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REVIEW

Advancements in Liposomal Nanomedicines: Innovative Formulations, Therapeutic Applications, and Future Directions in Precision Medicine

Zahra Izadiyan[1], Misni Misran[1], Katayoon Kalantari[2], Thomas J Webster[3,4], Pooneh Kia[5], Noor Ashyfiyah Basrowi[1], Elisa Rasouli[6], Kamyar Shameli[7]

[1]Department of Chemistry, Universiti Malaya, Kuala Lumpur, Malaysia; [2]Department of Chemical Engineering, Northeastern University, Boston, MA, USA; [3]Biomedical Engineering, Hebei University of Technology, Tianjin, People's Republic of China; [4]School of Engineering, Saveetha University, Chennai, India; [5]Institute of Bioscience, Universiti Putra Malaysia, Serdang, Selangor, Malaysia; [6]Department of Electrical and Electronics Engineering, Nanyang Technological University, Nanyang, Singapore; [7]School of Medicine, Institute of Virology, Technical University of Munich, Munich, Germany

Correspondence: Zahra Izadiyan; Kamyar Shameli, Email zahra_izadiyan@yahoo.com.my; kamyarshameli@gmail.com

Abstract: Liposomal nanomedicines have emerged as a pivotal approach for the treatment of various diseases, notably cancer and infectious diseases. This manuscript provides an in-depth review of recent advancements in liposomal formulations, highlighting their composition, targeted delivery strategies, and mechanisms of action. We explore the evolution of liposomal products currently in clinical trials, emphasizing their potential in addressing diverse medical challenges. The integration of immunotherapeutic agents within liposomes marks a paradigm shift, enabling the design of 'immuno-modulatory hubs' capable of orchestrating precise immune responses while facilitating theranostic applications. The recent COVID-19 pandemic has accelerated research in liposomal-based vaccines and antiviral therapies, underscoring the need for improved delivery mechanisms to overcome challenges like rapid clearance and organ toxicity. Furthermore, we discuss the potential of "smart" liposomes, which can respond to specific disease microenvironments, enhancing treatment efficacy and precision. The integration of artificial intelligence and machine learning in optimizing liposomal designs promises to revolutionize personalized medicine, paving the way for innovative strategies in disease detection and therapeutic interventions. This comprehensive review underscores the significance of ongoing research in liposomal technologies, with implications for future clinical applications and enhanced patient outcomes.

Keywords: liposomes, active targeting, targeted drug delivery, nano-carriers, cancer therapy

Introduction

Cancer refers to the uncontrolled growth of abnormal cells in the body and is the second leading cause of death from non-communicable diseases (NCDs) worldwide. Cancer is a significant societal, public health, and economic challenge in the 21st century, accounting for nearly one in six deaths (16.8%) and over one in five deaths (22.8%) globally.[1] It is responsible for 30.3% of premature deaths among individuals aged 30–69 and ranks among the top three causes of deaths in this age group in 177 out of 183 countries.[1] Cancer not only poses a significant obstacle to extending life expectancy but it also incurs considerable societal and macroeconomic costs, which differ depending on the type of cancer, geographic region, and gender.[2] A recent study highlighted the significant impact of disproportionate cancer mortality among women: in 2020, approximately one million children lost their mothers to cancer, with nearly half of these maternal deaths caused by breast or cervical cancer.[3] As shown in Figure 1, the top 10 cancer types in both men and women contribute to more than 60% of all new cancer cases and deaths.[4] Specifically, lung cancer is the most frequently diagnosed worldwide, representing 12.4% of cases, followed by breast cancer in women (11.6%), colorectal cancer (9.6%), prostate cancer (7.3%), and stomach cancer (4.9%). Lung cancer is also the leading cause of cancer-related deaths, accounting for 18.7%, with colorectal (9.3%), liver (7.8%), breast (6.9%), and stomach (6.8%) cancers following. In women, breast cancer is the most diagnosed and the leading cause of death, followed by lung and colorectal cancers in

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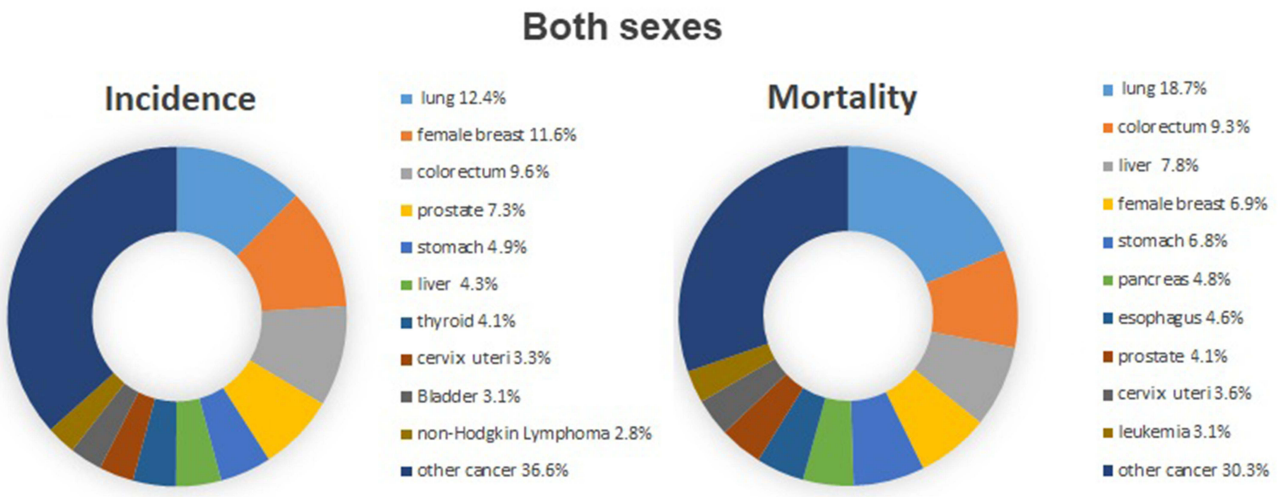

Figure 1 New cases and mortality rates for the top 10 leading cancers in 2022. Used from Bray F, Laversanne M, Sung H, et al. Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *Ca a Cancer J Clinicians*. 2024;74(3):229–263. © 2024 The Authors. CA: A Cancer Journal for Clinicians published by Wiley Periodicals LLC on behalf of American Cancer Society.[4]

both diagnoses and mortality. For men, lung cancer is the most common in terms of both cases and deaths, followed by prostate and colorectal cancers for new diagnoses, and liver and colorectal cancers for deaths.[4]

Cancer treatment involves various approaches, including surgery, chemotherapy, and radiation therapy.[5] However, these treatments can lead to side effects such as healthy cell damage, hair loss, infections, pain, nausea, mucositis, and vomiting. To mitigate these adverse effects and enhance treatment effectiveness, nanocarrier-based drug delivery systems have been introduced as alternatives to traditional cancer therapies. Among these systems, liposomes are one of the most widely used.[6,7] Liposomes offer significant advantages for cancer treatment due to their unique biological properties, including biocompatibility, biodegradability, cell-like membrane characteristics, low immunogenicity, and non-toxicity. They also help protect drugs from degradation and extend their biological half-life, while allowing for easy modification of size, charge, and surface properties.[8,9] Extensive research in recent years has advanced the development of liposomal drug delivery systems, leading to new formulations for cancer therapy.[10]

In a study published in 1964, Bangham et al provided the first description of liposomes.[11] The vesicles were once known as "banghasomes" or "multilamellar smectic mesophases", but Gerald Weissmann eventually dubbed these systems "liposomes" rather than "banghosomes", and he was granted the Nobel Prize for this discovery.[12] According to these investigations, liposomes are tiny vesicles that can be formed from cell membrane proteins, phospholipids, cholesterol, and nontoxic surfactants.[11] Bilayer vesicles are formed when phospholipids envelop the hydrophilic core of liposomes.[13] The hydrophobic tails of the phospholipids point inward (against the membrane), whilst the hydrophilic heads point outward (toward the aqueous phase). Because liposomes are amphipathic, they can be loaded with hydrophilic or hydrophobic medicines.[14] Liposomes can be classed according to their structure, composition, and manufacturing method. Liposomes can be classified as multilayer, monolayer, or multivesicular based on their structural makeup. Liposomes can be categorized as conventional, fusogenic, long-lived, pH-sensitive, ionic, magnetic, heat-sensitive, and immuno-liposomes based on their composition.[15] The physicochemical characteristics of the membrane components, the charge, and the dispersion medium are taken into consideration while selecting liposome manufacturing techniques.[6] The preparation of liposomes can be achieved through three methods: solvent dispersion (ethanol/ether injection, double emulsion, and reverse-phase evaporation), mechanical dispersion (hydration of lipid films, sonication, micro emulsification, French pressure cells, membrane extrusion, and freeze-thawing), and detergent solubilization (dialysis, column chromatography, and dilution).[15]

Liposomal formulations offer a number of advantages over drug solutions including reduced toxicity of the encapsulated drug,[16] prolonged systemic circulation when surface-modified (eg PEGylated liposome), improved pharmacokinetics,[17] controlled drug release kinetics,[18] and tumor targeting.[19] Drugs with various physicochemical characteristics can be delivered by liposomes due to their special capacity to encapsulate both lipophilic and hydrophilic substances. These characteristics

include overcoming multidrug resistance (MDR), improving the therapeutic index, enhancing drug solubility, sustaining drug release, decreasing drug adverse effects, increasing the concentration of the medication at the target site, and biocompatibility and biodegradability. They also include non-immunogenicity.[19] However, these systems' short half-lives, instability, heightened susceptibility to sterilizing procedures, and high production costs due to costly raw materials and the manufacturing equipment needed are major drawbacks.

The response of the immune system is another important issue. Liposomes are recognized as foreign materials by optogenins, which causes reticuloendothelial system (RES) macrophages to absorb them. Sterically stabilized liposomes (covered with PEG or other hydrophilic polymers) have been created as a solution to the RES absorption issue. The most popular method providing liposomes with a longer half-life in circulation is PEGylation. Additionally, silic acid, polyvinyl alcohol, and poly-N-vinylpyrrolidone—other PEG substitutes—have been employed for the same objective.[17]

One of the most popular nanocarriers for delivering anticancer drugs to tumor locations include liposomes. Targeting tactics alone or in combination can accomplish this. Because of the increased permeability and retention effect (EPR) brought on by the decreased lymphatic drainage and increased vascular permeability of the tumor microenvironment, liposomes are preferentially absorbed by solid tumors.[20]

Currently, liposomes are used as active and smart carriers helping bioactive agent accumulate in a specific part of the body.[21] Liposomes can be modified by the attachment of antibodies or ligands on their surface which can then be recognized by cellular receptors.[22] Because they lack selectivity, the majority of anticancer medications have harmful effects on both malignant and healthy cells. Although there have been attempts to choose treatments that eradicate tumor cells without endangering healthy tissue, the outcomes of chemotherapy typically fall short of these goals. Therefore, there is high scientific attention for the development of new anticancer therapies and new drug delivery strategies that can selectively deliver anticancer drugs to malignant tissues, thus increasing drug efficacy and possibly reducing their toxicity and adverse effects on normal cells.[23,24] Liposomal systems with a potential to enhance medication delivery for cancer therapy have made substantial progress in recent years. As a result, scientists have concentrated their efforts on making liposomal delivery systems for active cancer medication targeting to the tumor site, followed by organelle-specific targeting and triggered release of loaded pharmaceuticals that take advantage of the tumor's microenvironment.[25]

This review article aims to provide a concise overview of liposomes in modern drug delivery, particularly for cancer therapy. It covers liposomal structure, synthesis methods, drug encapsulation strategies, and their potential in targeted cancer therapies. The focus is on their biocompatibility, ability to overcome biological barriers, and applications in personalized cancer treatment. The goal is to enhance the understanding of liposomal drug delivery systems and their role in improving efficacy and reducing toxicity in cancer treatment.

Liposome Structure

Liposomes have a spherical bilayer structure which consistently includes one or more layers of a phospholipid that can be produced from cholesterol and natural/synthetic phospholipids. Lipophilic and hydrophilic materials are embedded in a lipid bilayer and interior aqueous region, respectively.[26] Liposomes with a phospholipid-based structure are known as amphipathic nanocarriers.[27] They have advantages such as extending the release of active pharmaceutical agents, biocompatibility, and biodegradability.[28] Liposomes are categorized based on the vesicle size and number of lipid bilayers (lamellae) which is presented in Figure 2. [29] The main types of liposomes can be considered as multilamellar (ML, 0.5 to 5 μm) and multivesicular (MV, > 1μm).[30,31] Unilamellar also can be classified into three different types of vesicles including small unilamellar (SU), large unilamellar (LU), and giant unilamellar (GU) of different sizes 20–200 nm, > 200 nm and ≥ 1μm, respectively. Unilamellar vesicles are described by the presence of a single bilayer, with the extra capacity for the enclosure of a hydrophilic material. Multilamellar vesicles are desirable choices for the enclosure of a lipophilic material and also, they represent two or more concentric lipid bilayers structured through an onion-like structure. Multi vesicular vesicles are perfectly suitable for the enclosure of a great number of hydrophilic substances. Besides that, they contain a few small non-concentric vesicles trapped inside a lipid bilayer.[31,32] Furthermore, the amount of encapsulation of the compounds in a liposome depend on the number of lamellae and the vesicle size.[15]

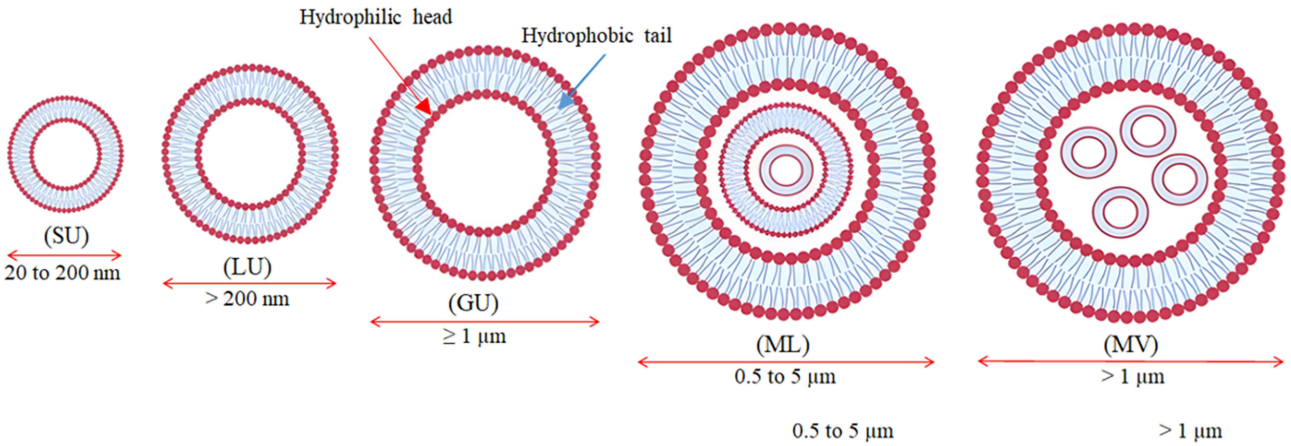

Figure 2 The classification of liposomes based on lamellarity and size. Their names are respectively: Small Unilamellar (SU), Large Unilamellar (LU), Giant Unilamellar (GU), Multilamellar (ML) and Multivesicular (MV).

Liposome Synthesis Methods

There are different methods to synthesize liposomes.[33] The most common methods such as thin film hydration, solvent injection, reverse phase evaporation, dehydration-rehydration, hydration in a packed bed of colloidal particles, pH jumping, freeze-thaw, and detergent removal are discussed in the following sections.[34,35] Table 1 provides a detailed overview of the advantages and disadvantages associated with different liposome synthesis methods, offering insights into their efficacy, scalability, and application-specific suitability which are further described below.

Thin Film Hydration

Liposome synthesis began with the Bangham method, also known as thin film hydration.[11] This technique involves dissolving lipids in organic solvents like chloroform, ether, or methanol. The solution is then evaporated in a round-bottom flask to yield a thin lipid film, which, upon hydration with an aqueous solvent, forms liposomes. The conditions during hydration play a pivotal role in shaping the liposome structures. Intense agitation leads to the creation of multilamellar vesicles with various sizes, while a gentler hydration process results in the formation of giant unilamellar vesicles.[36,37] However, this method comes with limitations: it tends to produce larger and more diverse liposomes, has restricted containment capacity, poses challenges in removing organic solvents, and can present scalability issues.[35]

Table 1 Comparative Analysis of Various Liposome Synthesis Methods Highlighting Their Advantages and Disadvantages

Methods	Advantages	Disadvantages
Thin film hydration	Simple, versatile, scalable	May require organic solvents, can produce mixed vesicles (MLVs and SUVs)
Solvent injection	Rapid, produces SUVs	Requires organic solvents, can lead to heterogeneous vesicle sizes
Reverse phase evaporation	Simple, versatile, scalable	Produces MLVs, may require organic solvents
Dehydration-rehydration	Simple, produces multilamellar vesicles (MLVs) and unilamellar vesicles (UVs)	Can lead to heterogeneous vesicle sizes
Hydration in a packed bed of colloidal particles	Simple, produces UVs	Requires specialized equipment, may not be suitable for all lipid formulations
pH jumping	Simple, produces UVs	Can lead to heterogeneous vesicle sizes
Freeze-thaw	Simple, produces UVs	Can lead to heterogeneous vesicle sizes
Detergent removal	Gentle, produces UVs	Slow, requires multiple steps
Extrusion techniques	Flexibility in selection of vesicle diameter, batch-to-batch reproducibility, absence of solvent and surfactant contamination	It may be difficult to supply similar ultrasonic energy in a large volume (scale-up), probe tip may be a source of metal contamination

Solvent Injection Techniques

The solvent injection technique, first detailed in 1973 by Batzri and Korn, offers an alternate method for liposome production.[38] Here, lipids are dissolved in an organic solvent like ethanol or ether and are injected into an aqueous phase, leading to the formation of liposomes.[39] It is widely used due to its scalability, reproducibility, simplicity, rapid implementation, and absence of oxidative changes or lipid degradation.[40] This method harnesses ethanol, acknowledged by the European Pharmacopeia for its suitability in diverse applications, including in vivo drug delivery.[41] However, this approach is not without drawbacks: ethanol might present challenges with lipid solubility, efficient removal from liposomes, agitation-related liposome heterogeneity, and low encapsulation efficiency (EE) for hydrophilic compounds.[32] Modifying parameters such as drug-to-lipid ratio, injection rate, the nature of the lipid, orifice diameter during injection, and ethanol lipid concentration allow for control over particle size and EE achieved in the ethanol injection process.[42]

Reverse Phase Evaporation

Reverse phase evaporation is another approach to prepare liposomes and the reverse-phase evaporation process was first described by Szoka and Papahadjopoulos.[43] The initial steps are similar to the thin film hydration. Firstly, phospholipids are dissolved in an organic solvent to produce the film, then the solvent is removed by evaporation. The new film is resolved in the organic solvent again which is normally diethyl ether and/or isopropyl ether, followed by an additional aqueous phase. The final output is oil in a water emulsion formulation.[37] Then, the new formulations are sonicated to generate inverted micelles, resulting in the formation of a homogeneous emulsion. In the final step, the organic solvent is evaporated under reduced pressure. The resulting liposomes are in the form of a viscous gel.[15,32] One of the main advantages of this method is high EE.[42] Also, this technique is defined as time-consuming.[35] On the other hand, the weaknesses of this method is based on the encapsulation of the mixture because of sonication and even the organic solvents used.[44]

Detergent Removal

Another method used to synthesize liposomes is the detergent removal technique. In this process, at the critical micelle concentration, phospholipids are solubilized with detergents.[36] After removal of the detergent by column chromatography or dialysis bags and with a suitable aqueous medium, the phospholipid molecules self-assemble into liposomes.[15,33] Some of the parameters can affect the homogeneity and the size of the liposomes, counting rate of detergent elimination and initial ratio of phospholipids to detergents.[32,42] The presence of contaminants in the final liposomal formulation, the possibility of interaction between the encapsulated drug and the detergent, and the fact that this procedure is time intensive are all disadvantages of the detergent removal process.[35,45]

Hydration in a Packed Bed

This method represents a one-step hydration-based approach to produce liposomes with a remarkably low polydispersity index (PDI 0.2) and eliminates the need for post-processing. Prior to hydration, lipid molecules, absorbed in a solvent, undergo drying within a densely packed bed of highly asymmetrical colloidal particles with rough surfaces, enabling the creation of liposomes within a specific size range. The resulting size distribution remains consistent regardless of the flow rate of the hydrating medium, indicating that extrusion does not influence the narrow size distribution of these liposomes. By subjecting a milky white dispersion of large and polydispersed liposomes (in the micrometer range) to drying in a packed bed and subsequent rehydration with an aqueous buffer, a monodispersed liposome dispersion below 100 nm can be achieved. Notably, the final size distribution remains unaffected by the size of the colloidal particles or the percentage of bed packing, emphasizing that the highly asymmetrical particles and porous packing structure dictate liposome size. Sundar et al highlight the robustness of this one-step hydration method, its lack of post-processing requirements, and the precise control it offers over liposome size, positioning it as suitable for point-of-care therapies utilizing liposomal drug delivery systems.[46]

Dehydration-Rehydration

Large unilamellar liposomes can be synthesized by dehydration-rehydration without using detergents or organic solvents. In this technique, lipid or amphiphilic molecules are dispersed into the aqueous phase at a low concentration along with

sonication to produce the liposome.[47] The drug for closure could be mixed with the formulated vesicles in the aqueous phase. Liposomes combine to form a multilayer film that traps drug molecules when water evaporates under the flow of nitrogen gas. After adding water, large vesicles are formed, encasing the active ingredient.

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pH Jumping

Another quick method for making liposomes is pH jumping, which does not require organic solvents. Small unilamellar structures form when a phosphatidic acid solution in water is exposed to a 3.5-fold increase in pH (from 3 to 10.5–11) for a short time period (less than 2 minutes).[48] When the same procedure is performed on a mixture of phosphatidic acid and phosphatidylcholine, similar results can be obtained, with a specific ratio of phosphatidic acid to phosphatidylcholine, a controlled percentage of small unilamellar versus large unilamellar structures can be obtained.[48]

Freeze-Thaw

Freeze-thaw cycles are commonly used in liposome production to improve lipid formation and unilamellar vesicle packing.[49] The freeze-thaw process might be included in any liposome manufacturing method. For example, following thin filming, the mixture is sonicated at ambient temperature and then frozen in a liquid nitrogen atmosphere at −196 °C. After that, the sample is kept at room temperature to melt. The above-mentioned cycles might be repeated up to ten times to get the desired outcome. The final result is a huge number of unilamellar vesicles. As a final point, if smaller vesicles are needed, the resultant solution can be resonated at room temperature. When lipid concentrations are high, freeze-thaw is not an appropriate approach.[15] By using this strategy, the drug enclosure efficiency was reported to be between 20% and 30%.[15,50] Freeze-thaw cycling is a common liposome synthesis strategy for increasing the EE.[51] The liposomes are usually frozen in liquid nitrogen (−196°C) and then thawed at a temperature above the lipid phase transition temperature.[52,53] Freeze-thaw cycling is used to reduce lamellarity,[54] reduce polydispersion, and/or tear the liposomal bilayer,[51] allowing drug molecules to enter the liposome and facilitate encapsulation.[55,56] The needed number of freeze-thaw cycles to encapsulate psychoactive compounds varies widely in the literature, with some papers claiming as many as ten.[57] The goal of performing a large number of freeze-thaw cycles is to achieve drug concentration equilibrium.

Extrusion Techniques

Most of the previous methods need additional steps to reduce liposome size such as extrusion, homogenization, and sonication.[58] Bath and probe sonication techniques are used to control the size of the liposomes.[15] The disadvantages of the sonication technique include that it may be difficult to supply similar ultrasonic energy in a large volume of liposomal suspension (scale-up), and the probe tip may be a source of metal contamination. Furthermore, there is a risk of phospholipid breakdown and subsequent compound enclosure, as well as reduced EE.[38,59] Liposomes can be driven through a high-pressure aperture to reduce their size in a homogenization process, resulting in a high-speed collision idea. Size reduction procedures include shear force-induced homogenization processes, micron fluidization, and homogenization.[42] Another way for reducing the size of liposomes is the extrusion procedure. Following liposome formation, extrusion cycles are passed a few times through a membrane with set pore size, which is typically a polycarbonate filter to ensure consistent size distribution.[35,60] Comparing the extrusion process with using homogenizers requires a lower volume of liposomes and a much lower pressure.[58]

Characterization of Liposomes

Once the liposomes are formulated and before they are used, they need to be evaluated for physical and chemical properties and they should be extensively characterized to guarantee their in vitro and in vivo performance.[61] The main characterization of liposomes are size, polydispersity index (PDI), and zeta potential which are related to the stability, shape, phase behavior, lamellarity, in vitro drug release, and EE.[33,36] In Table 2, a thorough list of lipid classification and respective characterization techniques are presented, detailing the merits and limitations of each method.

Size and Polydispersity Index (PDI)

Size and PDI are the most important characteristics of liposomes. Size is known to be a critical factor for inhalation and parenteral administration,[27] and finding the liposome's circulation half-life.[62] The small size of liposomes allows them to circulate in the organism for a long time period while larger liposomes are not suitable and are speedily eliminated from the blood circulation system.[63] The acceptable size range for liposomes in drug delivery is usually between 50 to 200 nm.[39] The PDI value indicates the size of the sample heterogeneity, which can be monodispersed or polydispersed. The PDI can be in a range from 0 to 1 and dimensionless while the desirable range of PDI in drug delivery should be below 0.3 or equal to this value.[64] The high PDI can be caused by a very wide range of size distribution or heterogeneity and also several populations of liposomes in the sample.[65] Based on the particle size, the PDI can be calculated with the solvent refractive index, the distribution variance, and the angle of measurement.[66] The measurement is mostly carried out by using dynamic light scattering (DLS) moreover being identified with photon correlation spectroscopy (PCS). DLS analysis is based on the continuous motion of dispersed particles in the solution, resulting in scattering of the incident light. Light scattering is proportional to the diffusion level of liposomes in suspension, implying that tiny particles diffuse more quickly than big ones. The quantity of light dispersed is used to compute the mean size of the liposome. DLS is considered a quick, straightforward, simple, and dependable method for determining the size of liposomes in their natural habitat. Furthermore, DLS can measure a wide variety of sizes from nanometers to micrometers.[33,36] Nevertheless, this technique has certain limitations, including difficulty in distinguishing individual particles from aggregates and it has a high sensitivity in detecting a small amount of impurities (contaminants) which can confound results.[67]

Recently, a new tool for size characterization called Nanoparticle Tracking Analysis (NTA) was presented to measure the diffusion coefficient of particles in a sample by determining size.[68] DLS calculates the diffusion coefficients of the particles based on the intensity change of the scattered light measurements. The dispersion coefficient of individual particle motions in successive optical video pictures can be determined by NTA. Because they measure the same physical attribute, NTA can be a useful approach for confirming the size as determined by DLS. As a result, the NTA size measurements should be the same as those obtained using the DLS approach.[69,70] The ability of NTA to simultaneously measure the size and intensity of particle dispersion allows, in addition to differentiating between particles with different refractive indices within the same sample solution, a direct estimation of the particle concentration.[71]

Zeta Potential

A "colloidal system" is formed when one of the states of matter is finely scattered in another. In aqueous media, the majority of colloidal dispersions carry an electric charge. The surface charges can come from a variety of sources, depending on the particles and their surroundings. The two most important mechanisms are ionization of the surface groups (acidic groups dissociate on a particle's surface, resulting in a negatively charged surface; conversely, a basic surface takes on a positive charge) and adsorption of a charged species (ie, surfactant ions may be precisely adsorbed on the particle surface, resulting in a positively charged surface in the case of cationic surfactants and a negatively charged surface in the case of anionic surfactants).[27] The total charge that a particle accumulates in a given medium is defined by its zeta potential. It is a physical property shared by all particles in suspension. The zeta potential has long been acknowledged as a reliable indicator of colloidal particle interaction. Zeta potential measurements are commonly used to estimate colloidal system stability. If all of the particles in the suspension have a large negative or positive zeta potential, they will not aggregate. If the particles' zeta potential values are low, however, there will be no force to keep them from flocculating. To determine the zeta potential, a laser is used to create a light source that illuminates particles within the

Table 2 Comprehensive Overview of Lipid Classification and Their Characterization Techniques, Highlighting the Advantages and Disadvantages of Each Method

Characterization Technique	Tools	Advantages	Disadvantages
Size and Polydispersity Index (PDI)	Dynamic Light Scattering (DLS)	Quick and easy to use, Non-invasive, Measures size distribution	Can be affected by impurities, Not suitable for large liposomes
Zeta Potential	Zeta Potential Analyzer	Accurate and reliable, Measures surface charge	Can be affected by conductivity, Not suitable for non-spherical particles
Shape	Transmission Electron Microscopy (TEM)	Provides high-resolution images, Can identify the shape of liposomes	Destructive, Requires sample preparation
Cryo-Transmission Electron Microscopy (Cryo-TEM)	Conserves the original structure of liposomes, Provides high-resolution images	Requires special equipment, Smaller sample size	–
Atomic Force Microscopy (AFM)	Provides real-time imaging of liposomes, Non-invasive, Does not require sample preparation	Lower resolution than TEM	–
Lamellarity	31P-NMR	Measures the distribution of phospholipids in the bilayer, Non-invasive, Provides quantitative information	Requires specialized equipment
Phase Behavior	Differential Scanning Calorimetry (DSC)	Measures the transition temperature of phospholipids, Quantitative	Requires specialized equipment
Encapsulation Efficiency (EE)	Direct methods	Directly measures the amount of encapsulated drug	Requires destruction of liposomes
Indirect methods	Measures the amount of free drug in the solution	Non-destructive	May not be as accurate as direct methods

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samples. At an angle of about 13 degrees, the incoming laser beam passes through the sample cell's center and is detected as scattered light.

Any particles moving across the volume measured cause the measured light to fluctuate at a frequency proportional to the particle speed when an electric field is applied to the cell. This information is sent to a digital signal processor, which then sends it to a computer, which calculates the potential zeta. Particle suspensions with a zeta potential greater than or equal to +30 mV or less than −30 mV are considered stable.[72] Each particle has a charge and overall this charge usually is stated as zeta potential or surface charge.[58] For nanoparticle surface characterization, zeta potential has become a common analytical measurement. Nanoparticle stability, circulation times, protein interactions, particle cell permeability, and biocompatibility can all be determined using the potential at the hydrodynamic shear boundary (also known as the sliding plane).[73] However, to draw meaningful conclusions from this data, it is necessary to grasp the technique's limitations and to define the measurement conditions properly. Zeta potential is influenced by temperature, pH, conductivity (ionic strength), and solvent (viscosity).[74] Small adjustments in one of these factors can have a big impact on the zeta potential readings. Zeta potential is considered a necessary physical property of liposomes to electrostatic interactions between particles in a sample solution.[75] The surface charge of liposomes are related to several parameters which include a head group of lipids, lipid composition, and ligands, also the surface charge could be present as different charges such as negative, neutral, or positive. Besides, ionic strength has an effect on zeta potential as well and one can consider this as an external environmental effect.[76]

Liposomal surface charge is a crucial property for tumor dissemination that must be carefully examined. Cationic liposomes accumulate in the tumor vasculature due to electrostatic interactions with angiogenic endothelial cells found in tumor blood vessels. However, due to the extracellular matrix and electrostatic attachment to cancerous cells, highly charged cationic liposomes do not diffuse efficaciously into the tumor site, whereas less cationic or neutral liposomes have shown more efficient penetration into tumor spheroids in vitro and extravasation of blood vessels in vivo.[77–79] Lipid functionalization with polyethylene glycol (PEG), according to a study, protects cationic groups from potentially damaging electrostatic interactions with tumor cells and the extracellular matrix. PEGylated cationic liposomes have clumped together in the tumor vasculature in vivo, resulting in a uniform tumor distribution. This method of PEGylation to maintain cationic liposomes (in a positive charge) may be a good way to make liposomes that can penetrate solid tumors and target sites effectively.[80,81]

Shape

Morphological analysis parameters are critical for effective liposome characterization.[71] Liposomal images can be captured using electron microscopy (TEM) and cryo-TEM methods.[36] Because the original environment of the liposomes must be removed, the TEM technique has several constraints in terms of sample preparation. Because this is a time-consuming procedure, it is not suitable for routine measurements. Furthermore, this approach has the potential to cause changes in the structure of the liposomes, such as vesicle shrinking, swelling, or the production of artifacts in the produced image.[33] There is another alternative technology, cryo-TEM, which can circumvent the sample preparation constraint. By adopting a liquid nitrogen flash-freezing process followed by direct visualization of the liposomes in a controlled environment, this technique retains liposomes near to their original condition and reduces shape distortion or shrinkage. Nonetheless, cryo–TEM typically produces better results with smaller particle sizes than with larger particle sizes because larger particle sizes may be removed from the samples during the preparation process. The AFM approach has been employed for direct investigation of liposomes in their natural environment without any alteration. This approach is thought to be non-invasive, powerful, and quick.[27] The main benefit of AFM over electron microscopy has been the greater resolution of three-dimensional micrographs offered by AFM, which can be down to the nanometer and Angstrom scales.[82]

Lamellarity

Lamellarity is also a property that due to its influence on EE and drug release profile, can influence subsequent liposomal applications. One of the most useful methods which provides valuable information, such as inter-bilayer and their bilayer thickness regarding liposome lamellarity, is 31P-NMR.[32] Other methods for accessing lamellarity information are based on variations in the visible signal or fluorescence of the lipid marker upon the addition of certain reagents.[36] The 31P-

NMR technique has also been used to estimate the liposomal lamellarity value, in particular, the ratio between the number of phospholipids in the outer layers and the inner layers. Paramagnetic ions (Mn^{2+}, Co^{2+} and Pr^{3+}) are often used to prepare an NMR sample to deactivate the 31P- NMR signal of the phospholipids. The interaction of ions within the bilayer can change the NMR spectrum. Thus, by comparing the two spectra before and after the paramagnetic ion incorporation, it is possible to estimate lamellarity.[83] Other techniques, such as SAXS and trapped volume, are also used to estimate the lamellarity of liposomes.[84]

Phase Behavior

In drug delivery applications, phase behavior is taken into account, since the permeability of the lipid bilayer for the hydrophilic active ingredients increases with the fluidity of the lipid membrane.[85] There are a few other properties that are dependent on the phase behavior of the liposomal membrane such as stability fusion, protein binding, and aggregation.[32] DSC is generally the most common method for studying and determining transition temperature (Tc). The thermal analysis method depends on estimating the heat flow differential between a reference sample and a real sample. Both samples are exposed to planned heating, cooling, or isothermal treatment while the environment, which is generally saturated with nitrogen gas, is carefully controlled.[36] The transition temperature of phospholipids (Tc) can be determined by other approaches like fluorescence probe polarization, TGA, NMR, electron paramagnetic resonance, FTIR and XRD.[86,87] Molecular dynamic simulations can be explored by calculating the phase behavior of phospholipids in the lipid bilayers.[88]

Encapsulation Efficiency (EE)

Optimal liposomal property studies can lead to the creation of liposomal formulations with optimal EE and medication release control. The composition of liposomes, the generation of liposomes, and the stiffness of the bilayer membrane can all have a significant impact on the EE of a particular medication.[32] The quantity of medication-loaded is an important point of therapeutic effectiveness in the medical industry.[89] The EE is the proportion of drug quantity incorporated into liposomes (encapsulated drugs) including the total amount of drug utilized in manufacturing liposomes (encapsulated and non-encapsulated drugs). The resulting liposomal formulation comprises a combination of encapsulated and non-encapsulated medication. As a result, separating the free (non-encapsulated) drug is the first step in assessing the amount of drug in liposomes and therefore evaluating EE. Many methods have been utilized for this purpose, including size exclusion chromatography based on size differences (liposome vs free drug), gravity or centrifugation, ultracentrifugation, and a dialysis membrane with an adequate cut-off.[27] The medication quantity is then measured to determine the encapsulated amount inside the liposomes in the following phase.

There are two recognized methods for the determination of EE: direct and indirect methods. The indirect technique focuses on determining the concentration of the non-encapsulated drug in the elution and subtracting it from the overall concentration of the medication employed in the liposome production. The direct approach, on the other hand, determines EE by physically breaking the liposomes with an organic solvent and then measuring the material liberated.[90] Traditional methods for estimating drug concentration in liposomes include UV-Vis and fluorescence spectroscopy, and enzyme or protein tests.[27] Furthermore, more advanced technologies such as UPLC, HPLC, gas chromatography-mass spectrometry (LC-MS or GC-MS), and liquid chromatography can be used to determine the quantity of medication.[91] Other techniques like 1H- NMR and ESR have also been utilized to quantify the drug amount.[92,93] Liposomes are water compartment-encapsulating vesicles made up of one or more lipid layers. Because of their high biocompatibility, liposomes have been used to deliver a wide range of chemicals. They improve the therapeutic indices of the pharmacological molecules enclosed in them dramatically. AmBisome (amphotericin B), DaunoXome (daunorubicin citrate), and Doxil were among the first commercial liposomal dose formulations (doxorubicin). Many more are now being tested in clinical studies.[94] Liposomes can be used as carriers in both lipophilic and hydrophilic medications due to their biphasic composition. Depending on their solubility and partitioning qualities, drug molecules are positioned differently in the liposomal environment and exhibit different entrapment and release behaviors (Figure 3). Based on these characteristics, the medications are divided into four categories: very hydrophilic, drugs with biphasic insolubility, amphiphilic pharmaceuticals with good biphasic solubility, and highly lipophilic. Only the watery compartments of liposomes contain very hydrophilic drugs with a log $P < -0.3$, such as cytosine arabinoside and CDP choline. The bilayer's composition affects the transport of such compounds through the liposomal membrane.[92] Highly lipophilic

medicines, such as cyclosporin, are virtually entirely entrapped in the lipid bilayer of the liposomes (log P oct > 5). Because they are very poorly soluble in water, difficulties like as entrapment drug loss during storage is minor with this category of drugs. Drugs having intermediate partition coefficients, such as 1.7 < log Poct < 4, offer a significant difficulty since they rapidly partition between the lipid and aqueous phases and are quickly lost from liposomes. Mitomycin C,[95] actinomycin D, and vinblastine are a few examples.[96] Only when these molecules form compounds with the membrane lipids can they generate stable liposomal systems. The most difficult choices for liposomal entrapment, however, are pharmacological compounds with low biphasic solubility. Because they are insoluble in either the aqueous or lipid phases, they are only a minor component of liposome absorption. 6-mercaptopurine, azathioprine, and allopurinol are typical instances.[96] Because the liposome was utilized as a biomembrane model, the EE of the drug was shown to be proportional to the partition coefficient between 1-octanol and water.[97] The log P octanol/water values of 5-fluorouracil (5-FU) (0.78), ibuprofen (3.72), flurbiprofen (4.11), and ketoprofen (2.81) were obtained from SciFinder Scholar and computed using Advanced Chemistry Development (ACD) Software Solaris V 4.67. The literature cited the log P octanol/water of diclofenac sodium as 0.70. The log P octanol/water ratio was substantially linked with the EE of the five medicines *(rs* = 0.97).[98]

Drug Loading and in vitro Release

Due to the ability of drug loading with different techniques, liposomes are a desirable system for drug delivery.[27] The choice of a suitable approach for drug enclosure into liposomes depends on numerous factors which are cost efficiency of EE, liposome stability, drug/lipid ratio, sterility, facility of production and scale-up, and drug leakage and retention.[32,99] Moreover, the volume of encapsulated drugs is related to the method used to produce the liposomes and also to the liposome's composition and type of drug.[33] There are two different methods to load the drug into liposomes which are active and passive.[15] In the passive method, the drug is encapsulated during the preparation of the liposome. Hydrophilic drugs were spread in the aqueous phase while hydrophobic drugs were placed in the bilayer of the liposome.[32] In this process, as liposomes form, they can capture the aqueous volume that contains the previously dissolved hydrophilic drug. Therefore, the drug concentration in the aqueous core is similar to the volume of water trapped by the liposomes. In the passive loaded drug, the EE can change due to a few characteristics such as production method, drug solubility, lipid concentration, liposome size, and zeta potential.[100] The drug and charged ions are not able to penetrate the liposomal membrane. Otherwise, uncharged drugs can diffuse through the lipid membrane, which can lead to drug leakage. Typically, this approach results in low EE, involving a large number of unencapsulated drugs and a large loss of drugs for drugs that are permeable to the liposomal bilayer.[101]

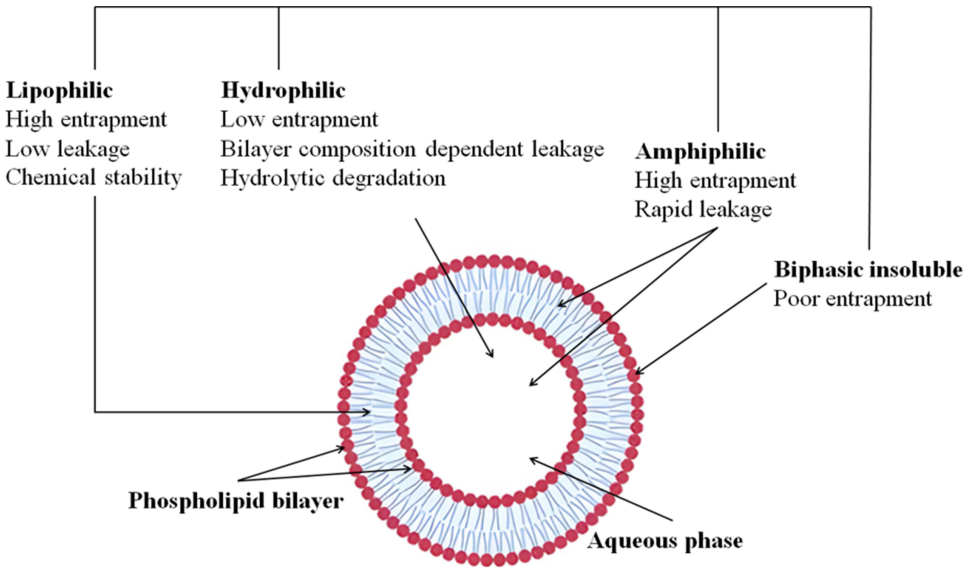

Figure 3 Different drugs and their location in the liposomal vesicle.

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There is another method of drug loading which is called active or remote loading, which contains the making of an ion gradient or transmembrane pH, which efficiently drives the drug over the lipid bilayer and in some drugs lead up to a 100% loading. After liposome preparation, this approach is applied. The gradient is created between the interior of intact (already formed) liposomes and the exterior of the liposome, the drug is solubilized in the aqueous medium. According to previous work, drugs can cross the lipid membrane, they convert them to a protonated form, prevent them from spreading outside the liposomes and also improving the EE and retention inside the liposome.[101] When the drugs are weakly acidic (pKa>3) or amphipathically a weak base (pKa ≤ 11), the desirable loading efficiency was achieved.[89] There are different methods to actively load drugs which includes following a calcium acetate gradient for weakly acidic drugs, ammonium sulfate transmembrane gradient for amphipathically weak bases, a phosphate gradient method, an EDTA gradient method and ionophore loading method.[101]

The in vitro drug release could be evaluated by using dialysis conditions. The choice of a dialysis bag should match the specifications of the drug. It must be freely penetrable to the drug and there must be no adsorption of the drug.[27] The liposome sample with a specific molecular weight is placed in the dialysis bags hermetically tied and cut off. Usually, the tubing membrane is put in the buffer at pH 7.4, and the system is kept at a 37 ◦C simulated environment, and under continuous stirring. At defined times, an aliquot of the sample is taken and analyzed according to standard drug quantification methods and the sample volumes need to be kept at a constant level. Hence, an equal volume is added to the system from fresh medium.[36] The cumulative release percentage is plotted against the selected time points to create the release profile. The results from drug release research are evaluated in the development of liposomes for the controlled release of pharmaceuticals as an extrapolation to in vivo liposome performance.[102]

Classification of Liposomes

Liposomes are small, artificial, enclosed spherical vesicles with a phospholipid bilayer separating one aqueous medium from another, capable of encapsulating hydrophilic molecules in the internal aqueous core or sequestering hydrophobic drugs in the lipid bilayer, and providing a controlled release system (Figure 4).[103–105] Liposomes are commonly employed as drug delivery nanocarriers because they may carry drugs to target areas while minimizing systemic exposure.[63,103,106,107] They are characterized as conventional, theranostic, PEGylated, and ligand-targeted,[63] as well as by size, lamellarity, and surface charge (Figure 4).[63,103,106,107] Liposome formulations are biocompatible and biodegradable,[103] because they are made of mammalian cell membrane-like constituents and may permeate biofilms,[108] and intracellular regions such as macrophages.[109,110] Liposomes are appealing for drug delivery for a variety of reasons, including their pharmacological inactivity, ability to self-assemble, possession of a large aqueous center to carry large drug "payloads", controlled drug release, and potential for enhanced pharmacokinetics and reduced toxicity;[111] they are particularly useful for drugs that require cell membrane penetration.[109,110] Liposomal behavior may be modified in vivo and liposomes can be directed to a specific area in the body. The stealth and targeted liposomes along with recent advancements in liposome design have resulted in a variety of liposomes, including immunological liposomes and stimulus-sensitive liposomes.[106,112]

Conventional liposomes were the first liposomes generated used for therapeutic applications.[113–115] To promote the stabilization of the liposomal bilayer, these liposomes can be made up of cationic, neutral, or anionic phospholipids in combination with CH.[63,116] Nevertheless, there are still several challenges with this type of liposome, such as plasma instability, which results in a short blood circulation half-life. RES captures liposomes quickly and removes them from the bloodstream,[115] The binding of opsonins to liposomes from serum proteins is the first signal for the elimination of liposomes. Conventional liposomes are recognized by opsonins as foreign particles and are therefore destroyed by mononuclear phagocytic system (MPS) phagocytes.[22]

To circumvent the limitations of regular liposomes, a second generation of liposomes was produced, resulting in the development of so-called stealthy, long-circulating, or PEGylated liposomes.[117] The capacity to coat the surface of the liposome membrane with biocompatible hydrophilic polymer conjugates such as chitosan, PEG, and others, therefore increases the repulsive forces between serum components and liposomes, and is fundamental to the stealth method.[118] As a result, macrophage immunogenicity and absorption are reduced, resulting in a longer circulatory half-life and lower toxicity of the encapsulated compound.[119] Physical absorption of the polymer onto the liposomes surface, integration of PEG-lipid conjugates during liposome production, and covalent coupling of reactive groups to the surface are all methods

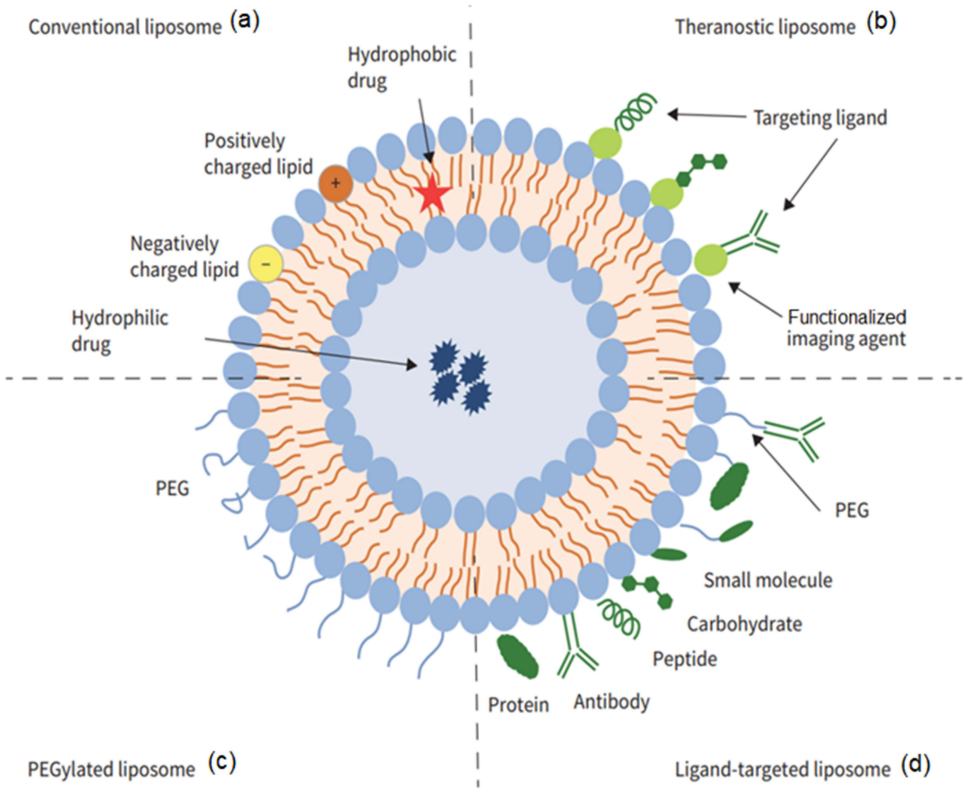

Figure 4 Structure of liposomes for drug delivery. (**a**) Conventional liposome, (**b**) Theranostic liposome, (**c**) PEGylated liposome, and (**d**) Ligand-targeted liposome. Reproduced Sercombe L, Veerati T, Moheimani F, Wu SY, Sood AK, Hua S. Advances and Challenges of Liposome Assisted Drug Delivery. Front Pharmacol. 2015 Dec 1;6:286. Copyright © 2015 Sercombe, Veerati, Moheimani, Wu, Sood and Hua. Creative Commons Attribution License (CC BY).[63]
Abbreviation: PEG, polyethylene glycol.

for attaching PEG to the liposome membrane.[115] Nonetheless, the high body bio-distribution of stealth liposomes is a serious drawback. As a result, drug encapsulation cannot be administered to a specific target location.[9]

Based on this constraint, ligand-targeted liposomes have been designed to transport drugs to specific tissues, allowing for more advanced and selective therapeutic activity.[115] Target liposomes are additionally functionalized with glycoproteins, ligands, or polysaccharides for specific receptors such as antibodies, peptides, or small molecules, in addition to PEG surface modification.[9,22] The ligand can target and bind to particular receptors overexpressed on diseased cell surfaces, with little off-target effects to healthy cells.[113,120,121] Antibody-functionalized liposomes (immune liposomes) and stimuli sensitive liposomes have been proposed based on the previous technique concepts.[106] Immune liposomes are made by chemically attaching antibodies or fragments of antibodies to the liposome surface, resulting in a more specific target antigen.[122] When specific physicochemical or biological stimuli, such as pH, temperature, redox potential, enzyme and electrolyte concentrations, ultrasonic, electric, or magnetic fields, alter in a stimulus-sensitive liposomal system, the medication is released.[123,124] The most prevalent stimuli responsive liposomes are temperature-sensitive and pH-sensitive liposomes.[125,126] In addition to medication delivery, liposomes may be used for various purposes, such as making minor changes to their composition and charge.[106] Cationic liposomes are a good example of a transfection vector utilized in gene therapy to carry genes. Gene encapsulation in liposomes allows nucleic acids to be protected from destruction during storage and circulation.[115] Due to the significant potential of multifunctional liposomes, surface modification approaches have recently been researched for performing a combination of diverse functionalities, resulting in liposomes with a wide variety of functions.[22] Another form of liposomes include theranostic liposomes, which can include imaging and therapeutic agents (diagnostic and therapy functions) within the liposome.[63,127] Dual targeting liposomes is another example of liposome that involves having two different ligands.[22]

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Targeting Strategies of Liposomes

The targeting strategy is an intense area for researchers to develop liposome formulations. One of the primordial functional properties of liposomes is specific targeting in drug delivery.[107] As a result, high attention on specific sites puts emphasis on both the discovery of novel diagnostic tools and the improvement of therapeutic agent efficacy.[128] Liposomes can currently be divided into two categories: active and passive targeting. Active tissue targeting is achieved with receptor-specific ligands on the surface of liposomes targeted for cellular uptake, while passive tissue targeting is accomplished primarily through the characteristics of the cancer or tumor vascular system, as illustrated in Figure 5.[129] The passive targeting method has been used mostly in the field of oncology due to the pathophysiological characteristics of cancer.[130] Liposomes passively target cancerous tissue or cancer cells by transporting and distributing them through the leaky tumor vasculature in the tumor interstitium via a molecular drive in the fluid.[131] Therefore, passive targeted liposomes with a size of 10 to 500 nm can accumulate preferentially in the tumor and inflamed tissues through the

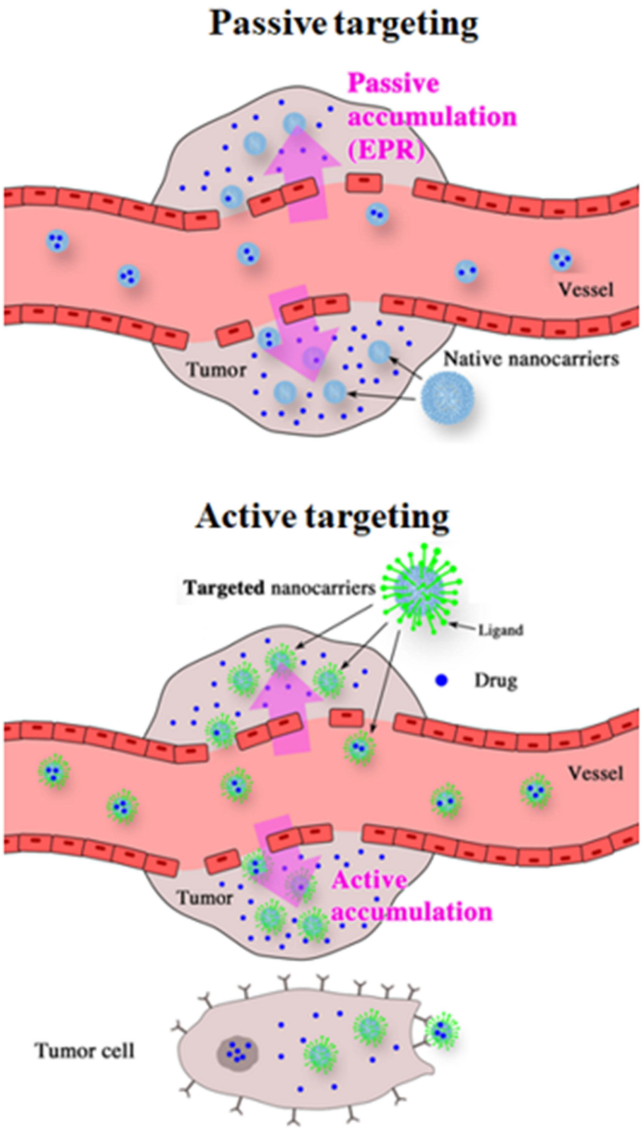

Figure 5 Targeting strategies for passive and active drug delivery using liposomes. Passive targeting relies on the natural distribution of drugs in the body, while active targeting uses targeting moieties on the liposome surface to bind to receptors on the target cells.
Notes: Repoduced from Attia MF, Anton N, Wallyn J, Omran Z, Vandamme TF. An overview of active and passive targeting strategies to improve the nanocarriers efficiency to tumour sites. J Pharm Pharmacol. 2019 Aug;71(8):1185-1198. [129]

permeability and retention effect RPE of the vascular system due to increased vasculature, vascular leaks, blood abnormalities, and dysfunction of the lymphatic vessels.[132,133]

Non-targeted liposomes prevent quick removal through the body's defense systems, such as phagocyte absorption or clearance by mononuclear phagocyte system (MPS) cells.[62] As a result, the production of stealth liposomes by PEG surface modification of liposomes can be an excellent example of applications in passive targeting techniques, and their circulation time can be increased.[107] This technique also makes use of liposome-specific features, such as charge, which can promote selective targeting of cancer cells. Cationic liposomes are another example. By electrostatic interactions, this class of liposomes has been shown to bind the head of the phospholipid that has a negative charge, especially expressed on tumor endothelial cells.[134] A targeting method based simply on the EPR effect is insufficient to prevent cytotoxic medication side effects. The variability of EPR effects in tumors, as well as their restriction to certain solid tumors, can have an impact on the efficacy of medications supplied by passive targeting.[62,135] As a result, researchers studied the creation for new targeting methods with expanded functionality, such as active targeting.[130]

Paul Ehrlich, who coined the term "magic bullet" to describe the need for precise drug delivery inside the body, introduced the first concept of active targeting in 1906.[129,136] Since then, researchers around the world have indeed been looking for a "miracle cure" which targets specific cells to make disease diagnosis and treatment easier.[137] For improved liposome delivery systems, active targeting includes applying a targeting ligand to the liposome surface.[22] Many target ligands, such as antibodies, peptides, nucleic acids, and whole proteins, as well as small molecules such as vitamins, are used for active targeting. For the identified target ligands, factors like the relative degree of overexpression or specific expression on the target, cellular uptake of the ligand-targeting formula, and degree of coverage of the target molecule have been considered.[138,139] These ligands must also be chosen in such a way that they can bind to target cells while avoiding healthy cells.[129] There are three methods to choose from when it comes to liposome functionalization. The first step is to attach the ligand to a lipid before mixing it with some other lipids in the liposomes. In the second method, the liposomes are activated immediately after preparation by targeting the required ligand (Table 3).[140] The PEG spacer-modified lipid group, which is activated with the amine at the end or using thiol, carboxylic acid, or malamide groups, demonstrates the options available for this method.[141] In another approach, it was suggested that functional lipids can be introduced into prefabricated liposomes. This approach is based on the spontaneous combination of activated lipids from the micelle phase to be prefabricated and even liposome-containing drugs. Derivation of the target molecule takes place in a separate step, as a way to prevent the activated lipids from interfering with other liposomal components, such as those compounds, which are present in the buffer.[142]

Active Targeting of Liposomal Anticancer Drugs

By using active targeting as a new approach, it is possible to overcome targeting barriers by adding a targeting moiety to the drug carrier's surface. It is expected that the inventory used for the target materials will detect tumor-associated receptors or antigens. As a result, drugs are targeted to the site of action because drug uptake in the target cells increases while drug uptake in non-specific, healthy cells is reduced. In addition, through the receptor-mediated endocytosis process, some ligands can cause drug release from liposomes into target cells.[143]

Table 3 Comparative Analysis of Three Liposome Functionalization Approaches, Including: Coupling Ligands Prior to Liposome Formation, Direct Activation with Target Ligands, and Incorporation of Functional Lipids into Preformed Liposomes

Method	Advantages	Disadvantages
Ligand coupling to lipids prior to liposome formation	Provides greater control over the ligand-lipid coupling process	More time-consuming and labor-intensive
Direct liposome activation with targeting ligands	Efficient and rapid method	Requires specialized equipment and can be challenging to control the degree of activation
Post-insertion of functional lipids into prefabricated liposomes	Simple and straightforward method	May require careful handling to prevent interference with other liposomal components

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As a result, the accumulation of drugs in the cells increases, and the overall effect of the therapies improve. Liposomes that target receptor internalization may also overcome drug resistance, at least in part.[144] Many different methods are used for liposome active targeting. Target ligands attached to the surfaces of drug delivery devices are used in active targeting. These target ligands can bind to the receptors that are expressed in the target locations. The ligand must bind to a receptor that is overexpressed by tumor vasculature or cancer cells but not by healthy cells. Similarly, the particular receptors generated by tumor cells must be distributed uniformly. The antibody fragments, monoclonal antibodies, and non-antibody ligands are the proper ligands used for targeting purposes. The degree of ligand binding plays a crucial role in tumor penetration. To specifically target cells that are easily accessible, typically due to the tumor vasculature, high affinity binding appears to be preferred due to the dynamic flow environment of the bloodstream.[145,146] Targeted liposomes for anticancer drugs will be discussed in the following section for different reported approaches (Table 4).

Receptor-Based Liposomal Anticancer Drug Targeting

Active targeting by cell surface receptors have been widely explored because many cancer cell types have tumor-specific receptors. Using receptor particular ligands or antibodies are some of the most common strategies to attack overexpressed cell surface receptors on cancer cells.[147] The next sections summarize the most frequently activated receptors for liposomal medication delivery against cancer that are over expressed by tumor cells.

Folate Receptor-Based Liposomal Anticancer Drug Targeting

Folic acid is required in one carbon metabolic process and also plays a vital role in nucleotide base synthesis. Overexpression of the folate receptor-α isoform is seen in around 40% of human cancer cells. However, activated macrophages and hematological malignant cells have been observed to overexpress the folate receptor-β.[148] Anticancer drugs are frequently targeted using folate-modified liposomes. Due to their site not on the apical surface of the epithelium but rather on the luminal side, targeting folate receptors (FRs) have been shown to be quite more efficient than targeting other receptors in reducing chemotherapeutic toxicities. The FR is a well-known tumor marker that binds with strong affinity to vitamin folic acid and folate-grafted drug carriers or folate drug conjugates, delivering them into cells via receptor-mediated endocytosis. When administered to multiple malignant cells by FR, doxorubicin (DOX) and daunorubicin (DUNO) have been demonstrated to have higher cytotoxicity.[149,150] In another research, all-trans retinoic acid activation of FR was combined with folate-modified liposomes loaded with DOX for the treatment of acute myelogenous leukemia.[151] Similarly, a diacid metabolite of norcantharidin, which is therapeutically effective against hepatocellular carcinoma and has been loaded into FR-modified polyethyleneglycolated liposomes, has been demonstrated to exhibit

Table 4 The Target Liposomes for Anticancer Drugs are Divided According to the Advantages and Disadvantages of Each of Them in the Listed Cases

Type of Targeted Liposomal Anticancer Drug	Advantages	Disadvantages
Receptor-based liposomal anticancer drug targeting	Highly specific targeting to tumor cells	Requires specific tumor receptors to be present
Folate receptor-based liposomal anticancer drug targeting	Folate receptors are overexpressed in many cancers	Drugs may bind to normal cells, leading to side effects
Transferrin receptor-based liposomal anticancer medication targeting	Transferrin receptors are highly expressed on tumor cells	Drugs may not be able to reach tumors in all parts of the body
Epidermal growth factor receptor-based liposomal anticancer drug targeting	EGFR is overexpressed in many cancers, including lung, breast, and ovarian cancer	EGFR inhibitors may cause side effects such as skin rash and diarrhea
Other receptor-based liposomal anticancer drug targeting	Various other receptors can be targeted, such as integrins and HER2	Each receptor has its own specific characteristics and limitations
Stimulus-responsive liposomal anticancer drug targeting	Drugs release when exposed to specific stimuli, such as pH, temperature, or enzymes	Can be more difficult to control precisely than receptor-based targeting

more cytotoxicity than plain PEGylated liposomes against the H22 hepatoma cell line. The researchers also observed that FR-modified liposomes were more effective in targeting tumors.[152] Another study looked at the use of FR-modified liposomes to deliver Paclitaxel (PTX) to specific areas of the body.[153]

Liposomal Anticancer Medication Targeting Based on Transferrin Receptors

Transferrin, a glycoprotein, carries iron through the bloodstream and into cells by attaching to the transferrin receptor (TR) and lastly, receptor-mediated endocytosis internalizes the iron. TRs are important proteins that regulate iron homeostasis and cell development. TRs are overexpressed on the surfaces of many tumor cells as a result of their high iron needs and their rapid growth. TR targeted tailored anticancer medication delivery has been a significant technique. These receptors can have a dual impact when used for medication targeting. They carry medications into cells while suppressing their normal function, depriving the cells of iron, when they are targeted for drug delivery. It is also suggested that they have a role in the transport of iron to the brain. This also provides a novel medication targeting method for passing medicines past the blood-brain barrier.[154,155] Transferrin coupled DOX-loaded liposomes bind and kill C6 glioma cells more effectively.[156] A TR-targeted DOX-loaded liposomal technology, on the other hand, enhanced DOX intracellular absorption, pharmacokinetic profile, and biodistribution, leading to improved efficacy of treatment against liver cancer.[157] Sharma et al developed a liposomal system that included transferring and poly-L-arginine. The strategy worked: the transferrin-modified liposomes targeted tumors, and poly-L-arginine increased cell penetration, allowing drugs to pass the blood-brain barrier endothelium.[158] Dual functioning liposomes have also been described for penetrating the blood-brain barrier and targeting tumors. When tested using bEnd3 blood-brain barrier models, DOX liposomes supplemented with transferrin and folate were found to be useful for bioavailability in cells, P-glycoprotein (P-gp) expression, and drug transfer throughout the blood-brain barrier. The dual targeting DOX liposomes were found to be able to cross the blood brain barrier and distribute primarily in brain gliomas during in vivo tests. This effectiveness of the dual targeting method has been established in terms of tumor size reduction and extended survival time.[159]

Epidermal Growth Factor Receptor-Based Liposomal Anticancer Drug Targeting

The epidermal growth factor receptor (EGFR) is a protein that controls cell growth, differentiation, and repair in non-cancerous cells. However, in cancer cells, EGFR is often overactive, leading to uncontrolled cell growth and division.[160] EGFR are overexpressed in a variety of solid tumors, including colon cancer, non-small cell lung cancer, ovarian, kidney, head, pancreas, neck, and prostate cancer, as well as breast cancer[161,162] This makes it a promising target for therapeutic drug delivery. Proliferation, angiogenesis, and metastasis are only a few of the mechanisms that EGFR controls in cancer cells. EGFR-targeted immune system liposomes have been shown to increase intracellular DOX delivery to tumor cells, as well as enhance cytotoxicity against targeted tumors in xenograft animal models.[163,164] The use of EGFR-targeted monoclonal antibodies in combination with liposomal systems has been studied extensively for signs of enhanced active targeted therapy. These antibodies, which act as targeting ligands on the surface of liposomes, have emerged as among the most effective drug candidate delivery techniques due to their high selectivity. At a DOX dose of 10 mM, cetuximab (an EGFR antibody)-biotin liposomes exhibited greater cytotoxicity for SKOV-3 cells than non-targeted biotin liposomes. On SKOV-3 cells, targeted liposomes demonstrated 22- to 38-fold greater binding than non-targeted liposomes.[25] These data point to the efficacy of this method in the treatment of ovarian cancer.

Other Receptor-Based Liposomal Anticancer Drug Targeting

In addition to the receptor-based liposomal anticancer drug delivery described previously, other receptors have been found and are being used for specifically targeting anticancer drug delivery. Vasoactive intestinal peptide (VIP) receptors have been studied as therapeutic targets because they are abundant on the surfaces of tumor cells. In mice, VIP-coated PEG liposomes with radionuclides were found to be more effective at suppressing breast cancer than uncoated PEG liposomes with radionuclides.[165] Immuno liposomes based on EGFR have also been reported for delivery to malignant cells overexpressing EGFR.[163] Many tumor cells also overexpress hyaluronan receptors (HRs),

which can be used to target liposomal anticancer treatments. When mitomycin C was encapsulated in long-circulating hyaluronan-targeted liposomes, it was more effective against tumors with HR overexpressed on their surfaces.[166] Liposome-loaded cisplatin has been effectively used in vivo to prevent tumor formation and metastasis by selectively binding to chondroitin sulfate, which is overexpressed in many tumor cells.[167] Furthermore, galactosylated liposomes have been shown to concentrate preferentially in parenchymal cells. They have been successfully used to transfer genes to these cells.[168] Another method for liposome targeting is surface functionalization using peptide amphiphiles.[169] Endothelial cells generate a variety of cell adhesion molecules (CAMs), which are necessary for the recruitment of leukocytes from the circulating blood to the endothelium following an inflammatory stimulus. CAMs are a natural target for anticancer therapy since they have a role in inflammatory disorders, including cancer.[25] VCAM-1 is one of the CAMs that is overexpressed in tumor vasculature and is a promising target for anticancer medication delivery.[170] Integrins, which are overexpressed in many cancers, aid in invasion and metastasis by allowing tumor cells to attach to the endothelium lining of blood arteries in various organs and tissues. Arginine-glycine-aspartate (RGD), a tripeptide, has a high integrin binding efficiency and has been shown to inhibit tumor cell adhesion and angiogenesis.[171] Targeted medication delivery has made use of tumor tissue-specific expression of integrin receptors. Chen et al created an integrin-targeted liposomal method for DOX administration. The liposomes were covalently linked with cyclic RGD. In the U87MG cell line, the RGD coupled liposomal system showed a 2.5-fold greater cellular absorption of DOX than the unmodified liposomes. The liposomes were internalized via an integrin receptor-mediated endocytic routes, according to a competitive binding assay.[172]

Stimulus-Responsive Liposomal Anticancer Drug Targeting

Anticancer medication accumulation in malignant cells is not enough for successful treatment. Furthermore, liposome surface modification with PEG can prolong their circulation duration in the bloodstream while decreasing cellular internalization owing to steric hindrance. This problem can be handled by utilizing both external and internal cues. After liposomal accumulation at the target locations, these stimuli can disrupt the PEG protective layer.[173] The notion of stimuli sensitivity is based on tumor microenvironmental features such as lower pH, greater temperature, and overexpression of various proteolytic enzymes.[174] The stimuli-sensitive liposomes maintain their shape and physical characteristics throughout circulation. When exposed to a specific tumor microenvironment, they are engineered to undergo rapid changes (aggregation, disruption, and permeability) that result in drug release.[25] The section that follows describes stimulus-responsive liposomal targeted delivery of anticancer medicines (Table 5).

Table 5 Overview of Targeted Anticancer Drug Delivery Methods via Liposomal Stimulation, Including pH, Temperature, Enzyme, Physical Adsorption, Magnetic, Ultrasound, and Light-Responsive Techniques

Stimulus	Targeted Delivery Mechanism	Characteristics
pH	pH-sensitive liposomes release encapsulated drugs within the acidic environment of tumor cells (pH 6.0–7.0)	Sensitive to acidic pH changes
Temperature	Temperature-sensitive liposomes release encapsulated drugs at elevated temperatures, mimicking tumor hyperthermia (40–42°C)	Sensitive to temperature changes
Enzyme	Enzyme-sensitive liposomes release encapsulated drugs in response to specific enzymes overexpressed in tumor cells	Targeted release based on enzyme activity
Physical Adsorption	Physically adsorbed liposomes target cancer cells by attaching to their negatively charged cell membranes	Simple and inexpensive method
Magnetic	Magnetic liposomes are guided to a tumor site using an external magnetic field	Targeted delivery based on external magnetic fields
Ultrasound	Ultrasound-responsive liposome release encapsulated drugs in response to ultrasound irradiation	Non-invasive and deep tissue penetration
Light	Light-sensitive liposomes release encapsulated drugs in response to light irradiation	High spatial imaging resolution

Targeted pH-Responsive Liposomal Anticancer Delivery

PEGylation of liposomes has been shown to be a promising method for extending their systemic circulation time. It does, however, prevent emulation-induced drug release and intracellular drug delivery. Because of the glycolytic conversion of glucose to lactate in tumor cells, the tumor microenvironment has been demonstrated to be somewhat acidic (pH 6.0–7.0), lowering the pH value from that of normal tissues (pH 7.4) and has been exploited to develop pH-sensitive drug liposomes.[175] The pH- sensitive breakdown of a liposomal carrier liberates encapsulated payloads in low-pH tissues like tumors, cell cytoplasm, or endosomes. Because of the low endosomal pH, liposomes containing pH-sensitive materials fuse with the endovascular outer layer during endocytosis and release their substances into the cytoplasm.[176] Wang et al employed modified liposomes to construct a pH-sensitive drug delivery device.[177] In a healthy environment (pH 7.4), the system releases slowly and gradually, but releases fast in a sub-acidic environment mimicking tumor tissue (pH 6.0). Tumor cells treated with pH-sensitive liposomes survived only 35% of the time after 48 hours, but normal cells survived 100% of the time, according to in vitro tumor cytotoxicity assays. Junior et al compared the tissue distribution of stealth pH-sensitive liposomes containing cisplatin to that of free cisplatin in solid Ehrlich tumor-bearing mice.[178] The longer the stealth pH-sensitive liposomes circulated, the more medication that was released in the blood and accumulated in the tumor.

Temperature-Responsive Liposomal Anticancer Targeted Delivery

Tumor tissues typically display hyperthermia due to their fast metabolism, and as a result, increased temperatures are frequently observed in tumor locations, similar to the inflammatory response. Temperature-sensitive liposomes were motivated by this phenomenon. During pathological hyperthermia or external warming, temperature-sensitive liposomes release anticancer medications at tumor areas. A controlled device can also be used to heat solid tumors using an external energy source, like infrared irradiation. This is because temperature-sensitive liposomes are made up of lipids, which may change phase from gel to liquid at a certain temperature. Following that, when the temperature rises, the phospholipid double molecular chain becomes more disordered and active, resulting in drug release from the liposome vesicles.[179] Temperature-triggered liposomal technologies are gaining popularity for targeted anticancer medication delivery. Also lipid temperature-sensitive liposomes have shown increased effectiveness in cancer-targeted medication delivery.[180] This formulation is now being studied in Phase III clinical trials for hepatocellular carcinoma, as well as Phase II trials for breast cancer and colorectal liver metastases. Temperature-sensitive liposomes containing cis-platinum were developed by Kakinuma et al for the treatment of animals with brain glioma. Furthermore, the researchers discovered a much greater quantity of cis-platinum in brain tumor locations.[181] Yatvin et al described temperature-sensitive liposomes that might release a hydrophilic medicine when heated to just a few degrees above physiological temperatures.[182]

Targeted Anticancer Enzyme-Responsive Liposomal Delivery

Over-expressed enzyme processes in the tumor environment, such as matrix metalloproteinases, have recently been used to improve the release of anticancer drugs from liposomes. Mura et al created enzyme-sensitive liposomes by using an MMP2-cleavable linker to connect a monoclonal antibody 2C5 to a PEG chain.[183] Furthermore, tumors have been reported to over secrete phospholipase A2 (sPLA2), which can be exploited to induce medication release from enzyme-sensitive liposomes. Human sPLA2 activity was found to be particularly sensitive to liposome phospholipid acyl-chain length and negative surface charge density, resulting in drug release of enzyme-sensitive liposomes, according to Hansen et al.[183]

Targeting Physically Adsorbed Liposomal Anticancer Drugs

By adsorbing onto the membrane of cancer cells, physical adsorption-mediated liposomes, which employ cationic materials to modify the surface of liposomes into positively charged liposomes, can have a targeted impact. Cancer cells have electronegative cell membranes and electropositive liposomes can attach to them. Cationic liposomes can also accumulate in living cell mitochondria in response to mitochondrial membrane potential,[179] after being taken up by cancer cells. Wang et al created mitochondrial targeting resveratrol liposomes by combining a dequalinium (DQA) molecule with polyethylene glycol stearoyl phosphatidylethanolamine (PEG2000- DSPE). The findings showed notable antitumor effects in both cancerous cells and drug-resistant cancerous cells.[184] Ma et al also created mitochondrial

targeting berberine liposomes by customizing DQA-PEG2000-DSPE-200.[185] Berberine liposomes that target mitochondria might penetrate across cancer stem cell membranes and accumulate preferentially in cancer cell mitochondria. When coupled with PTX liposomes, mitochondrial targeting berberine liposomes greatly boosted anticancer efficiency in human breast cancer stem cell xenografts in nude mice.

Liposomal Anticancer Targeted Delivery Using a Magnetic Response

Magnetic liposomes are nanoparticles of maghemite (γ-Fe_2O_3) or magnetite (Fe_3O_4) that have been placed into liposomes (MLs). They are used to target medicines to specific sites using an external magnetic field as a stimulus.[186] Such MLs have a wide range of applications in cancer. They are utilized in diagnostic applications such as MRI contrast agents. They are effective in the treatment of cancer using hyperthermia-based therapy. When an external magnetic field is supplied to MLs, they are used as heat mediators.[187] Furthermore, they are employed in combination therapy with medicines for triggered release to provide a more safe and more efficient customized treatment.[188] The toxicity of nanocarriers has long been a source of concern, limiting their application in medication delivery. When magnetic nanoparticles are encased in liposomes, the toxicity of the magnetic nanoparticles intended for targeted medication administration and diagnostic purposes is decreased or diminished.[189]

Magnetic nanoparticles are used in cancer treatments to increase medication accumulation at the tumor sites while minimizing the negative influence of chemotherapeutic medications on other normal tissues.[190] Furthermore, such systems are more effective at imaging the entire site and may efficiently transport medicines throughout the cell membrane, thereby maintaining the required concentration levels of pharmaceuticals or diagnostic agents for the diagnosis of brain leukemia.[191] Using magnetic gradients, 5-FU loaded MLs have been shown to improve biocompatibility and drug ability control. Surprisingly, the formulation was capable of exhibiting hyperthermia-triggered release of the drug, as well as an enhanced overall combination anticancer efficacy.[192] Another research group looked at the co-delivery of glutamic acid-chelated γFe_2O_3 and methotrexate in the aqueous core of liposomes. The investigation yielded intriguing results. When exposed to an external magnetic field, the formulation produced an increase in the concentration of the medication deposited in the targeted tumor tissues compared to the findings demonstrated by the same formulation without the application of a magnetic field.[193]

Liposomal Anticancer Targeted Delivery Using an Ultrasound Response

Because of its non-invasiveness, deep penetration into the body, and permeability of blood tissue barriers, ultrasound-based targeted delivery has received a great deal of attention.[194] Air is included in ultrasound-triggered drug release devices and is able to respond to ultrasonic stimulation to release a loaded material. The medication in ultrasonic-responsive liposomes can be released in line with ultrasound parameters. As a result, medication released from such stimuli sensitive liposomes may be tailored to meet the needs of the patient. If a medication burst release is desired, a high-intensity single ultrasonic pulse must be used. To induce sustained medication release, multiple low ultrasonic pulses are delivered over a long period.[187] It has been demonstrated that ultrasound-responsive liposomal formulations improve cellular transfection by increasing membrane permeability during drug administration into the artery wall.[195] A controlled release of DOX using an ultrasound-responsive liposomal formulation has been described. A perfluoropentane nanodroplet emulsion was used in the system, which was loaded with DPPC-based liposomes. The method was utilized for low-intensity DOX administration to the tumor. When the formulation was subjected to low-intensity ultrasound, it was able to release 80% of the loaded drug content, which was significantly higher than the basic emulsion of the same medication. When compared to free drugs, plain emulsions, and liposomal emulsions without ultrasound, the formulation also demonstrated greater anticancer efficacy of the medication against HeLa cells.[196]

Anticancer Delivery Using Light-Sensitive Liposomes

Because of their great spatial imaging resolution and the potential for targeted therapies, optical techniques for diagnosing and treating illnesses (such as skin wounds, inflammation, and cancer) are gaining scientific interest.[197] Light in the near-infrared range has been discovered to penetrate deeply into tissues, making it useful in the treatment of cancer. Photodynamic therapy is now widely used to treat superficial cancers. Photosensitizing compounds including

chlorins, porphycenes, porphyrin derivatives, and phthalocyanines, can produce radical oxygen species when exposed to light. As a result, they are utilized to sensitize and eradicate cancerous cells.[198] Temoporfin, an amphiphilic molecule, is one of the most widely used photosensitizers in clinical practice. Foscan is an FDA approved treatment for advanced squamous cell carcinoma of the neck and head. Temoporfin, a photosensitizer, is included in the formulation, along with ethanol and propylene glycol. Fospeg and Foslip are two further liposomal formulations based on PEGylated liposomes and DPPC, respectively.[199] Another liposomal formulation that is light and temperature sensitive was recently disclosed. It was made up of hollow gold nanospheres with DOX medication. When exposed to light, the formulation demonstrated light-triggered DOX release. When compared to control groups, this formulation based therapy demonstrated improved antitumor effectiveness.[200]

Therapeutic Applications of Liposomes

Liposomes have shown beneficial results as a drug delivery mechanism for several medications. Thus, rigorous studies of liposome use in medicine have led to the creation of diverse liposomal formulations for the control and management of a wide variety of illnesses, as well as a wide range of therapeutic applications such as fungal infections, analgesics, viral vaccines, photodynamic treatment, and cancer therapy. Because of alterations in pharmacokinetics and pharmacodynamics, encapsulating medications into liposomes increases their therapeutic effectiveness.[201] Modification of in vivo drug behavior and reduction of drug toxicity in organisms are essential elements in developing an effective liposomal formulation. In clinical applications, liposomes are used to treat and diagnose cancer. The pH-sensitive liposomal nanocarriers have shown great promise for the delivery of chemotherapeutic drugs to tumor locations, greatly increasing their efficacy in cancer suppression. Liposomes are often PEGylated to increase their blood circulation time.[202] Despite the fact that PEGylation reduces off-target toxicity in liposomes, PEGylated liposomes have low extravascular transport, limiting their survival advantage.[203] Furthermore, PEGylated liposomes cannot escape endosomes after endocytosis.[204]

To circumvent the aforementioned constraints, pH-sensitive PEGylated liposomes have been developed. Liposomes were created by thin-film hydration in an experiment, and then SN25860, a small chemical having anticancer activity, was loaded to increase its accumulation at the tumor location. The liposomes demonstrated a high drug loading efficiency (7.0 0.2% w/w) and could boost SN25860 cytotoxicity by up to 21- to 24-fold. pH-sensitive PEGylated liposomes increased anticancer drug internalization and cellular uptake, and they entered cancer cells via clathrin-mediated endocytosis. According to Figure 6, liposomes can be constructed to release their therapeutic cargo prior to cellular

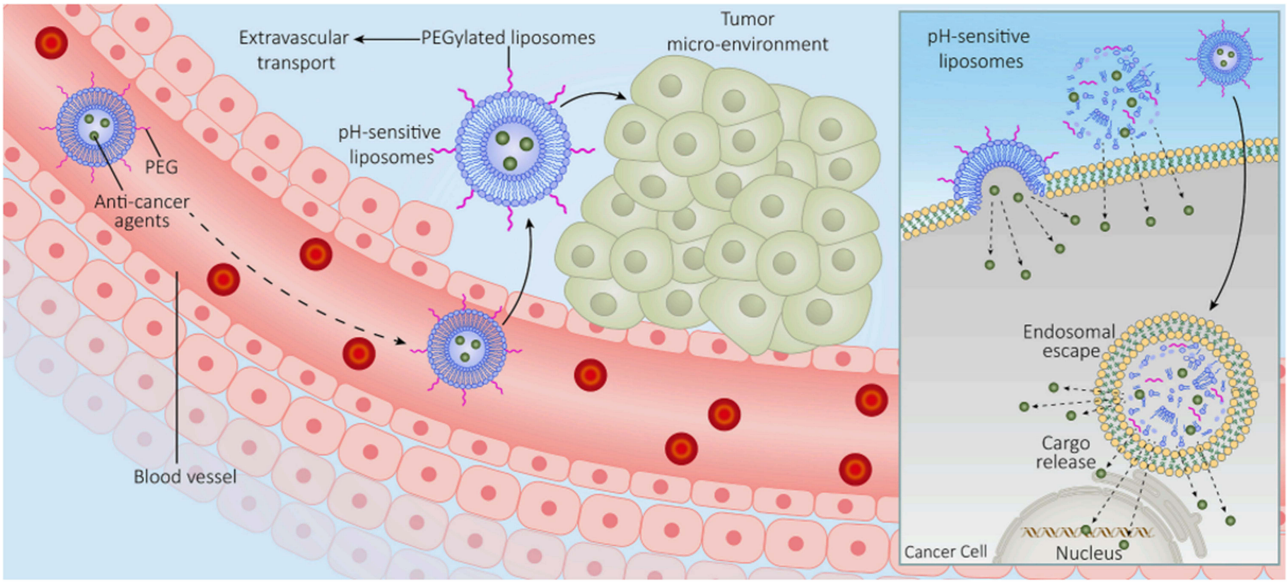

Figure 6 pH-sensitive liposomes exploit the lower pH in tumors versus normal cellular environments for drug release. They can release drugs before or after cell uptake, responding to the acidic tumor environment to target and treat tumors effectively. Adapted from Zhu L, Torchilin VP. Stimulus-responsive nanopreparations for tumor targeting. *Integr Biol.* 2013;5(1):96–107. © The Royal Society of Chemistry 2013.[198]

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uptake due to the intratumoral acidic pH, during cellular uptake by merging with a cell lipid membrane, or after endocytosis upon nanoparticle entry into the tumor location. In the latter situation, the pH-sensitive portion of the liposome degrades, resulting in medication release and targeted tumor suppression. The therapeutic potential of liposomes, however, is not limited to cancer therapy. Liposomes are regarded to be a very adaptable platform that may be used in a variety of research domains.[205] The next section will focus on liposomes and their application to the most prevalent cancers (Table 6 and Figure 6).

Breast Cancer

Breast cancer is classified clinically based on the presence of the estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor type 2 (HER-2). Breast cancer types that express receptors are treated with receptor-specific treatment. When the cells do not express any hormone receptor (triple negative), they become resistant, and chemotherapy is used to treat them.[206] Monotherapy is currently ineffective because of the risk of chemo-resistance and tumor recurrence. Thus, the combination of anticancer medicines has enabled the treatment to have a synergistic impact.[29] Nanoparticle-based drug delivery carriers have great promise as effective chemotherapeutic delivery methods for breast cancer.[207] Targeting drug delivery systems (TDDS) are becoming more important for improving the therapeutic effect against cancer diseases,[208] and several TDDS have been used in the research of therapies for breast cancer, including micelles, albumin, gold nanoparticles, and liposomes.[209]

Liposomes are highly researched and have been authorized for clinical use because of their greater biocompatibility, safety, and half-life in circulation compared to other formulations.[210,211] Surface ligand functionalization to increase targeting is a key liposomal development theme. Gkionis et al reported the physicochemical features and cytotoxic effects of a new co-loaded liposomal formulation made utilizing two different preparative methods: the classic thin-film hydration approach and the alternative and speedier microfluidic technology.[212] The size, zeta potential, stability, and drug loading capacity of liposomal formulations made using the microfluidic method were found to be equal to those created using the thin-film method. When generated utilizing microfluidic technology, lipid formulations were more homogeneous in size and shape, as well as more cytotoxic to the tested breast cancer cell types. Furthermore, the toxicity of all liposomal formulations was evaluated using a panel of human breast cancer cells (MCF-7, MDA-MB 231, and BT-474 cells) to determine the most powerful formulation per liposomal manufacturing technique and loaded chemical(s). Because of the delayed release of DOX from liposomes, the toxicity of DOX: umbelliprenin co-loaded liposomes were less than that of free DOX.[212] Table 7 shows the liposomal medicine delivery strategy for breast cancer.

As previously documented, the ICAM-1 antibody has been identified as a highly effective ligand for targeting TNBC in vivo. Through the conjugation of ICAM-1 antibodies to liposomes, we achieved specific delivery of encapsulated siRNA to TNBC tumors and cells. The engineered ICAM-Lcn2- liposomes were designed to hinder angiogenic activities in TNBC, as depicted in Figure 7A. The pH-responsive liposomal delivery system was composed of a blend of DOPC, DODAP, and DSPE-PEG-COOH. DODAP, incorporated into the liposome, responds to the acidic endosomal environment, enhancing its cationic character, facilitating fusion with the endosomal membrane, and delivering encapsulated

Table 6 Liposomes for Targeted Delivery of Chemotherapeutic Agents and Synergistic Tumor Targeting in Various Cancers

Cancer Type	Application of Liposomes	Key Findings
Breast Cancer	Targeted delivery of chemotherapeutic agents	Liposomes can be targeted to specific receptors on cancer cells, delivering drugs more effectively and with fewer side effects.
Lung Cancer	Targeted delivery of chemotherapeutic agents and sonosensitizers	Liposomes can be targeted to tumors and release drugs and sonosensitizers, which can be activated by ultrasound to kill cancer cells.
Prostate Cancer	Combination chemotherapy	Liposomes can be used to deliver a combination of chemotherapeutic agents to cancer cells, potentially improving treatment.
Colorectal Cancer	Targeted delivery of hydrophilic and lipophilic drugs	Liposomes can be targeted to tumors and release both hydrophilic and lipophilic drugs, which can have synergistic effects on cancer cells.

Table 7 Summary of Liposome Experimental Results for Breast Cancer

Active Agent	Method	Cell Line	Comment	Theranostic Agents	Reference
DOX & Sorafenibb	Thin layer evaporation	MCF-7 and MDA-MB-231 cells	The linear TT1 peptide was attached to the surface of therapeutic liposomes. In both positive estrogen receptor (MCF-7) and triple negative breast cancer (MDA-MB-231) cells, linear TT1-functionalized liposomes enhanced the therapeutic efficacy of two chemotherapeutic medications, DOX and Sorafenib.	Gold nanoparticles (GNPs) can be conjugated with liposomes for dual imaging and targeted drug delivery to both MCF-7 and MDA-MB-231 cells. The GNPs allowed for imaging using various techniques like CT or photoacoustic imaging, while also enhancing the targeting of drugs to the cancer cells.	[213, 214]
Azadiradione	Thin film hydration	MDA-MB-231 cells	Azadiradione loaded liposomes significantly increased Azadiradione for in vivo oral bioavailability. Azadiradione loaded liposomes considerably outperformed free Azadiradione in vitro for anticancer activities against triple negative breast cancer cells.	Iron oxide nanoparticles (IONPs) can be attached to liposomes, aiding in MRI-based imaging and serving as a targeting agent for MDA-MB-231 cells. These nanoparticles allowed for imaging and targeted drug delivery, enhancing the precision of treatment.	[215, 216]
PTX	Thin film hydration	4T1 and MCF7 cells	Liposomes containing Fru2-Chol ligand, Fru-Chol ligand, and 2-fold Fru-Chol ligand were used to target the glucose transporter 5 on breast cancer cells. All fructose- decorating liposomes outperformed the unmodified liposomes in terms of anti-proliferation effects on 4T1 and MCF7 tumor cells, as well as accumulated at the breast tumor site in vivo.	Quantum dots (QDs) conjugated with liposomes can provide both imaging and targeting capabilities for 4T1 and MCF7 cells. QDs enabled fluorescence-based imaging and, when conjugated with liposomes, enhanced the targeted delivery of drugs to these cancer cells.	[215, 216]
Tamoxifen and Raloxifene	Reverse-phase evaporation	MCF-7 and MDA- MB-231 cells	Liposomes were shown to be a superior formulation for increasing tamoxifen oral absorption, especially when combined with dimethyl-CD. Tumor-bearing rats were treated with tamoxifen + dimethyl—CD liposome formulations, which resulted in a 92.5% reduction in tumor area and a 50% drop in treatment efficiency. Caco-2 transport studies provided a highly useful platform for assessing medication absorption in vitro.	Gadolinium-based nanoparticles can be attached to liposomes to provide MRI-based imaging and targeted drug delivery to both MCF-7 and MDA-MB-231 cells. These nanoparticles offered imaging insights and improved drug localization at the targeted sites.	[217, 218]
Paclitaxel	Thin film hydration	MCF-7 and MDA-MB-231 cells	The cationic liposomal (CLs) delivery method will co-deliver the chemotherapeutic medication PTX and siPlk1 to cancer cells for successful breast cancer therapy. Findings showed that combining PTX with siPlk1 given by CLs can result in considerably greater cytotoxicity to breast cancer cell lines than either PTX or siPlk1 alone. CLs substantially enhanced the biological half-life of PTX following intravenous administration in rats. As a result, the co-delivery of anticancer medicines and siRNAs via CLs showed tremendous potential as a promising method for successful cancer treatment.	Liposomes conjugated with semiconductor nanocrystals (quantum rods) allowed for simultaneous imaging and targeted delivery to MCF-7 and MDA-MB-231 cells. The quantum rods enabled imaging using near-infrared fluorescence while aiding in targeted drug delivery.	[219, 220]

(*Continued*)

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Table 7 (Continued).

Active Agent	Method	Cell Line	Comment	Theranostic Agents	Reference
DOX hydrochloride and Sulforaphane	Thin film hydration	MDA-MB-231 and MCF-7 cells	Liposomes combining SFN and DOX have much greater anticancer activities against MDA-MB-231 cells than those containing DOX or SFN alone. A significant reduction in medication dose is achievable due to the synergistic interaction with malignant breast cancer MDA-MB-231 cells. In MCF-7 cells, the investigated combination had just an additive impact. Liposomal DOX/SFN combined liposomes internalized quicker than single-component DOX liposomes in MDA-MB-231 cells. The presence of DOX in lysosomes and mitochondria was shown by microscopic examination of these organelles. In the case of the DOX/SFN combination, the enhanced release of DOX from lysosomes was seen.	Liposomes coupled with superparamagnetic iron oxide nanoparticles (SPIONs) can offer imaging using MRI and targeted drug delivery to MDA-MB-231 and MCF-7 cells. The SPIONs contributed to both imaging and targeted treatment.	[221, 222]
TPGS* coated and PTX loaded	Thin film hydration followed by an ultrasonication method	MCF-7 and MCF-7/ADR cells	The cellular absorption of PTX from the TPGS-coated PTX-LP was found to be greater than that of the PTX-LP in the MCF-7/ADR cell. At 10 g/mL of the PTX concentration, the TPGS-coated PTX-LP was considerably more hazardous than the free PTX solution and PTX-LP in MCF-7/ADR cells. The idea that a TPGS coating on the liposome surface can improve cytotoxicity against MCF-7/ADR by decreasing p-glycoprotein production was validated by Western blot analysis.	Liposomes integrated with silica-coated magnetic nanoparticles can provide both magnetic resonance imaging and targeted drug delivery to both MCF-7 and MCF-7/ADR cells, improving imaging while aiding in overcoming drug resistance.	[223, 224]
Diethyldithiocarbamate and zinc phthalo cyanine	Reverse phase evaporation	NIH 3T3 and MDA-MB 231 cells	When diethyldithiocarbamate and zinc phthalocyanine were co-encapsulated in liposomes, the medicines were more protected when exposed to non-tumor cells. When exposed to human breast cancer cells, diethyldithiocarbamate increased phototoxic activity. The stronger phototoxic impact of the simultaneous treatment was caused by the suppression of antioxidant enzymes induced by diethyldithiocarbamate, which resulted in an increase in ROS formation and, as a result, increased cell death.	Liposomes conjugated with gold nanorods can be utilized for both photoacoustic imaging and targeted drug delivery to both NIH3T3 and MDA-MB 231 cells, enhancing imaging and treatment precision.	[224, 225]
MCF-7	Thin film hydration	L929 and MCF-7 cells	The 1.2-dipalmitoyl-sn-glycero-3-phosphocholine liposome-based PEGylated multi-responsive microgels based on a water-soluble 2-(N-morpholino) ethyl methacrylate microgel system were not cytotoxic to L929 cells but were considerably effective to MCF-7 cells. 1.2-dipalmitoyl-sn-glycero-3-phosphocholine liposome-based PEGylated multi-responsive microgels based on a water-soluble 2-(N-morpholino) ethyl methacrylate microgel system that responds to stimuli such as pH, temperature, and ionic strength, which are critical for medicinal applications such as novel drug delivery systems.	Liposomes combined with semiconductor quantum dots can provide fluorescence imaging and targeted drug delivery to both L929 and MCF-7 cells. These quantum dots assist in both imaging and precision drug delivery.	[226, 261]

Abbreviation: TPGS*, d-α-tocopheryl polyethylene glycol 1000 succinate.

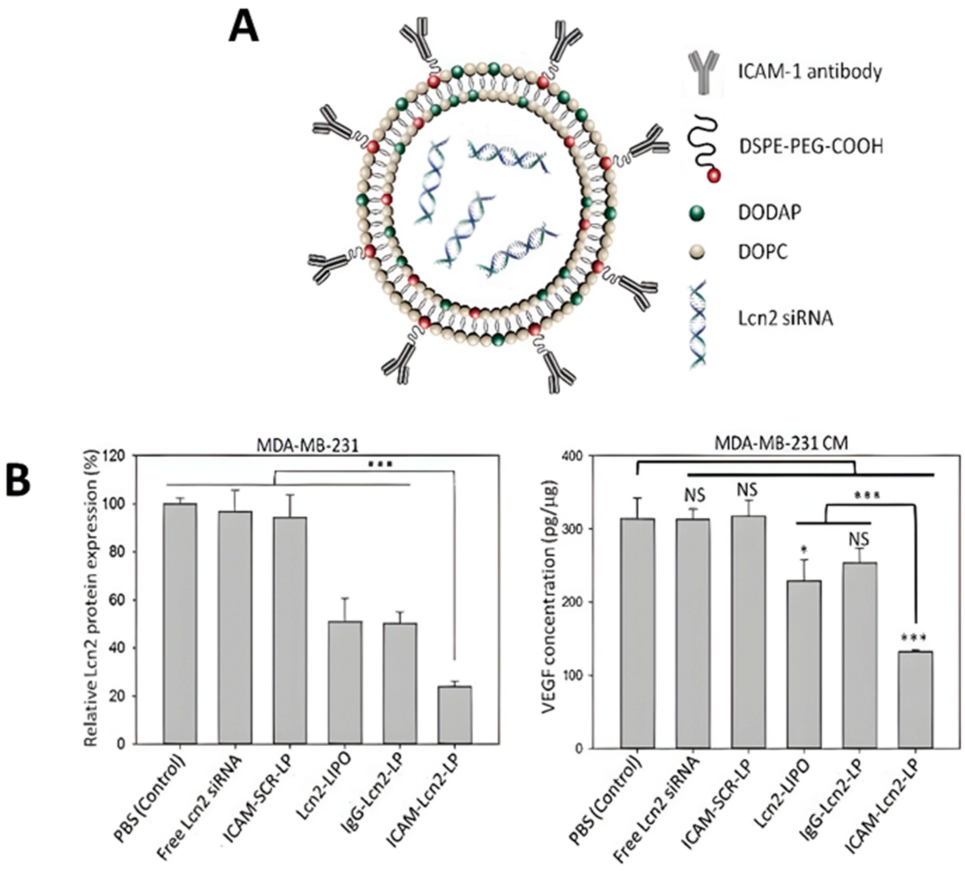

Figure 7 Example of ICAM-1 liposomal targeting for breast cancer. (**A**) Schematic of the Lnc2-encapsulating ICAM-1-functionalized liposomes (ICAM-1-Lnc2-LP). (**B**) Relative Lnc2 protein levels in MDA-MB-231 cells after Lnc2 gene knockdown by the ICAM-1-Lnc2-LPs, accompanied by VEGF concentration in the conditioned media (CM) collected from the knockdown MDA-MB-231 cells. ***: Very significant, P value < 0.001, *: Significant, P value 0.01 to 0.05, NS: Not significant, P value ≥ 0.05.
Notes: Reproducd from Nel J, Elkhoury K, Velot É, et al. Functionalized liposomes for targeted breast cancer drug delivery, *Bioactive Materials*, 24, 2023, 401-437. © 2022 The Authors. Publishing services by Elsevier B.V. on behalf of KeAi Communications Co. Ltd.[262]

siRNA to the cytoplasm (30,31). The 2 kDa PEG chain in DSPE-PEG-COOH demonstrated an ability to enhance liposome biocompatibility and circulation duration (32,33). The carboxyl group of DSPE-PEG-COOH serves as a site for conjugation with either the ICAM-1 antibody or the nonspecific immunoglobulin G (IgG). EDC/NHS chemistry was employed to covalently bond the carboxylic acid on DSPE-PEG-COOH to a primary amine group presented on the ICAM-1 antibody or the IgG. We examined the knockdown efficacy of ICAM-Lcn2-LPs by qRT-PCR. Lcn2 expression was measured after MDA-MB-231 cells were treated with PBS (control), free Lcn2 siRNA, ICAM-SCR-LPs, Lcn2-LIPO, IgG-Lcn2-LPs, and ICAM-Lcn2-LPs. As shown in Figure 7B, MDA-MB-231 cells treated with PBS (control), free Lcn2 siRNA and ICAM-SCR-LP demonstrated no change in their Lcn2 expression levels. Lcn2-LIPO and IgG-Lcn2-LP showed a reduction in Lcn2 of 41–56%. ICAM-Lcn2-LP was significantly more efficient than all other formulations, with a reduction in Lcn2 expression of 78.3 ± 1.7% (1.9-fold higher than IgG-Lcn2-LP). This was confirmed by immunoblot assays and densitometric analyses.

Combined qRT-PCR and immunoblot results indicated that engineered TNBC-targeted, siRNA-encapsulating immunoliposomes, significantly inhibited the expression of a specific molecular target in TNBC cells at both mRNA and protein levels. During angiogenesis, tumor cells release VEGF to promote new vessel growth, crucial for developing a blood supply supporting tumor growth and metastasis. Previous findings revealed that Lcn2 stimulates neovascularization in breast cancer, and silencing Lcn2 reduces VEGF production. In this study using specific ELISA for VEGF, ICAM-Lcn2-LP treatment significantly reduced VEGF by 58% in MDA-MB-231 cell conditioned media, compared to reductions of 27% and 19% with Lcn2-LIPO and IgG-Lcn2-LPs, respectively. No change in VEGF concentration occurred with free Lcn2 siRNA or ICAM-SCR-LPs treatment. This demonstrates that simultaneous targeting of overexpressed

ICAM-1 and silencing Lcn2 through ICAM-Lcn2-LP effectively suppresses VEGF secretion from MDA-MB-231 cells (Figure 7 and Table 7).

Lung Cancer

Lung cancer has become a major threat to human health, with an estimated 1.38 million cancer-related deaths in males and females in recent decades.[228] Non-small cell lung cancer (NSCLC) accounts for approximately 85% of all lung cancer diagnoses, with small cell lung cancer (SCLC) accounting for the remaining 15%.[229] Aside from initial lung carcinoma, lung metastases are likely to arise at a high incidence of 20–50% from other malignancies, such as colorectal cancer or breast cancer.[230] Lung metastases always appear as numerous lesions, limiting the efficacy of surgery or radiation treatment. Furthermore, because of widespread drug dispersion, conventional systemic chemotherapy has a poor clinical prognosis. As a result, a unique effective treatment for this lethal illness is urgently required.[231] Poor outcomes of lung cancer patients treated with traditional methods such as surgical resection, radiation, and chemotherapy have been reported.[232] The bulk of chemotherapy is administered intravenously, resulting in significant adverse effects owing to systemic drug distribution. Furthermore, first-pass metabolism typically reduces the bioavailability of orally administered anticancer drugs.[233] The cytotoxic effects of chemotherapeutic drugs against normal cells have been documented using dose response effects, resulting in patient frailty and mortality.[233] As a result, scientific studies have focused on the targeted delivery of anticancer medicines.

The treatment of non-small cell lung cancer can be improved by using a targeted administration of chemotherapeutic agents to inhibit the key signaling pathways implicated in lung cancer. Then, by delivering anticancer medicines directly into the lungs, they can accumulate in tumor cells while reducing unwanted side effects.[234] Price et al reported the combination of cationic liposomal hydroxycamptothecin (CLH) and 5-aminolevulinic acid (5- ALA) administration via intratracheal (i.t.) administration for the chemo-sonodynamic therapy of metastatic lung cancer. Hydroxycamptothecin and a lipid combination of soybean lecithin/cholesterol/octadecylamine were used to make cationic liposomal hydroxycamptothecin with a film technique. For sonosensitizer accumulation, ie, protoporphyrin IX, the metabolite of 5-ALA, an optimal pre-incubation period of 5-ALA with tumor cells before ultrasonic exposure was found at 4 h. In vitro investigations revealed that chemo-sonodynamic therapy had greater cytotoxicity than other therapies such as intratracheal cationic liposomal hydroxycamptothecin, intravenous cationic liposomal hydroxycamptothecin, and sonodynamic therapy alone.

The combination of pulmonary administration with chemo-sonodynamic therapy had the greatest anticancer impact on metastatic lung tumor-bearing mice, as determined by tumor appearance and pathological sections.[231] Chemo-sonodynamic treatment via primary anticancer mechanisms improved apoptosis of cancer cells and increased the generation of ROS, as well as the combination of chemotherapy and sonodynamic therapy.[231] A potential method for treating lung cancer is the pulmonary administration of chemotherapeutics and sonosensitizers.[231]

A drug delivery system (DDS) overcomes the limitations of liposomes and polymeric nanoparticles by introducing hybrid NPs. These self-assembled nanoscaled vehicles (<1000 nm) offer multiple benefits in cancer treatment, including enhanced sustained release, targeted delivery, biocompatibility, prolonged circulation time, and efficient surface modifications with ligands. Hybrid NPs consist of three primary components: (i) a hydrophobic polymeric core incorporating lipophilic drugs, (ii) a lipid layer serving as a biocompatible shell and enhancing drug retention within the polymeric core, and (iii) a hydrophilic PEG stealth layer surrounding the lipid shell. The lipid-PEG shell is crucial for enhancing stability, and PEG offers functional groups for additional modification with targeting ligands.[25,75] Figure 8 visually depicts the targeting of tumor cells using such hybrid NPs in the context of nanoparticulate formulations designed for lung cancer therapy.[229]

Polymeric materials (such as PLGA, dextran, albumin, and PCL) are commonly utilized as the core of hybrid NPs due to their non-toxic and biodegradable nature. The lipid shell of these NPs is typically composed of cationic, anionic, or neutral phospholipids.[76] While small interference RNA (siRNA) is a crucial advancement in cancer diagnosis and treatment, its lack of specific targeting due to instability and insufficient bio distribution is a challenge.[8,77] To address this, hybrid NPs have been investigated for delivering siRNA, employing PEGylated polyethyleneimine (PEI) with an Arg-Gly-Asp peptide ligand to inhibit vascular endothelial growth factor receptor-2. This approach enables tissue-specific and gene pathway-specific targeting of siRNA.[78] In a study by Lakshmikuttyamma et al (2014), hybrid NPs were employed to deliver Kirsten rat

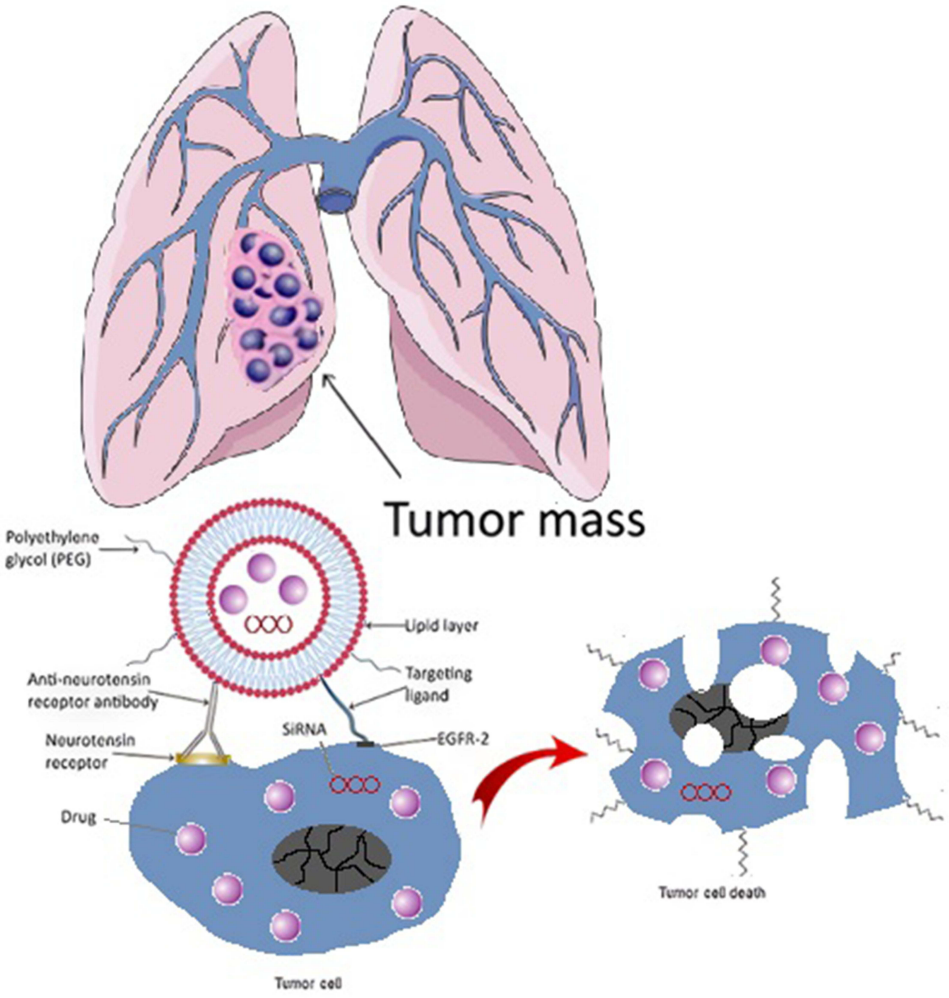

Figure 8 A schematic illustrating the targeting of tumor cells with hybrid NPs for lung cancer therapy.

sarcoma (KRAS) siRNA to A549 lung adenocarcinoma cells. Human IgG antibodies were also attached to the NPs to prevent immune activation associated with most NPs. The siRNA was efficiently delivered to the mutated KRAS cell line without being captured by the RES and without immune response activation.[79] Furthermore, the overexpression of neurotensin receptor 1 (NTSR1) in most non-small cell lung cancers (NSCLC) was targeted by modifying hybrid NPs with an anti-NTSR1 monoclonal antibody, facilitating the efficient delivery of anti-mutant KRAS siRNA to NTSR1-overexpressing tumor cells.[80] Table 8 summarizes the key experimental outcomes of liposomes.

Prostate Cancer

Prostate cancer (PC) has been the most frequently diagnosed cancer and the second leading cause of cancer death globally.[254,255] Anti-androgenic medications are beneficial in hormone-dependent prostate cancer, but tumors develop a hormone-refractory phenotype that is resistant to chemotherapy. This stresses the significance of establishing innovative therapeutic techniques for the treatment of PC.[256] Cancer stem cells (CSCs) initiate tumors, undergo epithelial-mesenchymal transition, and develop chemo resistance, culminating in metastatic dissemination.[257] As a result, for improving the treatment, a combination pharmacotherapy is recommended to target cancer stem cells with traditional cytotoxic agents capable of effectively eradicating cancer stem cells and bulk tumor cells at the same time,[258] and could potentially be applied to the field of cancer stem cells. Aside from delaying or suppressing cancer adaptability, mutation, and development, a combination therapy decreases individual medicine dosage, resulting in fewer adverse effects.[259,260] Liposomes are an excellent candidate for combinational chemotherapy because of their

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Table 8 Summary of Liposome Experiments Results for Lung Cancer

Active Agent	Method	Cell Line	Comment	Theranostic Agents	References
Telmisartan and Docetaxel	Modified hydration	NSCLC and H-460 WT cells	Docetaxel liposomes combined with Telmisartan were effective in 3D cultures of H460 cells and a xenograft model of Docetaxel-resistant lung cancers. The combination increased cytotoxicity in H460 WT 3D cells, enhanced liposomal absorption, reduced tumor volume, promoted apoptosis, and downregulated cancer stem cell markers in xenograft mice.	Gold nanoparticles conjugated with liposomes offer precise imaging due to their strong scattering and absorption of light in the near-infrared region with superior targeting in NSCLC & H-460 WT, provide precise imaging and drug delivery due to their exceptional surface plasmon resonance, allow for enhanced detection and therapeutic precision.	[235, 236]
PTX	Thin film hydration	A549 cells	A live drug-loaded carrier, PTX-in-liposome-in-bacteria (LPB), was developed for inhaling lung cancer treatment. Liposomal PTX (LP) was efficiently internalized into bacteria (E. coli or L. casei) to create LP-in-E. coli (LP E) or LP-in-L. casei (LP L) using electroporation without affecting bacterial growth. These drug-loaded bacteria deliver cargos into cells faster than other methods. Intratracheal injection of LP E had the most significant anticancer effect, downregulating VEGF and HIF-1, increasing cancer cell death, and boosting immune markers and cells.	Iron oxide nanoparticles with superparamagnetic properties conjugated with liposomes offer enhanced imaging capabilities in A549 cells, providing precise targeting and imaging through their magnetic properties, aiding in MRI for tumor localization and by binding to complementary receptors or antigens enhance the treatment efficacy.	[237, 238]
Afatinib	Thin film hydration	1H-1975 and HCC-827 cells	Cationic and pH-sensitive liposomes were created for Afatinib, a tyrosine kinase inhibitor, to enhance tumor targeting and efficacy against non-small cell lung cancer. In vitro studies showed prolonged drug release at pH 7.5 and rapid release at acidic pH 5.5, improving tumor targeting. These liposomes induced apoptosis in lung cancer cells (H-1975) and had a strong anticancer effect on both H-1975 and HCC-827 cells.	Quantum dots, renowned for their superior optical properties like broad absorption and narrow emission spectra, can be conjugated with liposomes for advanced imaging and targeting in 1H-1975 and HCC-827 with multicolor imaging for cellular visualization. Their size-tunable emission and high photostability enable precise and detailed imaging at the cellular level, aiding in both diagnostics and targeted drug delivery. Quantum dots allowed for multimodal imaging and therapy, providing a comprehensive view of the disease site and aiding in highly specific treatment approaches.	[228, 239]]
Folic acid and Docetaxel	Thin film hydration	A549 and SPCA1 cells	Docetaxel-loaded folic acid conjugated liposome physicochemical properties and pharmacokinetic behavior were compared to dry powder produced by co-spray-drying docetaxel-loaded folic acid conjugated liposomes. The particle size and particle density index (PDI) both increased. The re-dispersed liposomes enhanced cellular absorption by micropinocytosis and cytotoxicity compared to docetaxel-loaded folic acid linked liposomes.	Silicon nanoparticles, known for their biocompatibility and tunable properties, can be conjugated with liposomes for improved imaging and precise targeting in A549 and SPCA1 cells. Their flexible surface chemistry allows for specific functionalization, enhancing their attachment to the liposomal surface for highly targeted imaging and therapy. These nanoparticles assist in highly detailed and accurate imaging, enabling a refined and precise targeting approach for therapeutic intervention.	[240, 241]

PTX and pegylated	Thin film hydration	A549 and LL2 cells	PTX was contained in two forms of cationic liposomes, MultiLamellar and Small Unilamellar. In mice, MultiLamellar and Small Unilamellar liposomes with a polyethylene glycol (PEG) shell reduced tumor volume more than PTX (56.4 and 57.1% vs 36.7%). Interestingly, neither MultiLamellar-PEG-PTX nor Small Unilamellar- PEG-PTX induced mechanical or heat hypersensitivity, but free PTX did. In vivo studies employing induced tumor mice models revealed that MultiLamellar-PEG-PTX and Small Unilamellar-PEG-PTX may dramatically reduce tumor volume when compared to free PTX.	Gadolinium nanoparticles, known for their excellent MRI contrast enhancement, can be conjugated with liposomes with high relaxivity and strong paramagnetism for enhanced imaging and precise targeting for A549 and LL2 cells. Their high relaxivity allows for superior imaging contrast, aiding in the accurate delineation of tumor boundaries. The attachment to liposomes facilitates targeted drug delivery, ensuring high drug accumulation in the tumor site for effective therapeutic outcomes.	[242, 243]
Hyaluronic acid	Thin film hydration	BEAS- 2B, A549, CI-H1385, NCI- 1975, NCI-1650, NCI- H228 and Calu-3 cells	PEG-phospholipid conjugated with HA oligosaccharides of different sizes (DP4, DP6, and DP8) were used to create decorated liposomes. These decorated liposomes were taken up greatly (12 to 14-fold) in lung cancer cells with high CD44 expression, indicating receptor-mediated entry. HA-DP8 liposomes were taken up the fastest, followed by HA-DP6, and then HA-DP4 liposomes. They showed strong anticancer efficacy without cytotoxicity or inflammatory effects.	Carbon nanotubes, recognized for their high aspect ratio and unique electronic and mechanical properties, can be conjugated with liposomes for advanced imaging and precise targeting in human mesothelial and multiple cells and bronchial epithelial cells. Their exceptional properties enable multifunctionality for both imaging and therapy, allowing for highly precise and detailed imaging while aiding in targeted drug delivery for effective therapeutic interventions.	[244, 245]
PTX and Vinorelbine	Thin film-hydration	A549 and H1299 NSCLC cells	Folate-targeted, co-drug encapsulated, radiolabeled liposomes were developed for lung cancer detection and treatment. They had an appropriate particle size, EE, and zeta potential. Actively targeted liposomes absorbed better on H1299 cells. These liposomes co-delivered PTX and surviving siRNA to enhance treatment without toxicity. In vitro tests on NCI-H460 lung cancer cells showed that L-PTX-PSur had the highest cellular uptake, lowest cell viability, and strongest apoptosis. It also reduced protein expression.	Silver nanoparticles, known for their optical properties and biocompatibility, can be conjugated with liposomes for improved imaging and precise targeting in A549 & H1299 NSCLC cells. Their excellent optical properties enable enhanced imaging, aiding in accurate diagnostics, while their attachment to liposomes facilitated targeted drug delivery, ensuring high drug accumulation in the tumor site for effective therapeutic outcomes.	[246, 247]
Erlotinib, bovine serum albumin and zinc(II) phthalocyanine	Ethanol injection	A549 cells	Erlotinib and zinc (II) phthalocyanine liposomes were effectively synthesized and coated with bovine serum albumin. Phototoxicity of zinc (II) phthalocyanine on plasmid DNA was observed under light irradiation, particularly in the presence of H_2O_2. Liposomes containing zinc (II) phthalocyanine were more cytotoxic to A549 cells than liposomes containing erlotinib. Light irradiation enhanced the cytotoxic impact of zinc (II) phthalocyanine loaded liposomes. LP stability improved for 60 days at 4°C by covering with bovine serum albumin.	Polymer nanoparticles, due to their versatile surface chemistry and biocompatibility, can be conjugated with liposomes for advanced imaging and precise targeting in A549. Their adaptable surface properties enable functionalization for the precise attachment to liposomes, facilitating highly accurate imaging while aiding in targeted drug delivery for effective therapeutic outcomes.	[249, 250]

(*Continued*)

Table 8 (Continued).

Active Agent	Method	Cell Line	Comment	Theranostic Agents	References
Curcumin	Freeze-Thaw	BEAS-2B and A549 cells	Curcumin's anti-lung cancer mechanisms show that it has powerful anti-oxidative and anti- inflammatory effects, as well as improves apoptosis caused by curcumin, all contributing considerably to its high anticancer impact. Curcumin liposome absorption by human lung cancer A549 cells was significantly higher and quicker than free curcumin.	Liposomes may not require additional nanoparticles for advanced imaging and precise targeting in BEAS-2B and A549 cells due to curcumin's inherent properties. Curcumin exhibits remarkable anti-inflammatory and antioxidative effects, aiding in the prevention of cancer progression. It also facilitates efficient drug delivery, allowing for targeted and effective therapeutic intervention without needing additional nanoparticles.	[250, 251]
Paclitaxel	Lipid film hydration and ultrasound technique	A549/T cells	Liposomes modified with HA/TT targeted mitochondria. They had a size of 153 nm, a zeta potential of −30.3 mV, and 92.1% EE. HA-coated liposomes were stable and safe. HA improved PTX uptake in drug-resistant cells through CD44 receptor-mediated endocytosis and enhanced mitochondria-targeted PTX delivery. This disrupted cell function, increased ROS, decreased ATP, disrupted MMP, caused G2/M phase arrest, and boosted apoptosis for stronger anticancer effects.	PLGA nanoparticles have been conjugated with Paclitaxel through surface modification or encapsulation. The Paclitaxel-conjugated nanoparticles can then be then linked with liposomes, creating a stable connection. This strategy optimizes drug attachment, controlled release, and targeted delivery to A549/T cells. The combined therapeutic and diagnostic functions in this nanotheranostic platform enhance its efficacy for cancer treatment.	[252, 281]

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capacity to carry a wide range of medicines, high surface-to-volume ratios, and adjustable surfaces for targeting.[227] Vyxeos™ (daunorubicin and cytarabine) is a liposomal injectable authorized by the FDA for the combinatorial treatment for acute myeloid leukemia.[261] This opens the door to future effective and safe cancer treatments provided by nanomedicine-based synergistic medication combinations.[263]

Kroon et al showed that the increased expression of COX-2 and Glut-1 proteins is key in the initiation and progression of prostate cancer via altering related signaling pathways.[264] The combined action of these medicines causes prostate cancer cells to be more selectively induced to apoptosis than normal fibroblast cells. According to a mechanistic study, the major mechanisms behind the inhibition of prostate cancer cells include increased reactive oxygen species (ROS) production and a reduction in cellular glutathione concentration, as well as inhibition of COX-2 synthesis and Glut-1 receptors. Although the combination of celecoxib and genistein reduced prostate cancer cell growth by up to 90%, there was no significant damage to normal fibroblast cells, implying that perhaps the dosage of genistein and celecoxib required to eliminate prostate cancer cells seems to be non-toxic to healthy cells. A nanoliposomal formulation of celecoxib and genistein was shown to produce ROS, significantly decrease cellular glutathione concentration, and inhibit glucose uptake. When these events occur together, they successfully limit the growth of prostate cancer cells. Although the created nano-liposomes demonstrated promising in vitro results and thus have the possibility to be further improved for cancer therapeutic applications, more extensive research is needed to realize the full potential of this composition or the treatment of prostate and other types of cancer. Table 9 presents various liposomal medication delivery methods for prostate cancer.

Colorectal Cancer

In normal settings, human cells multiply and divide to generate new cells as the body needs. When cells get old or injured, they die, and new cells replace them.[282] Cancer is caused by the failure of this mechanism. Cancer is a condition in which some of the body's cells proliferate and spread into the tissues around them.[283] There are trillions of cells in the human body. As a result, cancer can begin everywhere in the body. When colorectal cancer is in its early stages, there may be no symptoms. In certain situations, diarrhea, constipation, blood in the stool, rectum bleeding, severe gas, stomach cramps and abdominal discomfort may be symptoms of colon cancer (CC). In the last 25 years, much progress has been achieved in understanding the molecular and biological aspects as well as stages connected with colon carcinogenesis. It has resulted in more reasonable and successful therapeutic approaches to colorectal cancer (CRC) therapy.[284] Traditional adenomas along with the traditional adenoma-to-carcinoma sequence and serrated adenomas via two different routes are both precursor polyps for CC.[285] The progression to CC is a multistep process; typical adenomas are caused by mutations in the APC gene. Serrated adenomas have an unusual main genetic abnormality.[286] Liposomes have become the most commonly employed nanocarriers for targeted medication delivery for CC.[287] Liposomes have several advantages, including biodegradability, biocompatibility, low toxicity, and the ability to entrap both lipophilic and hydrophilic medicines.[288,289] However, because hydrophilic medicines are more soluble in water and dissolve in the aqueous layer of liposome synthesis, formulation design and processing of hydrophilic pharmaceutical enclosures into liposomes is a significant challenge.[290]

Lip-F1 (non-PEGylated liposomes) and Lip-F2 (PEGylated liposomes) substances were created for in vivo as well as in vitro investigations, with interferon-gamma (IFN-) included measuring the impact on antitumor and macrophage activities. The liposomal substances LIP-F1 and LIP-F2 were 120 and 135 nm in size. LIP-F1 and LIP-F2 efficiency was 52.79 and 49.2%, respectively. These findings demonstrated that treatment reactions act as a moderator. LIP-F1 and LIP-F2 efficiency was 52.79 and 49.2%. These results indicated that the treatment reactions influenced by IFN-liposomes in the CRC animal studies were related directly to the lethal effects of IFN-liposomes on a C26 malignant cell line, which corresponded with the polarization of TAMs to exhibit antitumoral activity. IFN-produced PEGylated liposomes showed significant anticancer efficacy leading to enhanced drug delivery to the immune system and antitumor immune responses.[291] The goal of the study was to create and design 5-FU, including using tailored liposomes, to increase the drug's effectiveness and safety and to employ folic acid as a target ligand. CT26, HT-29, HeLa, Caco-2, and MCF 7 cell lines were tested in vitro for cytotoxicity from the formulations using the MTT assay; results showed that the targeted liposomes caused cell death via ROS. After giving the medication and the targeted 5-FU liposome, inhibition tests were performed, revealing that the optimized formulation's EE was 39.71%. The liposomes had a particle size of 174 nm, in spherical form, and a Differential Scanning Calorimetry (DSC) study demonstrated that the drug was present in the

Table 9 Summary of Liposome Experimental Results for Prostate Cancer

Active Agent	Method	Cell Line	Comment	Theranostic Nanoparticles	References
Cabazitaxel and Silibinin	Ethanol injection method	PC-3 and DU-145 cells	1,2-dipalmitoyl-sn-glycero-3-phosphocholine liposome-based PEGylated multi-responsive microgels based on a water-soluble 2-(N-morpholino) ethyl methacrylate microgel system were not harmful to the L929 cell line but were considerably effective at killing the MCF-7 cell line.	Iron oxide (Fe_3O_4) coated with liposomes that have surface ligands specifically attracted to receptors on PC-3 and DU-145 cells for MRI imaging and targeting.	[265, 266]
Docetaxel- loaded liposomes functionalized with transferrin	Thin film hydration	PC3 and PNT2 cells	Prostate cancer was examined using docetaxel-loaded liposomes functionalized with transferrin (LIP- DTX-TF). For liposomes with and without functionalization, DTX EE was about 69 and 37%, respectively. With TF, the functionalization efficiency was 31%. TF integrity was unaffected by the functionalization method. Docetaxel encapsulated in liposomes showed a delayed and sustained release in LIP-DTX and LIP-DTX-TF (51.70% and 31.97%, respectively). LIP-DTX-TF was shown to be more harmful to PC-3 cells than the commercial formulation in in vitro cytotoxicity assays on PC-3 and PNT2 cell lines.	Gold nanoparticles coupled with liposomes that attach specifically to PC3 and PNT2 cell surface markers for CT imaging.	[267, 268]
Sirolimus	Thin film hydration	LNCaP and DU145 P cells	The current work reveals the anti-proliferative impact of different blank and sirolimus-loaded LP formulations on both LNCaP and DU145 prostate cancer cells. In comparison to DU145, the produced LP nanoparticles had a greater anti- proliferative impact on LNCaP cell lines. LP nanoparticles containing dipalmitoyl-phosphatidylcho-line outperformed both traditional and stealth formulations regardless of cell line.	Quantum dots incorporated into liposomes bond with specific antigens expressed on LNCaP and DU145 cells for fluorescent real-time imaging and treatment response.	[269, 270]

Oleuropein loaded surface functionalized folate- targeted – PEG	Thin film hydration	22Rv1	The effects of oleuropein (OL) loaded surface functionalized folate – PEG liposomes (OL-FML) on 22Rv1 prostate cancer cells were studied and compared to a plain oleuropein solution. Phosphatidyl-serine externalization tests, TUNEL assays, mitochondrial membrane potential studies, and caspase-3 activation assays were used to investigate cell viability and apoptosis. OL-FML demonstrated a higher anti-proliferative effect and promoted apoptosis in 22Rv1 cells. In vivo pharmacokinetic experiments in mice revealed that OL-FML (AUC0 = 641.78,103.764 g/mL•hr) had a nearly 6-fold higher bioavailability than the OL solution (AUC0 = 104.11 18.374 g/mL•hr). In 22Rv1 tumor-bearing mice, OL-FML treatment resulted in enhanced tumor suppression, resistance to weight loss, and survival probability when compared to OL.	Carbon nanotubes linked with liposomes specifically bind to receptors overexpressed on 22Rv1 cells for NIR tumor imaging.	[271, 272]
P21 activated kinases-1 (PAK-1)	Thin film hydration	PC-3, LNCaP, and DU- 145	The demonstration and characterization of a new SSL-based nanoparticle formulation that is relatively stable and has outstanding effectiveness in vitro and in vivo. These findings showed that SSL- IPA-3 is a successful targeting method for reducing the growth of prostate cancer. The findings of this study further showed that IPA-3 action is cell dependent and is regulated by the amount of PAK-1 expression in cancer cells.	Silicon nanoparticles attached to liposomes bind to PAK-1 expressing receptors on PC-3, LNCaP, and DU-145 cells for photoacoustic properties and providing deep tissue imaging.	[273]
Liposomal 6BrCaQ*with DOX	Thin film hydration	PC-3 cells	The incorporation of this medication into liposomes addresses the problem of its poor solubility, facilitating the interpretation of in vitro studies. It was demonstrated that when this chemical is given via PEGylated liposomes, it does not cause a stress response that can lead to drug resistance.	Lipid nanoparticles encapsulated within liposomes specifically attach to receptors prevalent on PC-3 cells for dual drug delivery and imaging.	[274, 275]

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Table 9 (Continued).

Active Agent	Method	Cell Line	Comment	Theranostic Nanoparticles	References
Dexamethasone	Ethanol injection method	PC-3M-Pro4luc	Intravenously administered liposomes rapidly localize to bone metastases in vivo, and liposomal dexamethasone therapy of existing bone metastases resulted in a significant tumor growth decrease up to 26 days after treatment began. The favorable effects of DEX in a liposomal formulation are more likely to be mediated by the supportive tumor microenvironment.	Polymeric nanoparticles linked with liposomes specifically adhere to bone metastases on PC-3M-Pro4luc cells for enhanced bone-targeted drug delivery and imaging.	[276, 277]
1,2-distearoyl-sn-glycero- 3-phosphoethanolamine- N-methoxy (polyethylene glycol) (DSPE- mPEG2000), 1.2-distearoyl-sn-glycero-3- phosphoethanolamine-N- maleimide (polyethylene glycol) (DSPE-PEG2000-MAL), 1.2-distearoyl- sn-glycero-3-phosphoethanolamine-N- amino (polyethylene glycol) (DSPE-PEG5000-NH_2), folate and dicyclohexylcarbod-iimide	Thin film hydra-tion	PC-3 cells	A LP dual-modified by incorporating prostate-specific antigen-responsive and mediated liposomes may provide twofold selectivity for prostate cancer. Dual-modified (DM) liposomes containing small interfering RNA (siRNA) outperform control liposomes, including single-modified and non-modified liposomes. Cellular absorption was boosted by DMLPs, but PLK-1 expression was lowered and cell death was raised. DMLPs entered 22Rv1 cells by a variety of endocytic routes, including clathrin-mediated endocytosis and macropinocyto-sis, and were then effectively endocytosed. DMLPs were able to enter 22Rv1 cells by a variety of endocytic pathways.	Integrated with liposomes and bound to markers expressed on PC-3 cells for MRI and magnetic resonance targeting.	[278, 279]
Docetaxel	Hydra-tion of the lipid film	PC3 DU145 cells	The drug encapsulation process was successful, reducing drug crystallinity and enhancing drug release in acidic conditions. Conjugation with an anti-EGFR antibody maintained physicochemi-cal properties. Liposomes with DTX had an IC50 value of 65.74 nM in PC3 cells and 28.28 nM in DU145 cells. Immunoliposomes had even higher cytotoxicity with IC50 values of 152.1 nM for PC3 cells and 12.60 nM for DU145 cells, and were more rapidly internalized in cells with high EGFR overexpression. These findings demonstrate effective nanometric formulations with significant DTX encapsulation, leading to reduced viability in EGFR-overexpressing prostate cells.	Gold nanoparticles (GNPs) are surface-modified with ligands and conjugated with liposomes, which are further functionalized with targeting molecules for specific interaction with PC3 and DU145 prostate cancer cells. This hybrid nanosystem encapsulates Docetaxel within the liposomes, enabling targeted drug delivery, while the GNPs serve as imaging agents, allowing for real-time monitoring and diagnostics of the therapeutic response in the prostate cancer cell lines. The successful conjugation ensures a comprehensive theranostic approach, combining targeted drug delivery with imaging capabilities for enhanced efficacy in prostate cancer therapy.	[280, 297]

Abbreviation: 6BrCaQ*, 6-Bromo-3-[4-methoxyphenylcarboxamide]-quinoline-2-one.

amorphous state in liposomes. MTT findings showed that the targeted liposomes were more cytotoxic than 5-FU and liposomal 5-FU. In vivo, folate liposomal 5-FU suppressed tumors more effectively than the free medicine and control groups ($p < 0.05$). Please note that the control groups were the groups that received either free 5-FU and no treatment at all. Furthermore, as compared to the control group, the folate-liposomal 5-FU therapy group showed lower cell density in tumor tissue. As a result, folic acid-targeted liposomes might be the next drug carrier for selective drug delivery in CC cells.[292] The benefits of using nano- drug delivery systems include increased bioavailability by decreasing the dose and targeting the target cells with anticancer medications to decrease adverse effects. 5-FU in NPs provided an appropriate and safe treatment for CC with decreased side effects and dosage.[293] In vitro, folate- targeted liposomal 5-FU enhanced the absorption in B16F10 cells 11 times more than non-targeted liposomes; folate-liposomal 5-FU had a greater tumor inhibitory impact than free 5-FU.[294] A mouse model was used to detect ROS by accumulating liposomes in wounded colon regions, administering FL-labeled liposomes, and examining luminescence. Table 10 summarizes the applications of liposome medication delivery for colorectal cancer.

This passage discusses the use of liposomes as nanocarriers for the co-delivery of hydrophilic (OHP) and lipophilic (CUR) drugs, with a focus on enhancing their effectiveness through active targeting. Liposomes are lipid-based structures that can release drugs synchronously, reduce drug accumulation in tumors, and minimize toxicity to non-cancerous cells.[10,11] The hydrophilic core and lipid bilayer of liposomes make them suitable for encapsulating both types of drugs. Active targeting is employed to enhance the effectiveness of the liposomal nanocarriers by exploiting interactions between receptors on cancer cell surfaces and targeting groups on liposomes. Hyaluronic acid (HA) has been introduced as a targeting ligand due to its interaction with overexpressed HA receptors (CD44 and RHAMM) on cancer cells, especially in colorectal carcinoma. To address the potential degradation of liposomes in the gastrointestinal tract (GIT), the study proposed entrapping liposomes in alginate beads. These beads, coated with pH-sensitive polymer eudragit S-100 (ES-100), remained intact in the upper GIT and reached the colon. Upon dissolution of the coating in the ileocecal region, uncoated alginate beads entered the colon and underwent biodegradation, releasing surface-modified liposomes. These HA-conjugated liposomes have a higher affinity for HA receptors on colon cancer cells, achieving cell-specific targeting. The study's objective was to develop HA-anchored liposomes co-loaded with OHP and CUR, entrapped in ES-100 coated alginate beads, for specific delivery to colon cancer cells. The therapeutic efficacy and biocompatibility of these co-loaded liposomes were assessed using in vitro cytotoxic activity on OHP-resistant HT-29 cancer cell lines. Figure 9 shows a schematic of colon-specific targeting of eudragit coated bead encapsulating liposomes.[290,308]

Liposomal Formulations in the Clinic

Due to their appropriate size, biocompatibility, biodegradability, low toxicity, and immunogenicity, liposomes have proven to be one of the most mature nanomedicine platforms currently used in clinical settings.[309] Table 11 presents comprehensive information about different liposomal products, including details about their liposome composition, intended indications, and the preferred route of administration for clinical trials. Several of these liposomes have even been approved by the FDA for the treatment of cancer. Furthermore, the protective function of the liposomal encapsulation can lessen negative reactions, improve absorption, and ultimately improve the therapeutic impact of medications.

Future Research and Development

The future of liposomal nanomedicine is set to undergo transformative advancements, particularly through the incorporation of immunotherapeutic agents. Researchers are exploring the potential of liposomes not just as passive drug carriers but as active "immuno-modulatory hubs" capable of delivering antigens, adjuvants, and gene-editing tools. This paradigm shift in therapeutic approaches aims to evoke and regulate precise immune responses against cancer and infectious diseases. In addition to their therapeutic functions, these innovative liposomes are envisioned to possess theranostic capabilities, enabling simultaneous treatment and monitoring of disease progression. By engineering liposomes to track their accumulation in specific tissues and provide real-time imaging of immune cell dynamics, personalized medicine can be significantly enhanced. Furthermore, the development of "smart" liposomes that respond dynamically to the disease microenvironment holds immense promise. These liposomes could trigger drug release or immune activation based on specific environmental cues, thereby improving treatment precision and efficacy. The

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Table 10 Summary of Liposome Experimental Results for Colorectal Cancer

Active agent	Method	Cell Line	Comment	Theranostic Nanoparticles	References
Folic acid, 5-FU and phosphatidyl choline	Thin-film hydration	HT-29, Caco-2 HeLa, MCF and fibro-blast cells	The optimal conditions for the creation of 5-FU liposomes were PC:cholesterol ratio (2:1) and the quantity of medication (1.5 mg). The EE percent and particle size were 60.79 and 104 nm, respectively.	Nanoparticles named "Nanofol" coated with folate receptors have the potential for conjugation with liposomes. Folate on the liposome surface could bind with the overexpressed folate receptors in colorectal cancer cells, enhancing targeting and imaging capabilities. Molecular binding involved surface functionalization of liposomes with folic acid molecules for specific receptor interactions.	[292, 295]
PEGylated and DOX	Thin lipid film	C-26 cells	The capacity of cyclic Arg-Gly-Asp-PEGylated liposomal DOX to internalize into integrin-expressing HUVEC cells via receptor-mediated endocytosis was proven in vitro by liposome- cell interactions and cytotoxicity tests. The biodistribution experiments demonstrated that reducing the hydrophilicity of the peptide significantly lowered the blood clearance rate of cyclic Arg-Gly-Asp-PEGylated liposomal DOX and promoted its localization in the C-26 colon cancer tumor model.	Non-toxic, non-immunogenic polymers known as " PEGylated " coated with PEGylated ligands can be utilized. These nanoparticles can be conjugated with liposomes through surface PEGylation, facilitating prolonged circulation and tumor-specific accumulation. The binding process involves incorporating PEGylated ligands on the liposomal surface, enhancing the targeting effect towards tumors in colorectal cancer.	[281, 296]
Miltefosine, Pegylated and DOX	Thin lipid film	C26, MCF-7-ADR and B16F0 cells	Adding up to 2% molar ratio of HePC to Doxil had no effect on the particle size distribution or Dox release rate of the liposomes, but it greatly enhanced Dox absorption and toxicity in vitro, in vivo plasma clearance, and treatment in particular circumstances. Despite the fact that HePC 4% – PLDs varied from Doxil in practically every way, it was unable to outperform Doxil in terms of therapy efficiency due to its weak therapeutic impact and significant side effects.	Utilization of "MiltiNano" nanoparticles, which are PEGylated miltefosine-based nanoparticles, can be employed in conjunction with liposomes. These nanoparticles can be conjugated with liposomes by incorporating PEGylated miltefosine components onto the liposomal surface, enhancing the targeting and therapeutic efficacy for colorectal cancer. Molecular binding involves integrating PEGylated miltefosine moieties on the liposome, aiding in specific targeting towards the tumor microenvironment.	[297, 298]
PEGylated and DOX		BALB/c and C-26 cells	In comparison to Caelyx, successful post-insertion of LP31 as targeting ligands into the lipid bilayer of Caelyx had no influence on the physicochemical properties or release rate of the targeted formulations. The superiority of LP31 targeted formulations over Caelyx in terms of cell toxicity, absorption, and LP 31-Caelyx cell interactions was remarkable.	Nanoparticles bearing LP31 ligands can be utilized for conjugation with liposomes. These nanoparticles can be attached to liposomes by incorporating LP31 ligands onto the liposomal surface, enhancing specific interactions with colorectal cancer cells. The molecular binding process involves integrating LP31 ligands on the liposome surface, promoting cell-specific interactions for improved targeting and imaging capabilities.	[286, 299]

DOX 5-FU and Chitosomes	Thin lipid film	Caco-2 cells	The toxicity of DOX encapsulated in numerous modified liposomes was improved. Fluorouracil Chitosome in vivo HPLC analysis, physicochemical characterization, EE and drug loading and unloaded formulations and PBS did not exhibit substantial cytotoxicity, and >90% of cells were not killed. The chitosomes had a high EE and DL percentage, as well as strong physical stability and long-term 5-FU release.	Chitosan-based nanoparticles named "Chitosan Nano" can be used in conjunction with liposomes. These nanoparticles can be conjugated with liposomes through surface modification with chitosan components, augmenting the specific targeting and imaging capabilities in colorectal cancer. Molecular binding involves the incorporation of chitosan moieties on the liposomal surface, aiding in the enhancement of interactions with the tumor microenvironment.	[300, 301]
PEGylated (HSPC/DSPG/Chol, LIP single bondF1) PEGylated (HSPC/ DSPG/Chol/ mPEG2000– DSPE, LIP single bond F2) and Interferon- gamma	Thin film hydration	C26 cells	The sizes of the liposomal formulations LIP-F1 and LIP-F2 were 120 and 135 nm, respectively. LIP-F1 and LIP-F2 encapsulation efficiencies were 52.79 and 49.2%, respectively.	Lipo Nano" nanoparticles, derived from liposomes, can be utilized for conjugation with liposomes. These nanoparticles are an offshoot of liposomal structures and can be conjugated with liposomes through surface modification. Molecular binding involves the integration of these specialized LipoNano structures onto the liposomal surface, improving the targeting and imaging capacities specific to colorectal cancer.	[291, 302]
5-FU and PEGylated	Thin film hydration	HCT-116 cells	PH-sensitive PEGylated liposomal-5-FU had a mean size of 164.3 8.4 nm and an EE of 54.17%. While the cytotoxicity of 5-FU and 5-FU-loaded pH-sensitive PEGylated liposomal was dose-dependent, 5-FU-loaded pH- sensitive PEGylated liposomal was shown to be more effective against HCT-116 cells than 5-FU. A pharmacokinetic investigation revealed that 5-FU-loaded pH- sensitive PEGylated liposomes had a longer plasma circulation and a greater body exposure, while tumor accumulation of 5-FU-loaded pH-sensitive PEGylated liposomes was substantially higher than that of free 5-FU. A pH-sensitive PEGylated liposomal delivery method for 5-FU may be able to efficiently minimize 5-FU's unfavorable side effects while also improving its therapeutic index.	"PEG-5-FUNano" nanoparticles, designed to encapsulate PEGylated 5-FU, can be used in conjunction with liposomes. These nanoparticles can be conjugated with liposomes through surface functionalization with PEGylated 5-FU, augmenting the targeting and imaging capabilities for colorectal cancer. Molecular binding involves integrating PEGylated 5-FU moieties on the liposomal surface, promoting specific interactions with the tumor microenvironment for improved targeting and imaging abilities.	[303, 304]

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Table 10 (Continued).

Active agent	Method	Cell Line	Comment	Theranostic Nanoparticles	References
DOX	Thin film hydration	C26 cells	Mild hyperthermia produced by high intensity focused ultrasound (HIFU) and microbubbles (MBs) has been shown to improve tumor medication delivery from non-thermosensitive liposomes (NTSLs) and low temperature sensitive liposomes (LTSLs).	Nanoparticles termed "DoxoNano" coated with DOX can be employed in conjunction with liposomes. These nanoparticles can be conjugated with liposomes through surface modification with DOX components, enhancing the targeting and imaging capacities in colorectal cancer. Molecular binding involves incorporating DOX moieties on the liposomal surface, aiding in specific interactions with the tumor microenvironment for improved targeting and imaging capabilities.	[281, 305]
Gold nanoshell (NS)-based PTT and liposomal DOX	Freeze-Thaw	CT26 cells	Cancer treatments have limitations, so combining therapies has been explored. Traditional chemotherapy faces issues like drug delivery and resistance. Nanoparticle-based photothermal therapy (PTT) has heat distribution challenges. Combining PTT with chemotherapy shows promise, using compounds like gold nanosponges and liposomes. PTT followed by liposome treatment slowed tumor growth in mice. Clinical use needs optimization and consideration of side effects. Combining PTT with alternative drugs addresses resistance. Customizing nanocarriers and understanding the EPR effect in humans are crucial.	The gold nanoshells, with a gold core and silica shell, serve as imaging agents by absorbing near-infrared (NIR) light, enabling visualization and localized hyperthermia. Modified with CT26-targeting ligands, they selectively accumulate in colorectal cancer cells. Paired with LipoDox, a liposomal DOX formulation, the nanotheranostic agent enhances drug delivery and chemotherapy specificity. LipoDox, also modified for CT26 targeting, encapsulates DOX.	[306, 311]

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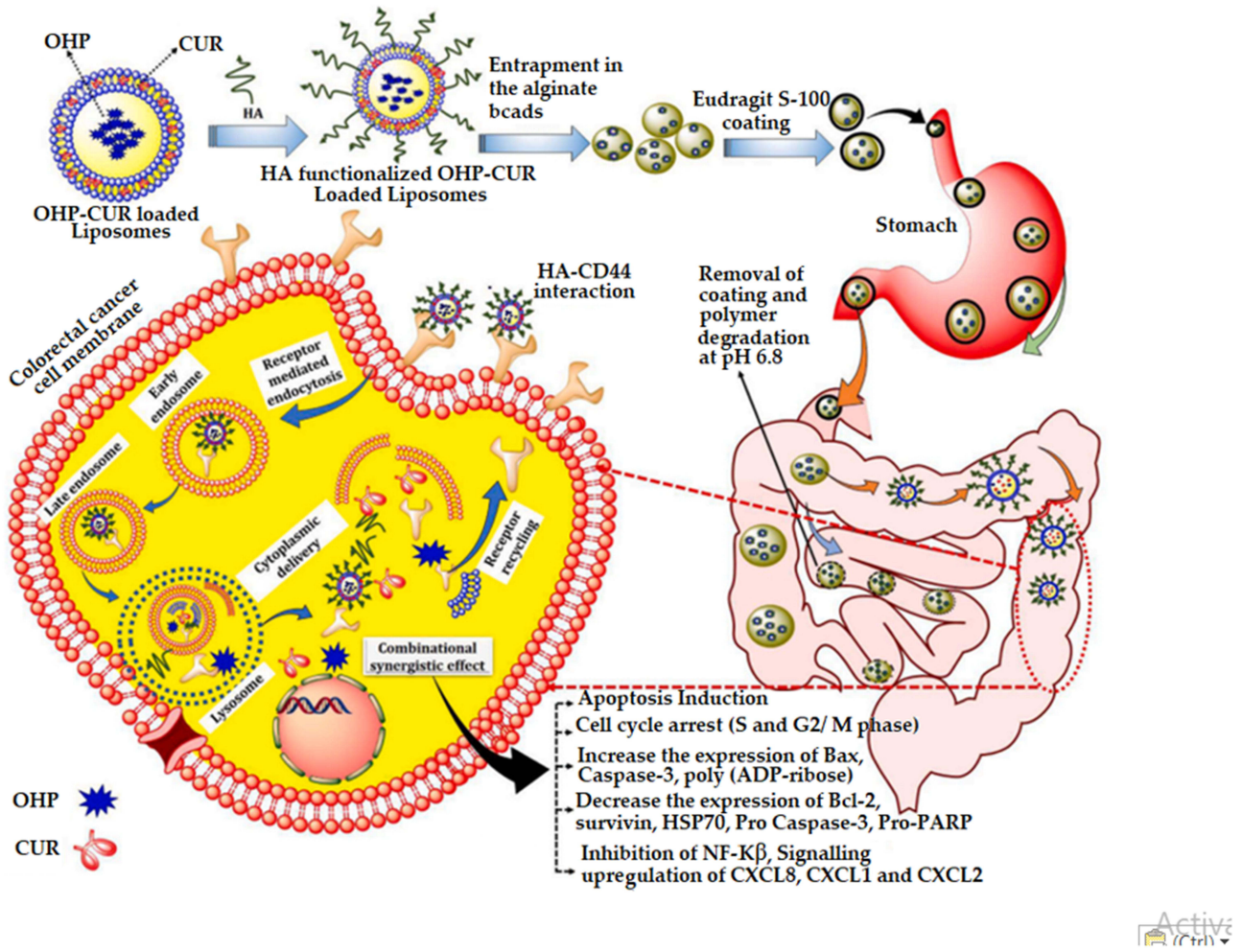

Figure 9 Schematic representation of colon-specific targeting of eudragit coated bead encapsulating liposomes.
Notes: Reproduced from Tiwari A, Gajbhiye V, Jain A, et al. Hyaluronic acid functionalized liposomes embedded in biodegradable beads for duo drugs delivery to oxaliplatin-resistant colon cancer. J Drug Delivery Sci Technol. 2022;77:103891. © 2022 Elsevier B.V. All rights reserved.[309]

COVID-19 pandemic has underscored the need for effective liposomal formulations in vaccines and antiviral agents, revealing challenges such as rapid clearance and suboptimal biodistribution that must be addressed through nanotechnology-based solutions. Incorporating artificial intelligence (AI) and machine learning (ML) into the design of liposomal

Table 11 Liposomal Formulations in Clinical Trials.

Drug	Company	Condition	Administration	References
Doxorubicin	Janssen Products, Johnson & Johnson	Breast cancer, ovarian cancer and Kaposi's sarcoma	Intravenous	[310]
Doxorubicin	Teva Pharmaceuticals	Breast cancer	Intravenous	[311]
Doxorubicin	Sun Pharmaceutical Industries	Ovarian, colon, and stomach cancers	Intravenous	[312]
Daunorubicin	Gilead Sciences	HIV-related Kaposi's sarcoma	Intravenous	[313]
0Irinotecan	Merrimack Pharmaceuticals	Pancreatic cancer	Intravenous	[314]
Cytarabine	Mallinckrodt Pharmaceuticals	Central nervous system lymphoma and leukemia	Lumbar puncture injection	[315]
Thalidomide	Mallinckrodt Pharmaceuticals	Specific types of non-Hodgkin's lymphoma	Intravenous	[316]
Patisiran	Alnylam Pharmaceuticals	Hereditary transthyretin-mediated amyloidosis	Intravenous	[317]
Paclitaxel	Teva Pharmaceuticals	Ovarian and breast cancer	Intravenous	[318]
Paclitaxel	Hisun Pharmaceutical	Non-small cell lung cancer	Intravenous	[319]

Note: Adapted from Bulbake U, Doppalapudi S, Kommineni N, Khan W. Liposomal formulations in clinical use: an updated review. Pharmaceutics. 2017;9(2):12. Creative Commons.[312]

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systems offers a pathway to optimize formulations, predict immune responses, and tailor treatment regimens to individual patients. This integration could lead to a revolutionary era in nanomedicine, enabling researchers to design liposomes that evade immune detection while delivering therapies directly to targeted tissues. Ultimately, the future of liposomal nanomedicines lies in their ability to evolve into sophisticated platforms that not only deliver drugs effectively but also actively participate in modulating immune responses. This advancement could lead to safer and more effective therapeutic options for a variety of diseases, paving the way for a new frontier in personalized medicine and immunology.

Conclusions

This manuscript explores the extensive potential of liposomal drug delivery systems, particularly in the realm of anticancer therapies. It highlights the advancements made in liposome design, characterization, and targeting strategies, emphasizing how these innovations have enhanced the precision and efficacy of drug delivery to cancer cells. By addressing challenges such as biodistribution, drug loading, and targeted delivery, liposomal formulations have shown great promise in improving therapeutic outcomes for various cancers, including breast, lung, prostate, and colorectal cancers.

The discussion extends to recent innovations, such as stimulus-responsive liposomes that react to environmental triggers like pH, temperature, or enzymes, thereby allowing for more controlled and effective drug release. Moreover, liposomes have moved beyond cancer treatment to applications in infectious diseases, as evidenced by their role in COVID-19 vaccines. These nanocarriers are increasingly being designed to modulate immune responses, with the potential to evolve into immuno-modulatory platforms capable of orchestrating targeted immune reactions.

The manuscript also emphasizes the future direction of liposomal nanomedicines, where integrating artificial intelligence and machine learning could optimize design and therapeutic outcomes. By coupling drug delivery with real-time diagnostic capabilities, liposomes have the potential to revolutionize personalized medicine. Ultimately, the conclusion underscores the vital role liposomal drug delivery systems will continue to play in both cancer therapy and broader medical applications, pushing the boundaries of precision medicine and nanotechnology.

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Disclosure

The authors declare no conflicts of interest in this work.

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