Surface modified liposomes for targeted drug delivery applications: A Review

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Abstract

Liposomes are self-assembled phospholipid bilayer vesicles that function as versatile drug delivery systems by accommodating both hydrophilic and hydrophobic therapeutic agents. This review examines the classification, preparation, characterization, and applications of liposomes, with particular emphasis on surface modification strategies that enhance targeting efficiency and therapeutic efficacy. Liposomes are categorized by size, lamellarity, lipid composition, and surface characteristics, with small unilamellar vesicles (SUVs), large unilamellar vesicles (LUVs), and multilamellar vesicles (MLVs) constituting the principal classes. Surface modifications, notably poly (ethylene glycol) (PEG) conjugation (PEGylation) and ligand functionalization, improve colloidal stability, prolong systemic circulation, and enable site-specific delivery. Passive targeting exploits the enhanced permeability and retention (EPR) effect, whereas active targeting employs antibodies, peptides, and aptamers to engage receptors overexpressed on target cells. Stimuli-responsive systems, including pH-sensitive, thermosensitive, magneto responsive, and near-infrared (NIR)-responsive liposomes, permit spatiotemporally controlled drug release. Nevertheless, PEGylation is limited by the accelerated blood clearance (ABC) phenomenon and anti-PEG immune responses, which have stimulated the development of alternative stealth coatings such as zwitterionic polymers and poly (2-oxazoline)s (POx). Recent advances in lipid–polymer hybrid systems have further expanded the functional repertoire of liposomal carriers, yet challenges of scalability, batch-to-batch reproducibility, immunogenicity, and regulatory approval persist. This review underscores the capacity of surface-modified liposomes to overcome key limitations of conventional drug delivery and to improve therapeutic outcomes, particularly in oncology, and outlines the characterization and quality-by-design (QbD) frameworks required for their clinical translation.

Keywords: liposomes, surface modification, targeted drug delivery, stimuli-responsive nanocarriers, active targeting

Introduction

Liposomes are spherical vesicles comprising one or more phospholipid bilayers enclosing an aqueous core (Akbarzadeh *et al*., 2013). Because their constituent phospholipids possess both a hydrophilic head group and hydrophobic tails, liposomes are amphiphilic, closely resembling the architecture of the cell membrane (Daraee *et al*., 2016), and the rigidity or fluidity of the bilayer can be tuned through the choice of lipid components (Akbarzadeh *et al*., 2013). Highly purified phospholipids, together with sterols such as cholesterol and its derivatives, are the principal materials used to prepare liposomes for pharmaceutical purposes (Maeda *et al*., 2016). The bilayer architecture permits the simultaneous encapsulation of hydrophilic drugs within the aqueous lumen and lipophilic drugs within the lipid membrane (Eloy *et al*., 2014, Eloy *et al*., 2017; Shah *et al*., 2020).

Owing to these properties, conventional liposomes remain among the most extensively studied drug delivery systems. Their advantages include the capacity to carry both hydrophobic and hydrophilic drugs, enhanced drug solubility, prolonged half-life, reduced systemic toxicity, and improved efficacy (Liu *et al*., 2022). Liposomes can be administered by oral, intravenous, and topical routes, and their utility extends beyond small-molecule drugs to nucleic acids and gene therapy (Venkata *et al*., 2022).

Conventional liposomes nonetheless suffer from important limitations. Their physicochemical instability can lead to drug leakage or vesicle fusion, diminishing the drug concentration delivered to the target and compromising targeting efficiency. Additional drawbacks include low drug-loading capacity, rapid clearance by the reticuloendothelial system (RES) (Lian & Ho, 2001), and the potential for immunogenicity (Carstens *et al*., 2011).

Surface modification of liposomes has emerged as a principal strategy for overcoming these limitations. Functionalization of the liposomal surface alters pharmacokinetic behavior and improves delivery efficiency, for example through the attachment of targeting moieties such as antibodies or peptides, or the conjugation of polymers such as polyethylene glycol (PEG) (Khan *et al*., 2020; Lestini *et al*., 2002; Nguyen *et al*., 2016).

The optimal modification strategy remains a subject of debate. Although targeting moieties can improve specificity, polymer coatings may impede drug release and cell interaction, thereby delaying the therapeutic response (Kim *et al*., 2017; Romberg *et al*., 2008). High surface densities of PEG can also destabilize liposomes and interfere with drug entrapment and loading (Nag & Awasthi, 2013). Moreover, PEGylated liposomes can elicit immunogenic responses manifesting as hypersensitivity reactions (Neun *et al*., 2018), and as the stealth effect of polymer coatings wanes, surface-modified liposomes are ultimately recognized and cleared by the mononuclear phagocyte system (MPS), limiting the *in vivo* stabilization conferred by PEGylation (Milani *et al*., 2019; Nag *et al*., 2013).

The rationale for surface modification is therefore rooted in the need to reconcile stability, circulation time, and targeting specificity (Milani *et al*., 2019). PEGylation, the conjugation of PEG to the liposome surface, creates a hydrophilic corona that reduces opsonization and recognition by the RES, thereby prolonging systemic circulation (Khan *et a*l., 2020). However, repeated administration of PEGylated liposomes may

provoke the accelerated ABC phenomenon, in which anti-PEG immunoglobulin M (IgM) antibodies promote rapid clearance of subsequent doses and diminish therapeutic efficacy. Consequently, alternative stealth coatings, including zwitterionic polymers and poly(2-oxazoline)s (POx), are being actively investigated because they combine low protein adsorption and reduced immunogenicity with prolonged circulation and do not elicit the ABC response (Knop *et al*., 2010; Abu Lila *et al*., 2013).

Beyond PEGylation, the attachment of targeting ligands to the liposomal surface has been extensively explored for active targeting of specific tissues or cells. Ligands such as antibodies, peptides, and small molecules can be engineered to bind receptors overexpressed on target cells, facilitating receptor-mediated endocytosis and enhancing intracellular drug delivery. This approach has shown particular promise in oncology, where ligand-functionalized liposomes directed at tumor-associated receptors achieve improved uptake and therapeutic outcomes (Liu *et al*., 2020).

Recent work has additionally focused on stimuli-responsive liposomes that release their payload in response to internal or external triggers such as pH, temperature, or light. Externally controlled systems have attracted particular attention: magneto-responsive liposomes incorporate magnetic nanoparticles and discharge their cargo upon exposure to an alternating magnetic field (AMF) through localized hyperthermia, enabling remote, site-specific drug delivery (Shahsavari *et al*., 2024). Likewise, near-infrared II (NIR-II)-responsive liposomes have emerged as promising nanocarriers because the NIR-II window (1000–1700 nm) offers deeper tissue penetration, reduced light scattering, and lower phototoxicity than visible or NIR-I irradiation, allowing precise, non-invasive, on-demand release (Aundhia *et al*., 2025). These next-generation systems afford enhanced spatial and temporal control of drug delivery and hold considerable promise for cancer therapy and precision medicine (Kobzev *et al*., 2026). For example, pH-sensitive liposomes have been designed to discharge their contents in the acidic tumor microenvironment, thereby increasing drug concentration at the intended site (Agrawal *et al.,* 2024).

The integration of polymers with liposomes has further broadened the functional capabilities of these nanocarriers. Polymer-coated liposomes have been developed to improve stability, control drug release, and confer stealth or mucoadhesive properties. Chitosan, for instance, has been widely employed as a surface-coating polymer to enhance the structural stability, mucoadhesion, and drug retention of liposomes, improving their performance in mucosal and oral drug delivery (Mohammed *et al*., 2021). By contrast, poly (lactic-co-glycolic acid) (PLGA) is used predominantly in lipid polymer hybrid nanoparticles (LPHNs) rather than conventional liposomes. These hybrid systems combine the structural integrity and sustained-release behavior of a PLGA core with the biocompatibility and biomimetic character of a lipid shell, affording improved drug loading, controlled release, and enhanced therapeutic efficacy (Danhier *et al*., 2012; Zhang *et al*., 2008).

Methodology

This narrative review was conducted to synthesize current evidence on the classification, preparation, surface modification, characterization, and clinical application of liposomes for targeted drug delivery. A structured literature search of PubMed, Scopus, Web of Science, and Google Scholar was performed for publications appearing between 2008 and 2025, with seminal earlier works (for

example, Bangham et al., 1965; Szoka & Papahadjopoulos, 1978) included where historically foundational. Boolean operators were used to combine the keywords "liposomes," "surface modification," "targeted drug delivery," "PEGylation," "ligand-conjugated liposomes," "stimuli-responsive liposomes," and "nanocarriers." Reference lists of retrieved articles were hand-searched to identify additional relevant studies.

Articles were eligible for inclusion if they: (i) were published in English in a peer-reviewed journal; (ii) addressed liposomal drug delivery, including liposome classification, preparation methods, surface-modification or functionalization strategies, physicochemical or biopharmaceutical characterization, or preclinical and clinical applications; and (iii) reported primary experimental data or constituted authoritative reviews, regulatory guidance documents, or pivotal clinical trials. Articles were excluded if they: (i) were published in languages other than English; (ii) were conference abstracts, editorials, commentaries, or preprints lacking peer review; (iii) concerned non-liposomal nanocarriers (for example, solid lipid nanoparticles, polymeric nanoparticles, or dendrimers) except where such systems were directly compared with liposomes; or (iv) did not address drug delivery, targeting, or pharmaceutical development. Titles and abstracts were screened for relevance, and full texts of potentially eligible articles were examined before inclusion. Given the narrative scope of this review, the evidence was synthesized qualitatively.

Liposomes

Liposomes are self-assembled phospholipid bilayer vesicles that have become versatile drug delivery systems by virtue of their capacity to encapsulate hydrophilic and hydrophobic agents. Their classification is essential for understanding their functional properties and applications in targeted delivery. Liposomes are broadly categorized by size, lamellarity, lipid composition, and surface modification, with surface modification playing a decisive role in targeting efficiency and therapeutic efficacy (Akbarzadeh *et al*., 2013).

Classification of liposomes

Classification based on size and lamellarity

Based on size and number of bilayers, liposomes are divided into three principal classes (Table 1):

Small unilamellar vesicles (SUVs), typically 20 –100 nm in diameter and bounded by a single phospholipid bilayer, are widely used for targeted delivery because of their tissue-penetrating capacity and relatively reduced uptake by the RES (Patil & Jadhav, 2014).

Large unilamellar vesicles (LUVs), ranging from 100 to 1000 nm, are likewise unilamellar and are frequently employed to encapsulate larger drug payloads (Tomnikova *et al*., 2022).

Multilamellar vesicles (MLVs), comprising multiple concentric bilayers, are generally larger than 500 nm and are well suited to sustained-release applications (Zhang *et al*., 2022).

Table 1. Classification of liposomes based on size and number of bilayers by Shah *et al.*, 2020

Type of liposome	Size range	Number of bilayers	Key characteristics
Small unilamellar vesicles (SUVs	20 – 100 nm	Single bilayer	Small size may reduce uptake by the reticuloendothelial system (RES); however, prolonged circulation and effective evasion of RES clearance generally depend on additional factors, particularly surface characteristics and functionalization such as PEGylation. Size reduction therefore complements, rather than replaces, surface modification in minimizing opsonization and RES-mediated clearance.
Large unilamellar vesicles (LUVs)	100 – 1000 nm	Single bilayer	Encapsulation of larger drug payloads, facilitating both passive and active targeting mechanisms.
Multilamellar vesicles (MLVs)	>500 nm	Multiple bilayers	High drug-loading capacity renders these vesicles suitable for sustained-release applications.
Giant unilamellar vesicles (GUVs)	>1000 nm	Single bilayer	Suitable for membrane biophysics studies and the modeling of cellular processes.
Oligolamellar vesicles (OLVs)	100–500 nm	Few bilayers (2–5)	Properties intermediate between SUVs and MLVs, suited to controlled drug-release applications.

Classification based on surface modification

Surface-modified liposomes are engineered to strengthen targeting capability and reduce immunogenicity. These modifications are conventionally grouped into passive and active targeting strategies.

Passive targeting depends on the EPR effect, whereby liposomes accumulate preferentially in tumor tissue because of its leaky vasculature and impaired lymphatic drainage. A PEG coating is commonly applied to prolong circulation by reducing opsonization and RES uptake; such PEGylated, or "stealth," liposomes have been studied extensively for the delivery of chemotherapeutic agents with minimal systemic toxicity (Suk *et al*., 2016).

Active targeting involves conjugating ligands, antibodies, peptides, or aptamers to the liposome surface to promote specific binding to receptors overexpressed on target cells. Folate-conjugated liposomes target folate receptors, which are highly expressed in many cancers (Wang *et al*., 2023), whereas

transferrin-modified liposomes exploit transferrin receptors upregulated in various malignancies (Li *et al.,* 2022). Such ligand-mediated strategies enhance the specificity and cellular uptake of liposomes and thereby improve therapeutic outcomes.

Classification based on functionalization

Liposomes can also be categorized by functionalization with stimuli-responsive elements such as pH, temperature, or enzyme-sensitive moieties. pH-sensitive liposomes destabilize in the acidic tumor microenvironment, enabling controlled release at the target site (Gupta *et al*., 2021), while thermosensitive liposomes release their contents upon exposure to mild hyperthermia, making them attractive for combination therapies involving localized heating (Needham *et al*., 2013).

Classification based on lipid composition

Conventional liposomes, composed primarily of natural phospholipids (such as phosphatidylcholine and phosphatidylethanolamine) and cholesterol, constitute the foundational category (Sharma *et al*., 2018). The incorporation of cholesterol enhances membrane rigidity and reduces drug leakage (Guimarães *et al*., 2021). Fusogenic liposomes, which promote fusion with cellular membranes, commonly contain dioleoylphosphatidylethanolamine (DOPE), a key lipid that improves intracellular delivery, particularly in pH-sensitive formulations (Zhu & Torchilin, 2013).

Cationic liposomes are widely used in gene delivery: the positively charged lipid 1, 2-dioleoyl-3-trimethylammonium-propane (DOTAP) facilitates efficient complexation with negatively charged nucleic acids, improving transfection efficiency (Ravula *et al*., 2024).

Stealth liposomes are surface-modified with hydrophilic polymers, most commonly PEG, to reduce opsonization, evade MPS recognition, and prolong circulation (Knop *et al*., 2010). However, repeated administration of PEGylated liposomes may induce the ABC phenomenon, in which anti-PEG IgM antibodies trigger rapid clearance of subsequent doses (Gaballa *et al*., 2024). Attention has therefore shifted toward alternative stealth coatings, particularly zwitterionic polymers, which resist nonspecific protein adsorption, display lower immunogenicity, and may mitigate the ABC phenomenon while sustaining long circulation (Ouyang *et al*., 2024). Both the molecular weight and the surface density of PEG critically influence the resulting pharmacokinetic profile (Guimarães *et al*., 2021).

Classification based on surface functionalization

Antibody-conjugated liposomes (immune liposomes) are highly specific delivery systems optimized to engage defined cell populations through antibody lipid conjugation (Eloy *et al*., 2017). Among ligand-conjugated liposomes, receptor-specific constructs such as transferrin-conjugated vesicles have demonstrated improved delivery across the blood brain barrier (Moreira *et al*., 2024).

Table 2. Classification of liposomes according to lipid composition and surface characteristics.

Type	Composition	Characteristics	References
Conventional liposomes	Phospholipids (e.g., phosphatidylcholine), cholesterol	Simple structure, biocompatible, widely used for drug delivery, limited stability in biological environments.	Sercombe *et al*. (2015)
PEGylated liposomes	Phospholipids, cholesterol, polyethylene glycol (PEG)	Enhanced circulation time, reduced immune recognition, improved stability.	Xing *et al*. (2016)
Cationic liposomes	Cationic lipids (e.g., 1,2-dioleoyl-3-trimethylammonium propane (DOTAP), N-[1-(2,3-dioleyloxy) propyl]-N, N, N-trimethylammonium chloride (DOTMA)), phospholipids	Positively charged, bind negatively charged nucleic acids, used for gene delivery.	Dizaj *et al*. (2014)
Anionic liposomes	Anionic lipids (e.g., phosphatidylserine, phosphatidylglycerol), phospholipids	Negatively charged, biocompatible; amenable to charge-mediated and tissue-specific targeting.	Riaz *et al*. (2018)
Thermosensitive liposomes	Phospholipids, lysolipids, cholesterol	Temperature-sensitive; release triggered at mild hyperthermia (39 - 42 °C), suited to combination with local heating.	Lindner *et al*. (2004)
pH-sensitive liposomes	Phospholipids, pH-sensitive lipids (e.g., 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE), cholesteryl hemisuccinate (CHEMS))	pH-responsive release in acidic environments (e.g., tumor interstitium, endosomes) facilitates targeted delivery.	Karanth & Murthy (2007)
Immunoliposomes	Phospholipids, cholesterol, conjugated antibodies or antibody fragments	Antibody-mediated specificity for defined cell populations (e.g., cancer cells).	Eloy *et al*. (2017)
Long-circulating liposomes	Phospholipids, cholesterol, PEG or alternative hydrophilic polymers	Prolonged blood circulation, reduced RES uptake.	Allen & Cullis (2013)

Stealth liposomes	Phospholipids, cholesterol, PEG	Evade immune detection, long half-life, established utility in targeted drug delivery.	Nag & Awasthi (2013)
Fusogenic liposomes	Phospholipids (e.g., DOPE), fusogenic peptides or pH-sensitive lipids	Promote membrane fusion, enhance cytoplasmic delivery of cargo.	Zhu & Torchilin (2013)

Emerging compositional innovations

Hybrid systems combining liposomal structures with other delivery platforms represent a recent advance. Lipid polymer hybrid systems merge the advantages of liposomes and polymeric nanoparticles, enhancing physicochemical stability, drug-loading capacity, controlled release, and overall delivery efficiency (Jain *et al.,* 2023).

Stimuli-responsive "smart" liposomes incorporate specialized lipids that undergo structural change in response to physicochemical cues such as pH, temperature, or enzymatic activity (Lee & Thompson, 2017; Puri *et al*., 2009). Externally triggered systems have attracted growing interest: magneto-responsive liposomes bearing magnetic nanoparticles can be activated by an AMF to induce localized hyperthermia and remotely controlled release (Shahsavari *et al*., 2024), and NIR-responsive liposomes designed for the NIR-II window (1000 – 1700 nm) enable deep-tissue penetration, reduced scattering, and combined imaging and on-demand release, improving therapeutic precision (Aundhia *et al*., 2025).

Surface-Modified Liposomes

Surface-modified liposomes (SMLs) represent an important advance in drug delivery, offering improved efficacy, specificity, and pharmacokinetic performance (Agrawal *et al.,* 2025). These spherical phospholipid bilayer vesicles can be engineered through surface modification to achieve targeted delivery, prolonged systemic circulation, and reduced off-target toxicity (Agrawal *et al*., 2025). These benefits, however, are accompanied by formulation and biological challenges, including altered cellular interactions, complement activation, and immunogenic responses associated with certain modifications. PEGylated systems in particular may induce anti-PEG antibodies, contributing to accelerated blood clearance and compromising the performance of repeatedly administered formulations (Tenchov *et al.,* 2023). Rational surface engineering therefore requires careful optimization of the type, density, and physicochemical properties of the surface modifier to balance circulation time and targeting against immunological safety (Jadhav & Garude, 2024).

A principal advantage of SMLs is their capacity for targeted drug delivery, typically achieved by attaching ligands or antibodies to the liposome surface so that the carriers are directed to cells or tissues overexpressing the cognate receptor. Folic acid modified liposomes, for example, effectively target cancer cells overexpressing folate receptors, and the resulting receptor-mediated endocytosis enhances accumulation of the therapeutic agent at tumor sites while sparing healthy tissue (Wang *et al.,* 2023). Similarly, transferrin modified liposomes exploit the transferrin receptor for targeted delivery in a range of cancers, illustrating the versatility of SMLs (Li *et al*., 2022). Surface modification also improves stability and circulation time:

PEGylation creates a hydrophilic barrier that limits opsonization, extending half - life and improving the pharmacokinetics of the encapsulated drug (Suk *et al.,* 2016). These benefits are nonetheless tempered by the formation of anti-PEG antibodies particularly IgM upon repeated administration, which accelerates clearance of subsequent doses (the ABC phenomenon) (Lin *et al*., 2023; Ouyang *et al*., 2024). This limitation has stimulated interest in alternative strategies, including zwitterionic polymers such as poly(2-methacryloyloxyethyl phosphorylcholine) (pMPC), which combine strong hydration and antifouling behavior with reduced IgM elicitation and diminished ABC relative to PEGylated liposomes (Kluzek *et al.,* 2025; Ouyang *et al.,* 2024).

The preparation methods for SMLs vary widely and include thin-film hydration, solvent evaporation, and microfluidic mixing, each affording control over size, morphology, and surface characteristics (Li *et al.,* 2022). As noted above, development and manufacture of SMLs should embed a QbD framework in which CQAs particle size and size distribution, PDI, surface charge, encapsulation efficiency, drug content, and release behavior are identified and controlled. Standardized, validated *in vitro* release assays are central to assessing formulation performance, batch consistency, and physicochemical comparability; such assays must maintain sink conditions, where necessary through a solubilizing or drug accepting agent, without disrupting liposomal integrity. Release medium composition, membrane characteristics, temperature, and pH can each substantially influence measured release profiles, underscoring the need for method optimization and standardization (Atre & Rizvi, 2024; Eneli *et al*., 2025; Juang *et al*., 2024). Consistent with this, microfluidic preparation has been shown to yield more uniform liposome sizes than conventional methods, improving stability and *in vivo* performance (Dong *et al*., 2019). Even so, challenges remain: the complexity of biological environments can compromise the stability and release behavior of modified liposomes, and consistent quality and reproducibility during manufacture are critical for clinical application (Modena *et al*., 2019).

Methods of synthesis of surface-modified liposomes

Thin-film hydration method

Thin-film hydration is among the most widely used techniques for liposome preparation. Lipids are dissolved in an organic solvent, the solvent is evaporated to deposit a thin lipid film on the wall of a round bottom flask, and the film is hydrated with an aqueous phase to yield multilamellar vesicles (Bangham *et al.,* 1965). Surface modification can be accomplished by incorporating functional lipids or polymers during hydration; for instance, adding PEGylated lipids to the lipid mixture produces PEGylated liposomes with prolonged circulation and reduced immunogenicity (Allen & Cullis, 2013).

The principal steps are as follows:

Lipid dissolution: lipids are dissolved in a suitable organic solvent, such as chloroform or methanol, in a round-bottom flask.

Film formation: the solvent is evaporated under reduced pressure on a rotary evaporator, leaving a thin lipid film on the inner wall of the flask.

Hydration: the film is hydrated with an aqueous solution, typically above the lipid phase-transition temperature, producing MLVs.

Size reduction (optional): MLVs are sonicated, extruded, or subjected to high

pressure homogenization to obtain SUVs or LUVs.

Surface modification in this method can be introduced during hydration or after vesicle formation. Common approaches include PEGylation, in which PEG conjugated lipids are incorporated into the lipid film to enhance *in vivo* stability and circulation (Allen & Cullis, 2013); ligand attachment, in which antibodies, peptides, or aptamers are introduced via ligand binding lipid derivatives or post hydration conjugation to achieve cell or tissue specific targeting (Torchilin, 2005), and charge modification, in which cationic or anionic lipids alter the surface charge and hence the interaction with biological membranes and cellular uptake (Wang *et al*., 2022).

The method is versatile (accommodating diverse lipids and functional components), simple (requiring relatively basic equipment), and scalable to large scale production. SMLs prepared by thin-film hydration are used extensively in drug delivery, gene therapy, and diagnostic imaging owing to their stability, targeting capability, and biocompatibility (Bozzuto & Molinari, 2015). In sum, thin-film hydration is a foundational technique that provides a flexible platform for generating SMLs with tailored properties for a broad range of biomedical applications.

Reverse-phase evaporation method

Reverse-phase evaporation begins with the formation of a water-in-oil emulsion by combining an aqueous phase with a lipid solution in an organic solvent, subsequent removal of the solvent under reduced pressure yields predominantly large unilamellar vesicles (Szoka & Papahadjopoulos, 1978). Surface modification can be introduced by incorporating targeting ligands such as antibodies or peptides (Torchilin, 2005).

The principal steps are as follows:

Emulsion preparation: lipids are dissolved in an organic solvent such as diethyl ether or chloroform, the aqueous solution containing the payload is added, and the mixture is sonicated or vortexed to form a stable water-in-oil emulsion.

Solvent removal: the organic solvent is gradually evaporated under reduced pressure; the aqueous phase coalesces and the lipids reorganize into bilayers.

Liposome formation: as the solvent is removed, liposomes form with the aqueous phase entrapped; the resulting vesicles are typically LUVs, which can be processed further to achieve the desired size distribution.

Purification: the suspension is filtered or centrifuged to remove unencapsulated material and excess lipid.

Surface modification of liposomes prepared by reverse-phase evaporation can be achieved in several ways: (i) incorporation of functional lipids PEGylated lipids or ligand conjugated lipids (for example, bearing antibodies or peptides) during emulsion preparation, yielding surface-modified vesicles directly upon synthesis (Allen & Cullis, 2013); (ii) post hydration modification, in which functional molecules are conjugated to reactive groups on the preformed liposome surface, or the surface charge is adjusted to enhance cellular uptake (Torchilin, 2005); and (iii) layer-by-layer assembly, in which polymers are deposited sequentially onto the liposome surface, allowing precise control of surface properties and functionality (Wang *et al.,* 2022).

The method offers high encapsulation efficiency for both hydrophilic and hydrophobic substances, versatility with respect to functional lipids and surface modifications, and scalability. SMLs

produced by reverse-phase evaporation find broad application in drug delivery, gene therapy, and diagnostic imaging, with corresponding gains in pharmacokinetics, targeting, and therapeutic efficacy (Bozzuto & Molinari, 2015). Reverse-phase evaporation thus remains a robust and versatile route to SMLs, and its efficiency in encapsulating diverse therapeutic agents makes it a valuable tool in nanomedicine.

Microfluidic method

The microfluidic method affords precise control over liposome size and composition through the controlled mixing of lipid and aqueous streams within microchannels. By adjusting critical process parameters, the flow-rate ratio (FRR), total flow rate (TFR), lipid concentration, and solvent composition, microfluidic systems regulate nucleation and self-assembly to produce liposomes with reproducible size and narrow distributions (Carugo *et al*., 2016; Rebollo *et al*., 2022). From a QbD perspective, these parameters should be linked to CQAs, notably particle size, PDI, encapsulation efficiency, and lipid and drug content. PDI is especially informative because it reflects the uniformity of the vesicle population and hence process consistency. Mass balance assessment should also be performed during process development to account for the recovery of drug and lipid across formulation and downstream steps, revealing material losses and supporting reproducible manufacture. Optimization of microfluidic conditions should therefore consider not only size and PDI but also drug and lipid recovery, encapsulation efficiency, and overall process yield (Atre & Rizvi, 2024; Rebollo *et al*., 2022).

The principal steps are as follows:

Solution preparation: lipids are dissolved in an organic solvent and an aqueous phase is prepared separately, with the solvent and lipid composition tailored to the desired liposome properties.

Device setup: the lipid and aqueous solutions are introduced through separate inlets of the microfluidic device, which contains a mixing region in which the two streams converge and liposomes form.

Liposome formation: rapid dilution of the organic solvent upon mixing drives lipid self-assembly into bilayers. Flow rate, FRR, lipid concentration, solvent composition, and channel geometry can be tuned to control vesicle formation and achieve reproducible CQAs (particle size, PDI, size distribution, encapsulation efficiency, and drug content) (Atre & Rizvi, 2024; Rebollo *et al*., 2022). Development should also incorporate *in vitro* release testing to characterize drug-release kinetics and support batch-to-batch consistency; *in vitro* release methods should maintain sink conditions and minimize artifacts from membrane diffusion or liposome disruption. Bio-Beads SM-2 resin, for example, has been used as a drug-accepting phase to maintain sink conditions in the *in vitro* release assessment of liposomal irinotecan, providing a robust approach for comparative evaluation and quality control (Juang *et al.,* 2026).

Collection and purification: liposomes are collected at the device outlet and may be purified further to remove unencapsulated material and residual solvent.

Surface modification in the microfluidic method can be integrated into synthesis or performed afterward. Common strategies include: incorporation of functional lipids, such as PEGylated or ligand-conjugated lipids, into the lipid solution so that surface-modified liposomes form directly during synthesis (van Swaay & deMello, 2013); layer-by-layer assembly of polyelectrolyte multilayers on the vesicle surface to modulate surface properties with precision (Wang *et*

al., 2022); and post synthesis conjugation of antibodies, peptides, or aptamers to reactive surface groups using standard bioconjugation chemistry to enhance specificity and targeting (Almeida *et al.,* 2021).

The method provides precision and reproducibility, enabling highly uniform vesicle populations whose consistency can be verified through CQAs such as particle size and PDI; assessment of drug and lipid mass balance (encapsulated and free fractions, and material recovery across steps) further supports process consistency and robust manufacture. Systematically relating microfluidic process parameters to these attributes through QbD principles strengthens the prospects for reproducible, scalable production (Atre & Rizvi, 2024; Buttitta *et al*., 2024; Rebollo *et al.,* 2022). The method is also scalable and adaptable to varied lipid compositions and surface modifications. SMLs synthesized microfluidically are broadly useful in drug delivery, diagnostics, and therapeutics, where precise control of size and surface properties enhances targeted delivery and mitigates off-target effects (Bozzuto & Molinari, 2015). Overall, microfluidics is an advanced synthesis technique offering precision, scalability, and versatility for engineering specialized liposomes.

In summary, SMLs can be synthesized by several complementary methodologies: thin-film hydration, reverse-phase evaporation, and microfluidic mixing each with distinct advantages, and each amenable to the incorporation of functional molecules that enhance liposome performance in targeted drug delivery and related applications.

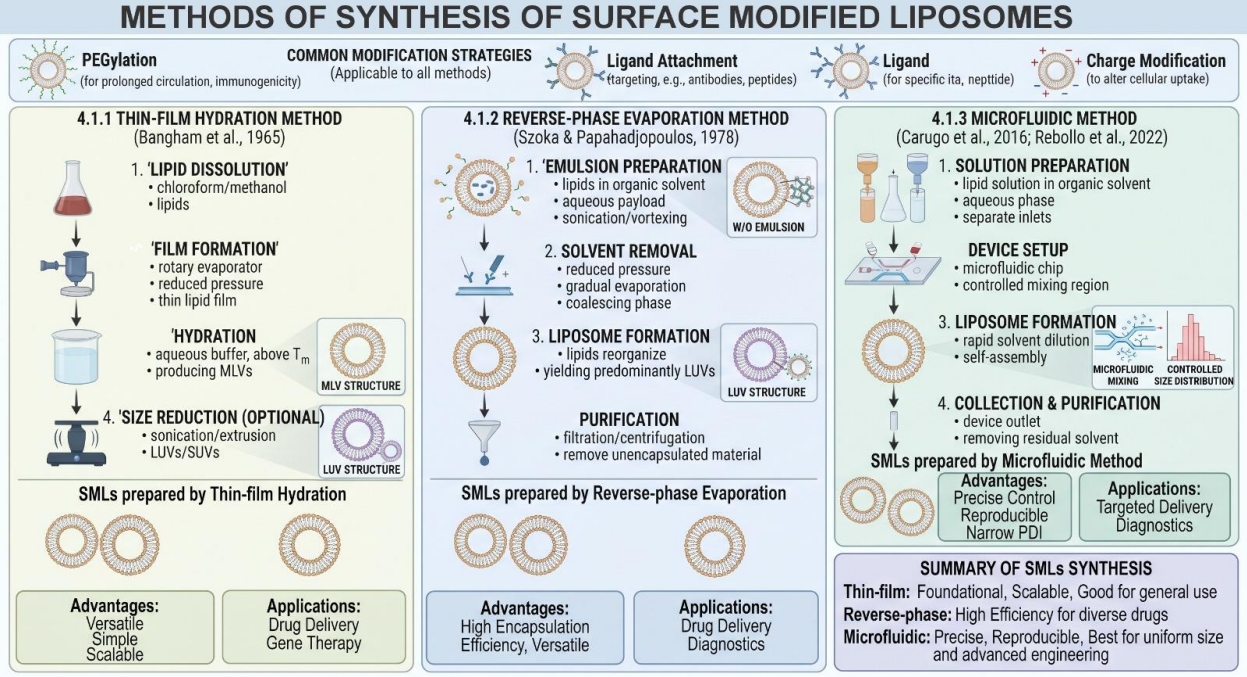

Figure 1: Methods of synthesis of surface modified liposomes

Targeting strategies of surface-modified liposomes

Liposomes can serve as drug carriers for numerous disorders, including cancer (Meissner *et al.,* 2015). For liposomes to be used specifically and effectively, it is essential to understand the chemistry of the target cell-surface receptors, to select appropriate ligands for attachment to the liposome surface, and to prevent rapid clearance by the RES (Bonde & Nair, 2017; Chudasma *et al*., 2023; Khan *et al*., 2020; Peng *et al*., 2022). Surface engineering such as PEGylation reduces opsonization and prolongs circulation, but it is not without limitations: repeated administration of PEGylated nanocarriers may induce anti-PEG IgM, causing ABC and reducing the persistence of subsequent doses (Ouyang *et al.,* 2024; Tenchov *et al*., 2023), a consideration of particular importance for formulations intended for chronic or repeated dosing. Emerging non-PEG strategies, notably zwitterionic polymers such as poly(2-methacryloyloxyethyl phosphorylcholine (pMPC), are being investigated for their strong hydration and antifouling properties; pMPC-functionalized liposomes elicit less antibody and a weaker ABC response than PEGylated liposomes while maintaining prolonged circulation, highlighting their potential for multi-dose regimens (Kluzek *et al*., 2025; Ouyang *et al*., 2024). Targeting of liposomes to diseased sites, particularly tumors, has been pursued through two broad strategies: passive and active targeting.

Passive targeting

Passive targeting of liposomes to diseased sites, especially tumors, rests primarily on the leaky character of tumor-associated vasculature (Haley & Frenkel, 2008). Whereas the junctions between endothelial cells in normal vessels are 5 - 10 nm wide, gaps in tumor capillaries range from roughly 100 to 780 nm depending on the cancer type

(Khan *et al.*, 2020; Sheikh *et al*., 2022). Together with the EPR effect, this architecture favors liposome accumulation at tumor sites, provided the vesicles are small enough to extravasate into tumor tissue (Kim & Huang, 2012; Lehtinen *et al*., 2012). An illustrative example is the use of sclareol-loaded solid lipid nanoparticles (approximately 88 nm) against human lung epithelial cancer cells, which exhibited sustained drug release over 48 h relative to the free drug (Paszko & Senge, 2012).

Active targeting

Actively targeted liposomes address the shortcomings of passive targeting by displaying targeting moieties on their surface to minimize off-target effects. Small molecules, peptides, aptamers, antibody fragments, and whole antibodies have all been used as targeting moieties (Guimarães *et al*., 2021). Effective cancer-cell targeting requires the attachment of a specific moiety in sufficient quantity to achieve adequate affinity for tumor cell-surface receptors (Eloy *et al.,* 2017). Recent efforts have concentrated on macromolecules overexpressed by particular cancer cell types, as outlined below.

Targeting cell-surface receptors

Targeting receptors expressed on cancer cells is a predominant strategy for liposomal drug delivery using antibody- or ligand-bearing vesicles (Deshpande *et al*., 2013). The principal receptor targets include the following:

Folate receptors are typically overexpressed in certain lung, ovarian, colon, breast, brain, and kidney tumors (Cheung *et al*., 2016). Because cancer cells generally overexpress these receptors relative to normal cells, they have been exploited successfully for liposome-mediated, tumor-selective drug delivery (Deshpande *et al*., 2013).

Transferrin receptors are upregulated in rapidly proliferating cancer cells because of their elevated iron requirement (Candelaria *et al*., 2021), and transferrin-targeted anticancer formulations have been developed accordingly (Dufès *et al*., 2013). Transferrin-targeted, doxorubicin-loaded stealth liposomes have shown improved pharmacokinetics, biodistribution, and intracellular uptake against liver cancer cells, enhancing therapeutic efficacy (AlSawaftah *et al*., 2021).

Epidermal growth factor receptors (EGFRs) regulate cell proliferation, survival, differentiation, angiogenesis, and metastasis and are frequently overexpressed in solid tumors, including non-small-cell lung cancer, colorectal cancer, and cancers of the breast, pancreas, ovary, kidney, and head and neck (Liu *et al*., 2023; Thomas & Zhang, 2019). Their high tumor-cell expression and surface accessibility make EGFRs attractive targets for receptor-mediated liposomal delivery. EGFR-targeted liposomes functionalized with antibodies, antibody fragments, peptides, or affibodies have demonstrated enhanced tumor-cell binding, receptor-mediated uptake, improved intracellular delivery, and greater antitumor efficacy than non-targeted liposomes in preclinical studies (Haeri *et al.*, 2016; Jia *et al*., 2022).

Targeting the tumor microenvironment

Targeting the tumor microenvironment offers several advantages over targeting tumor cells directly: destruction of the tumor vasculature can reduce tumor growth and metastatic spread; directing liposomes to the vasculature bypasses the barrier to diffusion into the tumor parenchyma; and the phenotypic stability of neovascular endothelial cells may limit the emergence of resistance. Moreover, for a given cancer type, the tumor vasculature is largely nonspecific, broadening the

applicability of vascular targeting (Deshpande *et al*., 2013).

Vascular endothelial growth factor (VEGF) and its receptor (VEGFR): VEGF/VEGFR signaling is a principal driver of tumor angiogenesis and therefore an important target within the tumor microenvironment (Patel *et al.*, 2023). For liposomal delivery, VEGF or VEGFR targeting can be achieved by conjugating antibodies or receptor-binding peptides to the liposome surface; bevacizumab-modified PEGylated cationic liposomes, for instance, showed enhanced cellular uptake and tumor-targeting potential relative to unmodified liposomes (Kuesters & Campbell, 2010). Bevacizumab is a US Food and Drug Administration–approved anti-human VEGF monoclonal antibody used as an anticancer agent (Liu *et al*., 2023). In addition, numerous tyrosine kinase inhibitors directed at VEGFRs and fibroblast growth factor receptors (FGFRs) have been incorporated into antineoplastic regimens (Thakur *et al*., 2024).

Vascular cell adhesion molecules (VCAMs): VCAMs are implicated in inflammatory conditions, including cancer, and are therefore candidate targets for antineoplastic intervention. VCAM-1 is overexpressed in the tumor vasculature, supporting its use for the delivery of anticancer agents (Kang *et al*., 2011; Khan *et al*., 2020).

B-rapidly accelerated fibrosarcoma (B-Raf): Vemurafenib is a BRAF inhibitor selective for the BRAF V600E mutation with proven activity against BRAF-mutant melanoma, although acquired resistance remains a major limitation. To improve vemurafenib delivery while mitigating the drawbacks of conventional administration, surface-modified liposomal formulations have been explored. Zou *et al*. (2018) developed peptide-modified, vemurafenib-loaded liposomes (Vem-TD-Lip) in which a TD peptide on the liposomal surface enhanced transdermal delivery; the formulation selectively inhibited BRAF-mutant melanoma cells, showed improved antitumor activity against subcutaneous melanoma in mice, and reduced systemic organ toxicity. These findings illustrate the capacity of SMLs to improve the site-specific delivery of a BRAF-targeted drug, although this approach should not be construed as directly overcoming acquired vemurafenib resistance. Resistance-focused liposomal strategies have also been reported: S100A9-functionalized liposomes targeting extracellular matrix metalloproteinase inducer (EMMPRIN), which is highly expressed in vemurafenib-resistant melanoma, achieved substantially greater cellular uptake and preferential accumulation in resistant tumors, suggesting a strategy for resistant disease that is mechanistically distinct from direct BRAF inhibition (Zeiderman *et al*., 2014).

Matrix metalloproteases (MMPs): these endopeptidases participate in diverse physiological and pathological processes, including metastasis, tumor invasion, tissue remodeling, and the acquisition of apoptosis resistance. In tumor-bearing mouse models, MMP-family inhibitors have reduced angiogenesis, and anti-MT1-MMP antibody–modified PEGylated liposomes have been used to target doxorubicin to tumors (Hatakeyama *et al.*, 2007).

Nanoscale delivery systems built on liganded liposomes offer an efficient means of improving disease therapy. The efficacy of ligand-targeted liposomal formulations may exceed that of free drugs, which are often cleared rapidly by the RES and rendered ineffective against tumors. PEGylated and other long-circulating liposomal formulations were developed to reduce opsonization and RES recognition, thereby prolonging circulation. Nevertheless,

PEGylation has important limitations during repeated administration: successive doses of PEGylated liposomes can induce anti-PEG IgM, which activates complement and hastens clearance of later doses—the ABC phenomenon (Takata *et al*., 2023; Tenchov *et al*., 2023). This has spurred the development of alternative stealth strategies, including zwitterionic polymers and POx, which aim to sustain long circulation while avoiding the immunological liabilities of PEG. In particular, pMPC-functionalized liposomes have recently shown prolonged circulation, lower IgM elicitation, and a reduced ABC response relative to PEGylated liposomes, underscoring their potential for repeated or multi-dose therapy (Kluzek *et al*., 2025). POx-based lipid conjugates have likewise been investigated as PEG alternatives owing to their PEG-like stealth properties and tunable physicochemistry (Holick *et al*., 2025).

The first anticancer drug approved by the US Food and Drug Administration in a liposomal formulation was doxorubicin encapsulated in PEGylated liposomes (Doxil) (Kraft *et al*., 2014). Surface modification with vitamins, carbohydrates, aptamers, and monoclonal antibodies or their fragments to engage cancer-associated antigens has since markedly enhanced targeting efficacy (Li *et al.*, 2019). With improved tumor accumulation and sustained exposure, such modified liposomes effectively deliver anti-angiogenic agents and antisense oligonucleotides. Numerous liposomal products are now marketed, most of them anticancer, antibacterial, or antifungal formulations.

The clinical impact of liposomal technology has extended well beyond early products such as Doxil®. In 2015, the FDA approved Onivyde® (nanoliposomal irinotecan), in combination with fluorouracil and leucovorin, for metastatic pancreatic adenocarcinoma following gemcitabine-based therapy; the pivotal NAPOLI-1 trial showed improved overall survival for the combination over fluorouracil and leucovorin alone (Wang-Gillam *et al*., 2016). In 2017, Vyxeos® (CPX-351), a fixed-ratio liposomal formulation of daunorubicin and cytarabine, was approved for adults with newly diagnosed therapy-related acute myeloid leukemia (AML) or AML with myelodysplasia-related changes; its phase III evaluation demonstrated improved overall survival over conventional 7+3 chemotherapy (Lancet *et al.*, 2018). Beyond oncology, liposome-based technologies have contributed to vaccine development: Shingrix®, the recombinant zoster vaccine, incorporates the AS01B liposome-based adjuvant system containing monophosphoryl lipid A (MPL) and QS-21 and demonstrated high efficacy against herpes zoster in older adults (Didierlaurent *et al*., 2017; Lal *et al*., 2015). These examples illustrate the broad clinical utility of liposomal platforms across drug delivery and vaccinology.

Liposomal formulations are also available for diseases of the eye, skin, and respiratory tract (Bonde & Nair, 2017; Cannito *et al*., 2022; Japiassu *et al*., 2022; Lestini *et al*., 2002). Multidrug liposomal carriers combining, for example, gemcitabine and paclitaxel have been explored, and some such formulations have exhibited greater cytotoxic activity than the individual free or singly encapsulated drugs. Engineered liposomal therapies based on diverse surface modifications continue to emerge as novel pharmacological tools, and future surface modification is expected to employ ever more effective and specific ligands for the targeted delivery of drugs against different diseases (Komeil *et al*., 2022).

Characterization of surface-modified liposomes

Like conventional liposomes, surface-modified formulations undergo rigorous characterization after preparation, using diverse analytical methods to define their properties and confirm the attributes required for effective *in vitro* and *in vivo* application (Guimarães *et al*., 2021).

Key CQAs include particle size and size distribution (notably the PDI), morphology, surface charge, lipid composition, drug content and encapsulation efficiency, surface characteristics, and drug-release behavior. For SMLs, characterization should additionally confirm the identity, density, and stability of the surface ligand or polymer. Assessing drug and lipid mass balance across formulation and purification steps is important for evaluating process consistency, material recovery, and potential losses. Appropriately validated *in vitro* release assays are likewise essential for characterizing release kinetics and comparing formulation performance; where appropriate, release methods should maintain sink conditions for example, with adsorbents such as Bio-Beads SM-2 to limit drug accumulation in the medium and provide a discriminatory, reproducible assessment of release (Atre & Rizvi, 2024; Juang *et al*., 2026).

Particle size and size distribution

Size and size distribution critically influence the biodistribution, cellular uptake, and targeting efficiency of liposomes. SMLs typically range from 20 nm to several micrometres in diameter, with smaller vesicles (for example, SUVs) preferred for systemic delivery because of their ability to evade the RES and penetrate tissue (Zhang *et al*., 2022).

Dynamic light scattering (DLS) is the most widely used technique for determining liposome size and PDI, providing rapid, non-invasive measurement of hydrodynamic diameter and distribution (Patil & Jadhav, 2014). Transmission electron microscopy (TEM) and scanning electron microscopy (SEM) are also used to visualize morphology and confirm nanoscale size measurements (Wang *et al*., 2023).

Surface modifications can alter size and size distribution. PEGylation, for instance, increases the hydrodynamic diameter through the polymer layer, whereas ligand conjugation may cause slight aggregation, necessitating careful optimization (Li *et al*., 2022).

Surface morphology and structural integrity

Surface morphology is a key indicator of liposome stability and functionality, and surface modifications such as PEGylation or ligand conjugation can change surface properties, warranting detailed characterization. TEM and SEM are widely used to visualize liposome morphology; cryo-TEM in particular yields high-resolution images of vesicles in their native state, enabling assessment of structural integrity and surface modification (Grüll & Langereis, 2012). Atomic force microscopy (AFM) provides three-dimensional topographical images of liposome surfaces, revealing surface roughness and the presence of ligands or polymers (Wang *et al*., 2023).

Zeta potential and surface charge

Zeta potential reflects the surface charge and colloidal stability of liposomes and governs their interactions with biological membranes and their propensity to aggregate. It is typically measured by electrophoretic light scattering; a zeta potential greater than +30 mV or less than −30 mV is generally

associated with good colloidal stability in electrostatically stabilized liposomal dispersions because of increased electrostatic repulsion between vesicles. Conversely, formulations with zeta potentials close to 0 mV may be more susceptible to aggregation, although stability also depends on lipid composition, ionic strength, pH, and the presence of steric stabilizers (Németh *et al*., 2022; Zhang *et al*., 2022).

Surface modification shifts zeta potential: PEGylation generally lowers it through the shielding effect of the polymer layer, whereas conjugation of cationic ligands raises it, enhancing cellular uptake through electrostatic interactions (Li *et al*., 2022).

Encapsulation efficiency and drug-release profile

Encapsulation efficiency (EE) and release kinetics are central to evaluating SML performance. EE is determined by separating unencapsulated drug from the vesicles by dialysis, centrifugation, or size-exclusion chromatography and quantifying the drug by spectrophotometry or high-performance liquid chromatography (HPLC) (Patil & Jadhav, 2014). *In vitro* release is commonly studied using dialysis bags or Franz diffusion cells under simulated physiological conditions; the release profile depends on liposome composition, surface modification, and environmental factors such as pH and temperature (Gupta *et al*., 2021).

In vitro and in vivo performance

The ultimate objective of SMLs is targeted delivery with minimal off-target effects, evaluated through *in vitro* and *in vivo* studies. *In vitro*, cellular uptake and cytotoxicity assays in cell lines assess targeting efficiency and biocompatibility, often with fluorescent or radiolabels to track uptake (Wang *et al*., 2023). *In vivo*, animal models are used to evaluate bio distribution, pharmacokinetics, and therapeutic efficacy, with imaging techniques such as fluorescence imaging, magnetic resonance imaging (MRI), and positron emission tomography (PET) enabling real-time monitoring of liposome behavior (Li *et al*., 2022).

Stability and storage considerations

The stability of SMLs is a critical determinant of clinical translation, and comprehensive assessment of physical, chemical, and biological stability under varied storage conditions is required.

Physical stability is monitored through changes in size, zeta potential, and drug leakage over time. PDI is an important attribute because shifts in the size distribution may signal aggregation or other physical instability during storage. Drug and lipid-mass-balance assessment helps track formulation integrity, material recovery, and potential losses during manufacture and storage, and rigorous *in vitro* release testing should be incorporated into stability evaluation to detect changes in release kinetics over time. Where appropriate, *in vitro* release methods should maintain sink conditions and minimize membrane-related artifacts; Bio-Beads SM-2 resin has been used to maintain sink conditions and measure release from a clinically approved liposomal product (Atre & Rizvi, 2024; Juang *et al*., 2026). Lyophilization is frequently employed to extend shelf life (Guimarães *et al*., 2021).

Chemical stability is assessed by monitoring the degradation of phospholipids and encapsulated drugs using HPLC and mass spectrometry (Suk *et al*., 2016).

Biological stability concerns the interaction of liposomes with serum proteins and their susceptibility to enzymatic degradation, both of which bear on *in vivo* persistence (Almeida *et al*., 2021). Particular attention should be paid to the ABC phenomenon associated with

repeated dosing of PEGylated liposomes: although PEGylation reduces protein adsorption and prolongs circulation, repeated exposure may induce anti-PEG IgM, activating complement and accelerating clearance of subsequent doses, thereby shortening circulation time and potentially compromising exposure (Takata *et al*., 2023; Wang *et al*., 2023). This "PEG dilemma" is an important consideration for the biological stability and clinical utility of repeatedly administered PEGylated formulations, and alternative surface-engineering strategies, including zwitterionic materials, are being explored to sustain circulation while limiting PEG-associated immunogenicity (Ouyang *et al*., 2024).

Clinical Applications of Surface-Modified Liposomes

Surface-modified liposomes are increasingly used in the clinic, where their enhanced stability, specificity, and efficacy address the inherent limitations of conventional liposomes and have enabled several successful applications across diverse diseases.

Cancer therapy

Surface-modified and other clinically established liposomal formulations have improved the delivery of chemotherapeutic agents. PEGylated liposomal doxorubicin (Doxil®) has been used to treat ovarian cancer, multiple myeloma, and AIDS-related Kaposi's sarcoma; PEGylation prolongs systemic circulation and alters the pharmacokinetic and biodistribution profile of doxorubicin. Clinical application has since expanded well beyond Doxil®. Onivyde® (nanoliposomal irinotecan) was approved in 2015 for use with fluorouracil and leucovorin in metastatic pancreatic adenocarcinoma after gemcitabine-based therapy; in the phase III NAPOLI-1 trial, the combination significantly improved overall survival relative to fluorouracil and leucovorin alone (Wang-Gillam *et al*., 2016). Similarly, Vyxeos® (CPX-351), a fixed-ratio liposomal formulation of daunorubicin and cytarabine, was approved in 2017 for adults with newly diagnosed therapy-related AML or AML with myelodysplasia-related changes; in a randomized phase III trial, CPX-351 produced superior overall survival to conventional cytarabine plus daunorubicin in older patients with secondary AML (Lancet *et al*., 2018). These successes illustrate the widening therapeutic utility of liposomal platforms beyond liposomal doxorubicin.

Infectious diseases

Ligand-modified liposomes can direct antibiotics to infected cells, potentially improving outcomes and reducing side effects. Mannose-modified liposomes, for example, have been investigated for targeting macrophages infected with Mycobacterium tuberculosis, enhancing intracellular delivery of anti-tuberculosis drugs (Shrivastava *et al*., 2021).

Cardiovascular diseases

Surface-modified liposomes are also being explored for cardiovascular therapy. PEGylated liposomes can deliver anti-inflammatory agents and thrombolytics with improved pharmacokinetic profiles and a reduced risk of systemic side effects (Liu *et al*., 2024).

Neurological disorders

The blood-brain barrier (BBB) poses a major obstacle to drug delivery to the brain. SMLs, for instance, those conjugated with transferrin or other BBB-crossing ligands, have been developed to deliver therapeutics for neurological conditions such as Alzheimer's disease and brain tumors (Moreira *et al*., 2024). These modifications improve liposome targeting and penetration into brain tissue, enhancing efficacy.

Gene therapy

Cationic liposomes are widely used in gene therapy to deliver nucleic acids such as DNA and siRNA into cells. Surface modifications, including PEGylation and ligand conjugation, improve the stability and targeting of these delivery systems; folate-modified liposomes, for example, have been used to target cancer cells overexpressing folate receptors, improving the delivery of therapeutic genes (Hoffman *et al*., 2022).

Immunotherapy

Liposomes are used in immunotherapy to deliver vaccines and immunomodulatory agents. Surface modifications such as the incorporation of immunostimulatory molecules or targeting ligands can enhance the immune response and direct delivery to specific immune cells. PEGylated liposomes bearing antigens or adjuvants have been investigated for cancer immunotherapy and for vaccines against infectious diseases (Alhamhoom *et al*., 2023).

Anti-inflammatory treatments

SMLs can target inflamed tissues, delivering anti-inflammatory drugs directly to the site of inflammation. Liposomes modified with hyaluronic acid, for example, have been used to engage CD44-overexpressing inflammatory cells, improving drug localization and efficacy (Cannito *et al*., 2022).

Surface-Modified Liposomes in Drug Delivery Applications

Surface-modified liposomes have become a transformative tool in drug delivery, enhancing therapeutic efficacy through targeted delivery and reduced systemic toxicity. Although phospholipid vesicles are inherently suited to encapsulating hydrophilic and hydrophobic agents, unmodified liposomes are limited by rapid RES clearance and nonspecific bio distribution (Beloqui *et al*., 2016). Surface modifications like PEG coating, ligand conjugation, and the integration of stimuli-responsive polymers have been developed to improve stability, prolong circulation, and enable site-specific delivery (Alavi *et al*., 2021).

PEGylation, the covalent attachment of PEG chains to the liposomal surface, remains a cornerstone strategy, generating a hydrophilic stealth layer that reduces opsonization and RES uptake. PEGylated liposomal doxorubicin (Doxil®) exemplifies the clinical benefit, showing prolonged circulation and reduced cardiotoxicity relative to conventional doxorubicin (Bulbake *et al*., 2020). However, anti-PEG immune responses have emerged as a limitation, prompting the exploration of alternative polymers such as poly (2-oxazoline) (POx) to mitigate immunogenicity (Suk *et al*., 2016).

Ligand-mediated targeting further enhances specificity: antibodies, peptides, or aptamers conjugated to liposomes enable active targeting of receptors overexpressed on diseased cells. Trastuzumab-modified immunoliposomes, for example, selectively deliver chemotherapeutics to HER2-positive breast cancer cells, with enhanced efficacy attributable to targeted delivery in preclinical models (Park *et al*., 2002). Similarly, transferrin-conjugated liposomes exploit upregulated transferrin receptors in glioblastoma, enhancing penetration of the blood–brain barrier (Ying *et al*., 2010).

Stimuli-responsive liposomes, engineered to release their payload in response to pH, temperature, or enzymatic triggers, offer spatiotemporal control. pH-sensitive liposomes destabilize in the acidic tumor microenvironment, ensuring intracellular release while minimizing off-target effects

(Karanth & Murthy, 2007); such systems are under evaluation in pancreatic and other cancer models (Wang *et al*., 2022).

The utility of nanoscale delivery extends beyond oncology to infectious disease, where lipid-based and polymeric nanocarriers have been investigated to improve the delivery and bioavailability of antimicrobials. These systems should, however, be distinguished from SMLs, because their structural organization and physicochemical properties differ from those of phospholipid-bilayer vesicles. Mannosylated solid lipid nanoparticle assemblies, for example, have been studied for targeted delivery of rifampicin to alveolar macrophages in pulmonary tuberculosis; surface decoration with mannose enhanced macrophage uptake and pulmonary retention, demonstrating the potential of solid lipid nanoparticles as an alternative lipid-based platform (Maretti *et al*., 2017; Truzzi *et al*., 2020). More recently, β-glucan-functionalized chitosan–PLGA nanoparticles have been examined as polymeric carriers for pulmonary rifampicin delivery, with weekly inhalation producing sustained pulmonary exposure and antibacterial efficacy in murine tuberculosis models (Kutscher *et al*., 2026). These findings highlight the broader potential of lipid-based and polymeric nanocarriers while underscoring the need to distinguish them from phospholipid-bilayer SMLs.

Despite notable advances, important challenges persist in clinical translation: scale-up complexity, batch-to-batch variability, and long-term instability must all be addressed. A further challenge is the ABC phenomenon associated with repeated dosing of PEGylated liposomes. Although PEGylation reduces opsonization and prolongs circulation after the first dose, repeated exposure may induce anti-PEG antibodies particularly IgM that activate complement and hasten clearance of later doses, reducing exposure and efficacy (Gaballa *et al*., 2024; Ouyang *et al*., 2024). This "PEG dilemma" has stimulated interest in zwitterionic polymers, whose strong hydration and antifouling properties make them candidate PEG replacements capable of sustaining circulation while limiting ABC (Ouyang *et al*., 2024). Future development of SMLs will therefore require not only scalable manufacture and adequate stability but also careful attention to immunogenicity and repeat-dose pharmacokinetics. SMLs remain a versatile platform for precision medicine, but successful translation will depend on balancing circulation time, immune compatibility, targeting performance, manufacturability, and long-term stability.

Continued innovation in polymer chemistry, ligand engineering, and stimuli-responsive design will further optimize therapeutic potential and address unmet needs across diverse diseases. In parallel, the regulatory development of complex liposomal products demands rigorous characterization of CQAs and appropriately validated, discriminatory *in vitro* release methods to ensure quality, reproducibility, and consistency. *In vitro* release method development should account for sink conditions, release medium, agitation, and separation procedures to minimize analytical artifacts and reliably distinguish formulation or manufacturing differences that may affect performance (Giordani *et al*., 2023; U.S. Food and Drug Administration [FDA], 2018). Sink-maintaining approaches such as Bio-Beads SM-2 resin have shown promise for the *in vitro* release testing of liposomal irinotecan and may prove useful for quality control and regulatory evaluation (Juang *et al*., 2026). Such advances will be important for translating SMLs from the laboratory into reproducible, clinically viable products.

Conclusion

Surface-modified liposomes hold considerable promise for transforming targeted drug delivery. Diverse modification strategies incorporating ligands, antibodies, peptides, and polymers improve delivery efficiency and enable targeted intervention, and they have been shown to enhance stability, prolong circulation, and increase cellular uptake, with corresponding gains in therapeutic efficacy. Recent advances, such as the integration of stimuli-responsive materials and externally triggered systems, offer further opportunities for controlled, site-specific release. At the same time, challenges like scalability, batch-to-batch reproducibility, biocompatibility, immunogenicity (including the ABC phenomenon associated with PEGylation), and regulatory approval must be resolved before widespread clinical translation can be achieved. Continued research into, and development of, surface-modified liposomes nonetheless hold great promise for overcoming the limitations of conventional drug delivery and improving the targeted treatment of a wide range of diseases.

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