., 2020).
- **Aptamer-mediated vectorization:** Aptamers are small segments of a single-stranded oligonucleotide molecule (DNA or RNA) that binds tightly to the surface and specifically to the target molecule with high affinity and specificity, folding into a unique three-dimensional structure. Nucleic acid aptamers have emerged as attractive carrier molecules. It has high chemical flexibility and tissue penetration, in addition to having stability, low immunogenicity, and simple synthesis (Li *et al*., 2019).
- **Folate-mediated vectoring:** folate is a water-soluble vitamin that induces receptor-mediated endocytosis. The high affinity of folic acid for the folate receptor has been used as a target in tumor cells due to the low level of expression in normal tissue and overexpression on the surface of cancer cells (Moghimipour *et al*., 2018).
- **Antibody-mediated vectoring (immunoliposomes):** the binding surface of antibodies to liposomes is a common approach used to produce systems with efficient targeting to match the target antigen (Eloy *et al*., 2017).
- **Vectorization mediated by other molecules:** other molecules can also be used to improve the drug delivery capabilities of liposomes, such as carbohydrates (Chen *et al.*, 2016), and small molecules such as porphyrins (Wang *et al.*, 2018).

THERAPEUTIC USES OF LIPOSOMES

- **Pain control action: DepoDur** is an FDA-approved extended-release morphine sulfate-filled liposome-based injection for the treatment of severe pain. The composition of the liposome includes DOPC, DPPG, cholesterol, tricapryline, and triolein (Large *et al.*, 2021).
- **Antibacterial action: Arikayce**, is an inhaled liposome suspension containing amikacin, approved by the FDA for the treatment of bacterial infection in the lungs by *Mycobacterium avium* complex (MAC) that can be caused by two non-tuberculous species: *Mycobacterium avium* and *Mycobacterium intracellulare*, which typically affect

immunocompromised patients. The drug is composed of the antibiotic amikacin and the DPPC and cholesterol wrapper (Zhang *et al.*, 2018).

- **Vaccines: Inflexal V**, is a trivalent inactive influenza vaccine, composed of virosomes, liposomes whose surfaces are decorated with viral antigens (hemagglutinin and neuraminidase, in this case influenza variants A and B), 150nm unilamellar composed of 70% lecithin, 20% cephalin, and 10% phospholipids (DOPC:DOPE, 75:25 ratio) (Bulbake *et al.*, 2017). In the case of **SARS-CoV-2 vaccines**, they have fragments of mRNA from spike proteins, which enable SARS-CoV-2 to be attacked and able to enter cells, encapsulated in lipid nanoparticles whose function is to protect the genetic material from being degraded by enzymes (Pardi *et al.*, 2018).

LIPOSOMES IN CANCER TREATMENT: NEW STRATEGIES FOR TUMOR THERAPY

DOUBLE-LIGAND LIPOSOMES

Double-ligand liposomes are two ligands modified into a single liposome. This method allows the simultaneous distribution of multiple targets on the surface receptors of tumor cells, significantly improving the selectivity of liposomes in target cells, resulting in a higher absorption and ability to kill tumor cells than passive targeting techniques or single-ligand-modified liposomes. An example of this type of strategy is by making a liposome with biotin and glucose to attack the sodium-dependent multivitamin transporter (SMVT), which is a key transporter of biotin overexpression on the surface of breast cancer cells (4T1 and McF-7) as well as GLUT1, which is also overexpressed in several tumor cells (Huang *et al.*, 2020).

LIPOSSOMAS DE CO-DELIVERY

Combination chemotherapy refers to the combination of two or more antitumor drugs to improve the induction of the mechanism of drug resistance and reduce toxicity. However, different drugs with different pharmacokinetics may have uneven distribution. Therefore, with the design of liposomes that carry these drugs, they can increase the half-life in the systemic circulation and better accumulation of these in the tumor, preventing different stages of cell growth (Sen *et al.*, 2019).

STIMULUS-SENSITIVE LIPOSOMES

Stimulus response systems emerged as an emerging mode of drug delivery and delivery at specific sites. There are two different categories of approach: the first explores the differences between the tumor microenvironment and normal tissues, such as elevated temperature, low pH, elevated local enzyme activity, and elevated redox potential inside and outside cells. The second

approach to drug delivery is through external stimulation, such as ultrasound exposure and magnetic localization (Wang *et al.*, 2021).

- **PH-sensitive liposomes:** Since the pH of the tumor microenvironment is < 6, unlike normal cells, pH-sensitive liposome formulations can be an effective way to improve the liposomes' ability to distribute and increase efficacy. However, it requires that the nanoparticle arrive intact until it reaches the tumor site (Lee *et al.*, 2017).
- **Temperature-sensitive liposomes:** Temperature-sensitive liposomes are used to improve the permeability of tumor cells by increasing local temperature, thus allowing more accumulation in the tumor. The ideal temperature to activate liposomes under internal and external influence is above 37°C (Lee *et al.*, 2017; Wang *et al.*, 2021).
- **Ultrasound-sensitive liposomes:** Ultrasound-sensitive liposomes can be activated by external stimuli to activate drug release. An example is the ultrasound-activated folic acid-linked liposome drug delivery system with oridonine as a model being activated by ultrasound device (Wang *et al.*, 2021).

MAIN LIPOSOMAL DRUGS PRESENT ON THE MARKET

Among the various mechanisms made possible by using liposomes as a drug vehicle, about 14 drugs authorized by the FDA and EMA are currently proposed (Table 1), without taking into account generics and lipid complexes. Thus, the main therapeutic focus of these drugs is in the treatment of cancer, but they also cover other functionalities such as infection, lung diseases, as well as anesthesia, vaccines and photodynamic therapy encompassing several routes of administration such as intravenous infusion, intramuscular and/or intrathecal injection, epidural, local infiltration and inhalation of the components (Liu et al., 2022).

Table 1: Liposomal drugs available on the market

Name of the product	Drug	Indication	Composition and type liposome
Doxil/Caelyx	Doxorubicin chloridate (DOX-HCl)	Câncer ovariano, sarcoma de Kaposi, melanoma mieloide	HSPC, MPEG-DSPE, Colesterol Type: SUV
Mepact	MTP-PE	Osteossarcoma	POPC, OOPS Type: MLV
DaunoXome	The Writer of the	Sarcoma de Kaposi	DSPC, Chol Type: SUV
Myocet	DOX-HCl	Breast cancer	EPC, colesterol Type: MLV
Marqibo	Vincristine Sulfate	Leukaemia	SM, colesterol Type: SUV
Vyxeos	Daunorubicin, recombinant cytarabine	Leukaemia	DSPC, DSPG, Chol Type: Bilamellar
Onivyde	Hydrochloreth Trihydrate	Pancreatic adenocarcinoma	DSPC, MPEG2000-DSPE, colesterol Type: SUV

Source: Liu *et al.*, 2022 (adaptado)

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