

Nanoliposomes for Site-Specific Drug Delivery in Cancer Therapy: A Comprehensive Review

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ABSTRACT

Cancer remains one of the leading causes of mortality worldwide, and conventional chemotherapy often suffers from lack of specificity, systemic toxicity, and multidrug resistance. Nanoliposomes—submicron vesicular systems composed of lipid bilayers—have emerged as a promising nanocarrier for targeted cancer therapy. This review focuses on the advancements, mechanisms, and applications of nanoliposomes for site-specific drug delivery in cancer, highlighting design strategies, targeting approaches, therapeutic efficacy, and future prospects.

KEYWORDS: Nanocarrier, Liposomes, Sitespecific, Phospholipid, Cancer

I. INTRODUCTION

Cancer remains one of the leading causes of mortality worldwide, and chemotherapy continues to be a mainstay in its treatment. However, conventional chemotherapeutic agents often suffer from several drawbacks, primarily due to their non-specific biodistribution, which leads to severe toxicity in healthy tissues, low therapeutic indices, and poor patient compliance [1]. Moreover, the development of multidrug resistance further complicates effective treatment [2]. To overcome these challenges, nanotechnology-based drug delivery systems have garnered significant attention for their ability to enhance therapeutic efficacy while minimizing systemic side effects [3].

Among these, nanoliposomes have emerged as one of the most promising nanocarriers. Nanoliposomes are submicron-sized, phospholipid-based vesicles, generally ranging between 50 to 200 nm in diameter, capable of encapsulating both hydrophilic and lipophilic drugs [4]. Due to their structural similarity to biological membranes, they offer excellent biocompatibility, biodegradability, and reduced immunogenicity [5]. Furthermore, nanoliposomes enhance drug solubility and stability, prolong systemic circulation time, and allow for controlled and sustained drug release [6].

Importantly, nanoliposomes can facilitate both passive and active targeting strategies. Passive targeting relies on the enhanced permeability and retention (EPR) effect, which allows nanoparticles to accumulate preferentially in tumor tissues due to their leaky vasculature and poor lymphatic drainage [7]. In contrast, active targeting involves the functionalization of liposome surfaces with ligands such as antibodies, peptides, or aptamers, which bind specifically to overexpressed receptors on cancer cells, thereby improving cellular uptake and therapeutic specificity [8].

The convergence of liposomal drug delivery technology with advances in molecular targeting and bioengineering has opened new frontiers in personalized and precision oncology. Several liposomal formulations have already gained regulatory approval or entered advanced clinical trials, reinforcing the translational potential of this approach [9]. This review highlights the current progress in the development of nanoliposomes for site-specific drug delivery in cancer therapy, emphasizing their design strategies, targeting mechanisms, clinical applications, and future directions.

II. NANOLIPOSOME STRUCTURE AND COMPOSITION

Nanoliposomes are nanoscale lipid vesicles composed of one or more concentric phospholipid bilayers that enclose an aqueous interior. Their ability to encapsulate both hydrophilic and hydrophobic drugs makes them highly versatile for cancer therapy. Hydrophilic drugs reside in the aqueous core, while hydrophobic or amphiphilic drugs integrate within the lipid bilayer. This dual-drug loading ability makes nanoliposomes ideal carriers for combination therapies. Their small size—typically 50 to 200 nm—facilitates enhanced permeability and retention (EPR) in tumor tissues, promoting passive accumulation at tumor sites [8].

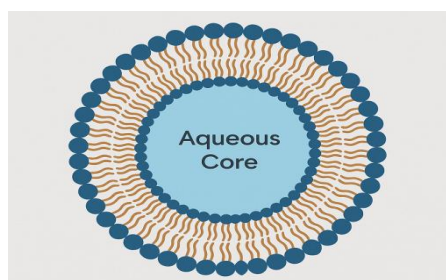


Figure 1: Nanoliposome

heads and hydrophobic tails, allowing spontaneous bilayer formation in aqueous environments. Common examples include phosphatidylcholine (PC), phosphatidylglycerol (PG), and synthetic lipids such as distearoylphosphatidylcholine (DSPC) and dipalmitoylphosphatidylcholine (DPPC) [9]. The lipid composition affects bilayer stability, fluidity, and drug encapsulation efficiency. Natural phospholipids offer good biocompatibility, while synthetic variants provide greater control over membrane properties and transition temperatures [10].

Phospholipids

Phospholipids are the foundational components of nanoliposomes. They are amphiphilic molecules consisting of hydrophilic

Table: Common Phospholipids Used in Nanoliposomes

Phospholipid	Type	Source	Phase Transition Temp (°C)	Key Properties
Phosphatidylcholine (PC)	Natural/Semi-Synthetic	Egg, Soy, or Synthetic	-5 to -20 (natural)	High biocompatibility, forms stable bilayers, widely used in drug delivery
Phosphatidylglycerol (PG)	Natural	Bacterial/Soy	~-10 to 20	Negatively charged; improves stability and surface charge of liposomes
Distearoylphosphatidylcholine (DSPC)	Synthetic	Chemically Synthesized	~55	High T _m ; provides rigid bilayer, good for sustained drug release
Dipalmitoylphosphatidylcholine (DPPC)	Synthetic	Chemically Synthesized	~41	Thermoresponsive, used in temperature-sensitive liposomes
Hydrogenated Soy PC (HSPC)	Semi-Synthetic	Hydrogenated Soy	~52	High stability, long-circulating formulations, used in Doxil®
Phosphatidylethanolamine (PE)	Natural/Synthetic	Bacterial/Soy	~-16 to 63 (varies)	Often used in pH-sensitive liposomes;

				fusogenic lipid enhancing endosomal escape
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Notes:

- **Phase Transition Temperature (T_m)** indicates the temperature at which the lipid transitions from a gel to a liquid-crystalline phase, impacting membrane fluidity and drug release.
- **Natural lipids** (like egg-PC) are typically more fluid and biocompatible but less stable.
- **Synthetic lipids** (like DSPC, DPPC) offer better control over membrane rigidity and drug retention.

Cholesterol

Cholesterol plays a crucial role in enhancing the structural integrity of liposomal membranes. By embedding itself within the phospholipid bilayer, it modulates membrane fluidity, reduces permeability, and prevents premature leakage of encapsulated drugs [11]. Cholesterol helps maintain bilayer stability at physiological temperatures and during systemic circulation. The typical cholesterol-to-phospholipid molar ratio ranges from 1:2 to 1:1, depending on the desired rigidity and release profile [12].

PEGylation

To improve circulation time and evade immune system detection, nanoliposomes are often modified with polyethylene glycol (PEG)—a strategy known as PEGylation. PEG chains form a hydrophilic, steric barrier around the liposome, reducing opsonization by plasma proteins and subsequent uptake by the mononuclear phagocyte system (MPS) [13]. This modification allows the liposomes to circulate for extended periods, increasing their chance of accumulating in tumors via the EPR effect. PEGylated liposomal doxorubicin (Doxil®) is a clinically successful example of this approach [14].

Ligand Conjugation

Active targeting of tumor tissues can be achieved by decorating the liposomal surface with ligands that bind specifically to receptors overexpressed on cancer cells. Common targeting moieties include monoclonal antibodies (e.g., trastuzumab for HER2), peptides (e.g., RGD peptide for integrins), folic acid (for folate

receptors), and aptamers [15]. Ligand-receptor interactions facilitate receptor-mediated endocytosis, allowing selective internalization of the nanoliposomes by tumor cells. This approach enhances intracellular drug delivery and reduces systemic toxicity [16].

III. METHODS OF PREPARATION

The method of preparation plays a critical role in determining the physicochemical characteristics of nanoliposomes, including their size, lamellarity, surface charge, encapsulation efficiency, and stability. Different techniques are selected based on the nature of the drug to be encapsulated, the desired release profile, and the scale of production. Below are the major methods used for nanoliposome synthesis:

Thin-Film Hydration Method (Bangham Method)

The thin-film hydration method is the most widely used and classical technique for liposome preparation. In this method, lipids (such as phosphatidylcholine and cholesterol) are dissolved in an organic solvent like chloroform or methanol and then evaporated under reduced pressure using a rotary evaporator to form a thin, dry lipid film on the flask wall. This lipid film is then hydrated using an aqueous buffer containing the drug, leading to the spontaneous formation of multilamellar vesicles (MLVs). These are then downsized to nanoliposomes through sonication or extrusion through polycarbonate membranes with defined pore sizes [17]. The advantages of this method include simplicity, scalability for laboratory use, and versatility for both hydrophilic and lipophilic drugs. However, it may suffer from low encapsulation efficiency for hydrophilic drugs and batch-to-batch variability [18].

Ethanol Injection Method

The ethanol injection method involves the rapid injection of a lipid solution (lipids dissolved in ethanol) into an aqueous phase containing the drug under constant stirring. Upon contact with water, the lipids self-assemble into small unilamellar vesicles (SUVs). This method is simple, rapid, and does not require high

temperatures, making it suitable for thermolabile drugs [19]. The major advantage of this method is its ability to produce liposomes with relatively small sizes and narrow size distribution without the need for post-processing like sonication. However, the residual ethanol needs to be removed to avoid cytotoxicity, and the method is generally more suitable for hydrophobic drugs [20].

Reverse-Phase Evaporation Method

The reverse-phase evaporation technique is used to achieve high encapsulation efficiency, particularly for water-soluble drugs. It involves the formation of a water-in-oil (w/o) emulsion by sonicating an aqueous drug solution with a lipid solution in organic solvent (such as diethyl ether or isopropyl ether). The organic phase is then gradually removed by rotary evaporation under reduced pressure, leading to the formation of a gel-like phase that collapses into large unilamellar vesicles (LUVs) [21]. This method provides high aqueous drug entrapment and is suitable for

forming unilamellar vesicles. However, the use of organic solvents may raise toxicity and regulatory concerns, especially in pharmaceutical applications [22].

Microfluidic Synthesis

Microfluidic methods have gained prominence for the controlled, continuous, and scalable production of nanoliposomes. In this technique, lipids in organic solvent and the aqueous drug solution are introduced into microchannels where controlled mixing occurs under laminar flow conditions. This results in the rapid self-assembly of nanoliposomes with precise control over size, distribution, and encapsulation efficiency [23]. Microfluidics offers reproducibility, high-throughput capability, and minimal batch variation—features essential for clinical translation and commercial production. Moreover, the process can be automated and integrated with in-line quality control tools [24].

Table: Comparison of Nanoliposome Preparation Methods

Method	Key Principle	Advantages	Limitations	Suitable For
Thin-Film Hydration (Bangham Method)	Lipid film hydration followed by sonication or extrusion	Simple, widely used; accommodates hydrophilic/lipophilic drugs; scalable	Low encapsulation for hydrophilic drugs; batch variability	Lab-scale research, general-purpose
Ethanol Injection	Rapid mixing of lipid ethanol solution into aqueous phase	Fast; no heat needed; small vesicles with narrow size range	Ethanol must be removed; not ideal for large-scale; limited to lipophilic drugs	Thermolabile/lipophilic drug delivery
Reverse-Phase Evaporation	Water-in-oil emulsion followed by solvent evaporation	High encapsulation of hydrophilic drugs; forms large unilamellar vesicles	Organic solvent toxicity concerns; complex process	Water-soluble drug delivery
Microfluidic Synthesis	Controlled laminar flow of lipid and aqueous streams in microchannels	Precise size control; scalable; reproducible; suitable for automation	Requires microfluidic setup; optimization may be complex	Clinical-scale, high-throughput production

IV. PASSIVE AND ACTIVE TARGETING MECHANISMS

Targeted drug delivery is one of the most advantageous features of nanoliposomes in cancer therapy. By exploiting the unique characteristics of tumor tissues and combining them with molecular targeting strategies, nanoliposomes can achieve

site-specific drug accumulation and improve therapeutic efficacy while minimizing damage to healthy tissues.

4.1 Passive Targeting

Passive targeting is based on the Enhanced Permeability and Retention (EPR) effect, a

physiological phenomenon unique to solid tumors. Tumor vasculature is typically disorganized, with large fenestrations and poor lymphatic drainage. These abnormalities allow nanoparticles like nanoliposomes to extravasate and accumulate preferentially in tumor tissues [25]. Due to their nanoscale size (50–200 nm), nanoliposomes can exploit the EPR effect to achieve substantial intratumoral accumulation, thereby enhancing local drug concentration while reducing systemic toxicity. Moreover, PEGylation of the liposomal surface helps prolong circulation time, which further facilitates accumulation at the tumor site through passive mechanisms [26]. However, the EPR effect can be highly variable among tumor types and between patients, posing challenges to reproducibility and therapeutic predictability [27].

4.2 Active Targeting

Active targeting enhances the specificity of nanoliposomes by decorating their surface with ligands that bind selectively to overexpressed receptors on cancer cells. This ligand-receptor interaction facilitates receptor-mediated endocytosis, leading to higher intracellular drug delivery and improved therapeutic outcomes. Several classes of ligands have been successfully employed for this purpose:

- **Monoclonal antibodies:** For example, trastuzumab is a humanized monoclonal antibody used to target HER2 receptors, which are overexpressed in certain types of breast and gastric cancers. Trastuzumab-conjugated liposomes have shown increased specificity and therapeutic benefit in HER2-positive tumors [28].
- **Peptides:** The RGD (Arg-Gly-Asp) peptide is widely used for targeting $\alpha v \beta 3$ integrins, which are commonly upregulated in angiogenic tumor vasculature. RGD-conjugated nanoliposomes enhance targeting to tumor endothelial cells and facilitate deeper penetration into tumor tissue [29].
- **Small molecules and aptamers:** Folic acid is a popular targeting moiety due to the overexpression of folate receptors in various cancers, including ovarian, breast, and lung cancer. Nanoliposomes functionalized with folic acid exhibit enhanced uptake in folate receptor-positive cells. Similarly, aptamers—short, single-stranded nucleic acids—offer high binding affinity and specificity with low immunogenicity, making them suitable for targeting specific tumor markers [30].

Active targeting significantly improves the cellular internalization of nanoliposomes and facilitates intracellular drug release, especially when combined with pH-sensitive or stimuli-responsive delivery systems. Nevertheless, the success of active targeting depends on the density and accessibility of target receptors and may require personalization for optimal results [31].

V. FUNCTIONALIZATION STRATEGIES

Surface functionalization of nanoliposomes plays a crucial role in improving their biological performance, including enhanced circulation time, tumor-specific accumulation, and controlled drug release. Advanced surface engineering techniques allow nanoliposomes to evade immune detection, penetrate solid tumors more effectively, and respond to unique tumor microenvironmental cues, thereby enhancing therapeutic efficacy and reducing systemic toxicity.

PEGylation

One of the most widely used surface modification techniques is PEGylation, which involves grafting polyethylene glycol (PEG) chains onto the surface of liposomes. PEG forms a hydrophilic, steric barrier around the nanoliposome, which reduces protein adsorption (opsonization) and subsequent recognition by the mononuclear phagocyte system (MPS) [32]. This stealth behavior prolongs systemic circulation time, allowing more nanoliposomes to accumulate in tumor tissues via the enhanced permeability and retention (EPR) effect. Clinically approved formulations like Doxil® have demonstrated the success of PEGylation in achieving long-circulating, tumor-targeted delivery [33]. However, repeated administration may lead to accelerated blood clearance (ABC) due to anti-PEG antibodies, which has prompted further research into PEG alternatives [34].

Stimuli-Responsive Liposomes

To achieve on-demand drug release, stimuli-responsive liposomes have been developed to respond to specific internal or external stimuli. Internal triggers include pH, enzymatic activity, and redox potential, while external triggers may involve temperature, ultrasound, or light [35]. For instance, redox-sensitive liposomes are functionalized with disulfide bonds that cleave in the high-glutathione environment inside tumor

cells, triggering drug release. Similarly, enzyme-sensitive liposomes incorporate peptide linkers that are cleaved by matrix metalloproteinases (MMPs), which are overexpressed in many tumors [36]. These smart liposomes enhance therapeutic precision by ensuring that drug release occurs only at the tumor site.

pH-Sensitive Liposomes

Among internal stimuli, pH-sensitivity is one of the most exploited features in cancer drug delivery. The extracellular pH of tumor tissues is typically more acidic (pH ~6.5) than that of normal tissues (pH ~7.4), and the endosomal/lysosomal compartments within cancer cells have even lower pH values (pH ~5.0–5.5). pH-sensitive liposomes are designed with lipids or polymers that destabilize or undergo conformational changes in acidic conditions, leading to membrane disruption and rapid drug release [37]. For example, liposomes containing phosphatidylethanolamine (PE) combined with mildly acidic pH-labile polymers such as CHEMS (cholesteryl hemisuccinate) have demonstrated efficient release of encapsulated agents in the acidic tumor environment [38]. This selective release profile enhances cytotoxicity toward cancer cells while sparing healthy tissues.

VI. THERAPEUTIC APPLICATIONS IN CANCER

Nanoliposomes have emerged as powerful nanocarriers for the delivery of a wide range of anticancer agents. Their ability to encapsulate both hydrophilic and hydrophobic drugs, improve pharmacokinetics, and reduce systemic toxicity makes them highly attractive in oncology. Liposomal formulations can also bypass drug resistance mechanisms, enhance intracellular uptake, and deliver therapeutics specifically to the tumor microenvironment, improving overall treatment outcomes.

Doxorubicin (e.g., Doxil®)

One of the most prominent examples of nanoliposomal cancer therapy is Doxil®, the first FDA-approved PEGylated liposomal formulation of doxorubicin. Doxil® significantly reduces the cardiotoxicity commonly associated with conventional doxorubicin therapy by altering the drug's biodistribution and limiting its exposure to healthy cardiac tissue [39]. The PEGylated surface of Doxil® prolongs its circulation time, allowing passive accumulation in tumor tissues through the

EPR effect. It has demonstrated clinical success in treating breast cancer, ovarian cancer, and Kaposi's sarcoma, highlighting the potential of nanoliposomes to transform conventional chemotherapeutics into safer and more effective therapies [40].

Paclitaxel, Cisplatin, and Curcumin

Several other chemotherapeutic agents have also benefited from nanoliposomal delivery. Paclitaxel, a poorly water-soluble drug, faces formulation challenges and dose-limiting toxicity. Liposomal encapsulation enhances its solubility and enables safer intravenous administration with reduced hypersensitivity reactions [41]. Similarly, cisplatin, a widely used platinum-based chemotherapeutic, can be incorporated into liposomes to reduce nephrotoxicity and improve tumor selectivity [42]. Natural compounds like curcumin, known for their anti-inflammatory and anticancer properties, suffer from poor bioavailability and rapid systemic clearance. Liposomal formulations of curcumin have demonstrated improved stability, tumor targeting, and anticancer efficacy in preclinical models [43].

siRNA and Gene Therapies

Nanoliposomes also offer a protective and efficient platform for delivering small interfering RNA (siRNA), plasmid DNA, and other gene-based therapeutics. These molecules are highly susceptible to degradation by nucleases in the bloodstream and exhibit poor cellular uptake. Liposomal encapsulation protects nucleic acids from enzymatic degradation and facilitates endosomal escape after cellular uptake [44]. Functionalized liposomes can also deliver genetic material specifically to tumor cells via ligand-mediated targeting, improving transfection efficiency and therapeutic outcomes. For instance, targeted nanoliposomes carrying siRNA against oncogenes such as VEGF, Bcl-2, or KRAS have shown promising results in silencing gene expression and inhibiting tumor progression in experimental cancer models [45].

In summary, nanoliposomes have proven to be versatile and effective carriers for a wide range of therapeutic agents in cancer treatment. Their success in clinical and preclinical studies underscores their potential to improve efficacy, reduce side effects, and enable the delivery of advanced biologics like gene therapies.

VII. CHALLENGES AND LIMITATIONS

Despite the promising potential of nanoliposomes in cancer therapy, several challenges and limitations hinder their widespread clinical use. One major issue is drug leakage during storage, especially for lipophilic or amphiphilic drugs, which may compromise therapeutic efficacy and shelf stability. This instability is influenced by lipid composition, temperature, and the physicochemical properties of the encapsulated drug [46].

Another significant concern is rapid clearance by the reticuloendothelial system (RES). Although PEGylation helps reduce RES uptake, some liposomes may still be recognized and cleared by macrophages in the liver and spleen, particularly after repeated dosing due to the “accelerated blood clearance” (ABC) phenomenon [47]. This shortens circulation half-life and limits therapeutic accumulation in tumors.

Scale-up complexities also pose technical barriers. While nanoliposomes can be efficiently produced on a laboratory scale, translating these methods to industrial production requires precise control of size, lamellarity, and encapsulation efficiency. Batch-to-batch consistency remains a critical issue due to variations in processing parameters like temperature, shear force, and lipid hydration time [48].

Moreover, regulatory hurdles complicate the path to commercialization. Regulatory agencies require detailed characterization of particle size, zeta potential, release kinetics, and sterility. The complexity of liposomal systems, including their dynamic behavior in biological environments, makes batch-to-batch reproducibility challenging and necessitates robust quality control protocols [49].

VIII. CLINICAL STATUS AND REGULATORY OUTLOOK

Several nanoliposomal formulations have advanced to clinical trials or achieved regulatory approval, validating the feasibility of this drug delivery approach. Notable examples include Doxil® (liposomal doxorubicin), which was the first nanomedicine approved by the U.S. FDA, and Onivyde® (liposomal irinotecan), approved for metastatic pancreatic cancer. These products have demonstrated superior pharmacokinetics, reduced off-target toxicity, and improved patient outcomes compared to conventional chemotherapy [50].

Regulatory agencies such as the FDA and EMA emphasize the importance of comprehensive physicochemical characterization, including size distribution, encapsulation efficiency, sterility, and in vitro release kinetics. Guidelines also require demonstration of stability under different storage conditions, as well as reproducibility between manufacturing batches [51]. Regulatory science is still evolving to adapt to the complexity of nanomedicines, but the successful approval of liposomal products has paved the way for future innovations in this space.

IX. FUTURE PERSPECTIVES

The field of nanoliposome-based drug delivery is rapidly evolving, with emerging trends focused on increasing precision, functionality, and therapeutic versatility. One major direction is the development of multifunctional nanoliposomes, which integrate imaging agents (e.g., fluorescent dyes, MRI contrast agents) and therapeutic compounds for theranostic applications, enabling real-time tracking and treatment [52].

There is also growing interest in personalized nanomedicine, where nanoliposomes are customized with patient-specific ligands or biomarker-guided payloads to target tumors more precisely and reduce variability in treatment response [53]. Advances in molecular biology have enabled the loading of nanoliposomes with gene-editing tools such as CRISPR/Cas9, siRNA, and mRNA, offering new avenues for correcting oncogenic mutations at the genetic level [54].

Moreover, artificial intelligence (AI) and machine learning are being integrated into liposome design and optimization. AI-driven platforms can predict optimal lipid compositions, drug-lipid ratios, and targeting ligand configurations to enhance efficacy and reduce trial-and-error during formulation development [55]. As the field matures, these innovations are expected to overcome existing limitations and usher in a new era of smart, personalized, and adaptive cancer nanotherapy.

X. CONCLUSION

Nanoliposomes offer a highly versatile and effective platform for targeted cancer therapy, with the potential to improve therapeutic outcomes and reduce systemic toxicity. With continued advances in material science, drug loading technologies, and targeting strategies, nanoliposomes are poised to play a central role in

the next generation of personalized oncological treatments.

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