RESEARCH PAPER

Formulation And In Vitro And In Vivo Evaluation Of Ligand-Functionalized Liposomes For Targeted Cancer Therapy

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Abstract

The traditional chemotherapy has weaknesses on non-specific biodistribution, severe systemic toxicity, and optimum tumor accumulation. In a bid to overcome these obstacles, the present research was aimed at developing and in vitro assessing PEGylated liposomes as a core system to target cancer therapy, where Irinotecan hydrochloride, a prototype chemotherapeutic agent, was used in the study. The liposomes were prepared successfully through the thin-film hydration and sonication technique using egg phosphatidylcholine, cholesterol and methylated polyethylene glycol (MPEG). The prepared vesicles had ideal nanoscale properties with a mean particle size of 152.3 ± 4.7 nm, polydispersity index of 0.18 ± 0.02, and zeta potential of -12.5 ±1.8 mV that are favorable to passive tumor targeting through Enhanced Permeability and Retention (EPR) effect. Efficiency of drug entrapment of 85.4 ± 2.1 percent was attained. The in vitro drug release studies indicated a sustained profile of up to 48 hours and stability studies showed that storage at 4degC was essential to the maintenance of integrity of formulation during a period of one month. A comparative cellular uptake study was modeled as a demonstration of active targeting using the HER2-positive (MCF-7/HER2+) and the HER2-negative (MDA-MB-231) breed cancer cell lines. The flow cytometry and confocal microscopy were performed to observe low and non-specific uptake of the non-targeted PEGylated liposomes in both cell lines, which demonstrates the property of stealthiness of the liposomes and the need to functionalize their surface. Finally, an Irinotecan liposomal nanocarrier characterized and stable was established. These findings support the platform as an emerging basis of future conjugation with any desired targeting ligand (e.g. anti-HER2 antibodies) to undergo receptor-mediated active targeting, which is a key step to improving therapeutic efficacy and specificity in oncology.

Keywords: Targeted drug delivery, Liposomes, Irinotecan hydrochloride, PEGylation, Nanomedicine, Cancer therapy

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Introduction

The undying quest to identify more effective and less toxic treatments to cancer has been an eminent challenge of contemporary medicine.[1][2] Although the traditional chemotherapy is revolutionary in its creation, it is essentially undermined by the lack of specificity. These powerful cytotoxic agents target all rapidly multiplying cells and thus yield catastrophic systemic toxicities including myelosuppression, nephrotoxicity, cardiotoxicity, and neurotoxicity that significantly reduces the quality of life of a patient and usually sets the maximum dose limit. Moreover, their pharmacokinetics is not generally favorable their small molecule drugs are quickly excreted by the kidneys or broken down, and their disordered distribution tends to lead to poor accumulation in the tumor tissue, This has to be high and frequent dosing to ensure that a therapeutic effect is attained at the site of disease further increasing the off-target damage.[3][4] The main issue, then, is one of delivery: how to focus a deadly dose on the right place and leave the healthy tissues intact.[5][6]

The challenge of this delivery triggered the development of nanomedicine, and one of the market leaders was a first-generation solution, liposomes. Liposomes are vesicles that are self-assembled, spherical and made up of a single or more layers of phospholipid bilayers with an aqueous interior. [7][8] They were biocompatible and biodegradable, which prequalified them as the ideal carriers of hydrophilic drugs (in the core) and hydrophobic drugs (within the bilayer). The breakthrough was the seminal one with the realization of the Enhanced Permeability and Retention (EPR) effect, which is a type of passive targeting. Tumours, to promote their intensive growth, form permeable, leaky vasculature interstitial between endothelial cells, and many do not have good lymphatic drainage.[9][10] This pathological anatomy permits particles of nano-size, such as liposomes (usually 50-200 nm) to extravasate and accumulate in the tumor interstitium, with the small size preventing their rapid renal clearance. Liposomes engineered alternative (stealth liposomes) by the additional polyethylene glycol (PEG) coating that extended the circulation time as opsonization and mononuclear phagocytic system uptake were avoided.[11]

Nevertheless, EPR effect is a heterogenous and inefficient phenomenon, whereby, the effects differ greatly between tumor types, individual patients, and even over different regions of the same tumor.[12] Passive accumulation is non-specific and is dependent on leakage and can still occur in inflammatory sites or other tissues with fenestrated vasculature. Furthermore, the liposomes confined in the tumor interstitium might not be absorbed by the cancer cells thus low rates of drug release and drug resistance are likely to be encountered. The urgent necessity of targeting the carrier to the cancer cell surface in a specific way and initiating the process of effective cellular uptake gave rise to the rationale of active targeting. It is a technique to label the surface of nanocarriers such as liposomes with targeting ligands (highly affinity receptors or antigens overexpressed on the surface of the cancer cell). The aim is to move the paradigm of collection to that of particular molecular recognition, which facilitates receptor-mediated binding and subsequent internalization (endocytosis) of the whole vehicle, therefore, delivering its cargo right into the cellular cytoplasm.[13]

This theoretical development leads to the second-generation Smart nanocarriers, which is denoted as ligand-functionalized liposomes. They are highly targeted liposomes that incorporate the passive targeting properties of the EPR effect and long circulation with the highly specific homing properties of a biological ligand.[14] The benefits are manifold: they can dramatically improve the effects of the therapy, as they will cause a higher concentration of drugs to accumulate within the cancer cells, they can severely decrease the level of systemic side effects, as they will eliminate off-target binding, and they may in fact overcome some forms of drug resistance, bypassing efflux pumps.[15] The clinical implication is deep and there are a number of candidates that have been taken through trials. As an example, an anti-HER2 antibody-targeted liposomal doxorubicin, MM-302, was created to tackle HER2-positive breast cancer potentially with enhanced specificity as compared to trastuzumab (Herceptin). Although the clinically useful development of targeted liposomes has not been smooth sailing, such as immune response and the biology of tumor cells, it still stands as the foundation of precision oncology, as a complex effort to transform chemotherapy into a more discriminating tool.[16]

These advanced therapeutic devices are developed in the form of multi-stage work, and each component is optimized carefully. It is based on the core liposome, which is a delivery vessel and the nature of which is determined by its lipid composition. The most commonly occurring backbone lipid is phosphatidylcholine (PC) which is a natural product such as egg yolk or soy that creates a biocompatible bilayer.[17] At molar ratios typically ranging between 30-50% that is essential to adjusting membrane fluidity and stability, cholesterol is required to occupy interstitial space between phospholipid chains, stabilizing permeability to small molecules (preventing premature drug leakage) and enhancing membrane rigidity against osmotic pressure and serum

proteins. PEGylated lipids are necessary in long circulating liposomes which are stealth liposomes.[18] PEG is a hydrophilic polymer which forms a dense hydrating cloud on the liposome surface hindering the adsorption of opsonins in a steric effect, increasing the circulation half-life by many days. The properties such as the phase transition temperature and stability also vary according to the choice of the length of the lipid chain and saturation.[19]

These components are assembled into the uniform drug-carrying vesicles using the well-established methods of preparation. The hydration of thin film is a traditional method in which lipids in organic solvent are evaporated in vacuum to create a thin film on the sides of a flask. When an aqueous buffer is added, the film becomes hydrated and spontaneously swells to give large, multilamellar vesicles (MLVs). [20] These MLVs need to be reduced in size to achieve the clinically necessary size of 80-120 nm. It is done by extrusion, in which the suspension is pressed repeatedly through polycarbonate membranes of specified pore size, producing small, unilamellar vesicles, or SUVs, with a slender size distribution, a key factor towards reproducible pharmacokinetics and tumor accumulation with EPR. Other techniques such as ethanol injection, whereby a lipid ethanol solution is injected into an aqueous layer might also allow the creation of liposomes in a mono step although further extrusion can be required to control size.[21]

After the empty liposome (or liposome) is made, the therapeutic agent has to be loaded. Passive loading is where the drug is loaded by incubating with liposomes during the process, a process that is easy but often the process has low encapsulation efficiency and high leakage that is only useful in a few drugs highly lipophilic drugs. Active loading is the gold standard in the case of hydrophilic drugs, especially weak base drugs such as doxorubicin or cisplatin.[22] It is a clever method that takes advantage of transmembrane gradients to entrap the drugs with high efficiency within the liposome. The pH-gradient technique involves acidification of liposome interior (e.g. citrate buffer, pH 4.0) and neutrality of the external medium. The non-polarized and membrane-pervious drug molecule breaches inward and becomes protonated, charged, and hence imprisoned in the acidic interior. A similar mechanism is employed in the ammonium sulfate method, where the intra-liposomal ammonium sulfate dissociates to ammonia (diffuses out) and protons to form an acidic interior with also outstanding encapsulation efficiencies (more than 90) and also stable retention in circulation.

These superior systems are characterized by the targeting ligand, which is a molecule selected based on its specific high-affinity binding to a tumor-associated antigen or receptor. Selection is the most important and it is based on aspects such as specificity in target expression, binding affinity, immunogenicity, size, and conjugation simplicity. The most specific is comprised of antibodies and their fragments. Full monoclonal antibodies (mAbs), including anti-HER2 (trastuzumab) or anti-EGFR (cetuximab) are highly specific, but large (about 150 kDa), which can affect liposome pharmacokinetics and can also cause immune response. In a bid to overcome this, smaller engineered fragments such as antigen-binding fragments (Fab'), single-chain variable fragments (scFv), or even nanobodies are used. These are binding-specific and have increased penetration and reduced immunogenicity.[23]

Another popular type of ligand is peptides, due to their small size, simplicity in production, and flexibility. This is achieved by the spacecraft-like motif of Arg-Gly-Asp (RGD), which specifically targets avb3 integrin, which is a receptor on the tumor endothelial cells and various cancer cells and is overexpressed in tumor endothelial cells, thereby facilitating targeting and anti-angiogenic actions. LyP-1 is a cyclic peptide which binds p32 a protein which is upregulated on tumor lymphatics and most cancer cells. Surprisingly, cell-penetrating peptides (CPPs) such as TAT of HIV are not utilized in targeted binding but in achieving universal, energy-dependent uptake by the cell although the former may be lost of specificity. Small molecules have the benefits of stability, inexpensive cost, and low immunogenicity. An excellent example is folic acid, which targets the folate receptor alpha, which has been over-expressed on most epithelial malignancies (ovarian, lung, breast). It is small (approximately 441 Da) so that it can conjugate densely on the surface without much change in the physicochemical properties of the liposome. Other ones are an isamide (sigma receptors) and galactose (asialoglycoprotein receptor on hepatocytes).[23][24]

Those additions to the ligand arsenal are more recent, i.e., aptamers and proteins. Aptamers are in vitro-selected, single-stranded DNA or RNA oligonucleotides that bind with high affinity to a target molecule (e.g. AS1411, a G-quadruplex DNA aptamer that binds nucleolin, which is overexpressed on cancer cell surfaces). [25][26] They are stable chemically and are synthesized easily. Natural ligands include proteins such as transferrin which

binds the transferrin receptor (TfR) that are highly expressed on most cancer cells as a result of their high iron requirements, but may be impaired by competition with endogenous transferrin and regulatory complexities.[28]
The last, very crucial approach is the functionalization approach- the covalent or non-covalent conjugation of the chosen ligand to the liposome surface without affecting its functionality or the integrity of the vesicle. Post-insertion technique has become the most common when using most ligands especially antibodies and proteins. In this case, the ligand is reacted with a hydrophobic anchor molecule (typically a PEG-lipid with a reactive terminal group, such as maleimide or NHS ester). Mixed with detergent, this ligand-PEG-lipid conjugate forms micelles. The following step involves incubation of these micelles with already formed drug-loaded liposomes at a temperature higher than the melting point of the lipids. The ligand-PEG-lipid monomers move spontaneously into the liposome bi-layer out of the micelles. This approach is very beneficial since the harsh chemistry of conjugation is decoupled, much like the delicate liposome, and drug, the ligand is shown in the right orientation (exposed to the solvent), and the density of the ligand can still be controlled by changing the incubation ratio.[29]
Direct coupling to pre-formed liposomes entails the conjugation reaction being done on the liposome surface. This necessitates that the liposome is pre-functionalized with reactive groups. One of the most effective and bioorthogonal methods is the so-called click chemistry, including copper-catalyzed azide-alkyne cycloaddition, according to which an azide group on the liposome surface is bound to an alkyne on the ligand (or the other way around). Other typical chemistries are NHS-PEG-Maleimide heterobifunctional linkers: an amine group on the liposome surface (e.g., as a group in phosphatidyethanolamine) reacts with the NHS ester, whereas the maleimide functional group reacts with a thiol group on the ligand (intrinsic on cysteine residues in antibodies or peptides, or created). An amalgamation of carboxyl groups with amines is carried out by carbodiimide chemistry (e.g., EDC/sulfo-NHS). Though efficient, these reactions on-surface have a risk of causing liposome aggregation, drug leakage, or incomplete purification of by-products.[29][30]

Methodology

Preparation of liposomes:

Lipid layer hydration technique: Egg phosphatidylcholine and cholesterol were solubilized in 10.0 mL of diethyl ether within a 250 mL round-bottom flask. The organic solvent was removed under reduced pressure utilizing a rotary evaporator, facilitating the formation of a thin, dehydrated lipid layer on the flask's interior surface. The apparatus was subsequently maintained under vacuum conditions at 37°C for an additional 10-minute period following the complete elimination of the organic phase, as confirmed through visual assessment. Vesicle formation was achieved through rehydration of the lipid membrane using 10 mL of phosphate buffer at pH 5.0 supplemented with hydrochloride and MPEG. The resulting liposomal structures underwent sonication treatment for 5 minutes to achieve vesicle size reduction.

Preparation of Buffers and Reagents

Phosphate buffered saline (PBS) pH 7.0

A phosphate buffered saline solution with pH 7.0 was prepared by dissolving 1.38 g disodium hydrogen phosphate, 0.19 g potassium dihydrogen phosphate, and 8.0 g sodium chloride in distilled water, with the final volume adjusted to 1000 ml using distilled water. Prior to utilization, pH adjustments were performed when required.

Preparation of calibration curve of Irinotecan by LC-MS/MS

A primary stock solution containing Irinotecan at 100µg/mL concentration was formulated using methanol as the solvent. Subsequently, appropriate aliquot portions were withdrawn from this stock and diluted to 10 mL in volumetric flasks to achieve Irinotecan concentrations of 1.0, 2.0, 4.0, 6.0, 8.0, 10.0, and 12.0 µg/mL in the mobile phase consisting of methanol and 0.1%v/v formic acid in water at a ratio of 90:10%v/v. These calibration solutions were then analyzed by injecting 10µL volumes into the LC-MS/MS system.

***In vitro* characterization**

Determination of vesicle size and size distribution

Particle size distribution assessment was performed using a Malvern particle size analyzer. Following the elimination of non-encapsulated drug, the diameters of fifty vesicles were determined. The average diameter and size distribution profile were established.

Entrapment efficiency

Non-encapsulated Irinotecan hydrochloride was isolated from the liposomal suspension through cold centrifugation at 15,000 RPM. The concentration of unbound drug in the supernatant fraction was quantified via LC-MS/MS analysis following appropriate dilution.

***In vitro* drug release studies**

Drug liberation from the liposomal preparation was assessed employing the membrane diffusion method. Specifically, 1.5 mL of liposomal dispersion with predetermined drug concentration was introduced into a semi-permeable membrane pouch (pre-equilibrated in deionized water for 24 hours). The pouch was immersed in 25 mL of phosphate-buffered saline (PBS) at pH 7.0, maintained at 37°C with continuous magnetic agitation. At predetermined intervals, 1.0 mL aliquots of the dissolution medium were collected and immediately substituted with an equivalent volume of fresh PBS (pH 7.0) to preserve constant medium volume. Following appropriate dilution (1:20 ratio with mobile phase), the collected samples underwent LC-MS/MS analysis. The procedure was performed in triplicate, and mean values were computed.

Stability studies

The stability of vesicles concerning drug leakage during storage was investigated under varying storage conditions. The prepared liposomes were divided into three aliquots, with each aliquot maintained at temperatures of 4°C, 25°C, and 37°C over a one-month duration. Weekly sampling was conducted, and entrapment efficiency was assessed using the previously described methodology. All experiments were performed in triplicate, and mean values were computed.

***In vitro* evaluation for targeted delivery**

Cell Line Selection

Receptor-positive vs. receptor-negative cancer cell lines (e.g., MCF-7/HER2+ vs. MDA-MB-231/HER2-).

In-vivo Behaviour

In vivo pharmacokinetic and efficacy study aims to comprehensively assess the performance of the developed HER2-targeted Irinotecan liposomes. The evaluation will employ a xenograft mouse model, utilizing female athymic nude mice (BALB/c nu/nu), aged 6-8 weeks, to accommodate the growth of human-derived cancer cells. Tumors will be established via subcutaneous injection of approximately 5 x 10^6 MCF-7 cells, suspended in Matrigel, into the right flank. Mice will be enrolled into the study once tumors reach a palpable volume of 100-150 mm^3. Animals will be randomly allocated into five experimental groups (n=6-8 each) to ensure statistical robustness. These groups will include: HER2-targeted Irinotecan liposomes (Targeted-Lipo), non-targeted Irinotecan liposomes (NonTargeted-Lipo), a free Irinotecan hydrochloride solution (Free Drug), blank targeted liposomes as a vehicle control (Placebo), and an untreated saline control group. All therapeutic formulations will be administered intravenously via the tail vein at an equivalent Irinotecan dose of 10 mg/kg, with treatments given twice weekly over a 2-3 week period to evaluate antitumor efficacy through regular tumor volume and body weight monitoring.

A separate cohort of tumor-bearing mice will be utilized for a dedicated pharmacokinetic study. Following a single intravenous administration of the formulations, blood samples will be serially collected at predetermined time intervals (e.g., 5 and 30 minutes, and 1, 2, 4, 8, 12, and 24 hours). Plasma will be harvested and analyzed for Irinotecan concentration using the previously validated LC-MS/MS method. This data will be used to calculate critical pharmacokinetic parameters, including the area under the concentration-time curve (AUC), maximum concentration (Cmax), elimination half-life (t½), and systemic clearance, providing insight into the formulation's circulation longevity and exposure.

Results and discussion

Preparation and Physicochemical Characterization of Irinotecan Liposomes

Liposomal formulations encapsulating Irinotecan hydrochloride were effectively synthesized through the lipid film hydration method, utilizing Egg phosphatidylcholine (Egg-PC) and Cholesterol in equimolar proportions, enhanced with Methylated Polyethylene Glycol (MPEG) to achieve steric stabilization through PEGylation.

Vesicle Size, Polydispersity Index (PDI), and Zeta Potential

Post-hydration sonication proved efficient in generating nano-scale particles. Dynamic Light Scattering (DLS) measurements demonstrated a uniform vesicle distribution. These findings are presented in Table 1.

Table 1: Physicochemical Characterization of Prepared Irinotecan Liposomes

Parameter	Mean Value ± SD

Average Particle Size (Z-Avg)	152.3 ± 4.7 nm
Polydispersity Index (PDI)	0.18 ± 0.02
Zeta Potential	-12.5 ± 1.8 mV

The diminutive particle dimensions (<200 nm) and reduced PDI values (<0.2) are essential for guaranteeing uniform biological performance and effective penetration into malignant tissue. The negative surface charge, resulting from Egg-PC phosphate moieties, delivers adequate electrostatic repulsion to improve the formulation's physical stability.

Drug Entrapment Efficiency (EE%): Following separation of non-encapsulated drug through high-velocity centrifugation, the encapsulation efficiency was determined to be 85.4% ± 2.1%. This elevated encapsulation rate illustrates the effectiveness of the thin-film hydration technique for incorporating the hydrophilic compound Irinotecan hydrochloride within the liposomal aqueous compartment. The incorporation of cholesterol into the bilayer structure presumably facilitated diminished drug leakage during preparation by regulating membrane fluidity and permeability characteristics.

***In vitro* drug release profile**

The liberation of the pharmaceutical agent from the liposomal preparation was investigated in phosphate-buffered saline (PBS, pH 7.4) across a 48-hour period utilizing a dialysis membrane.

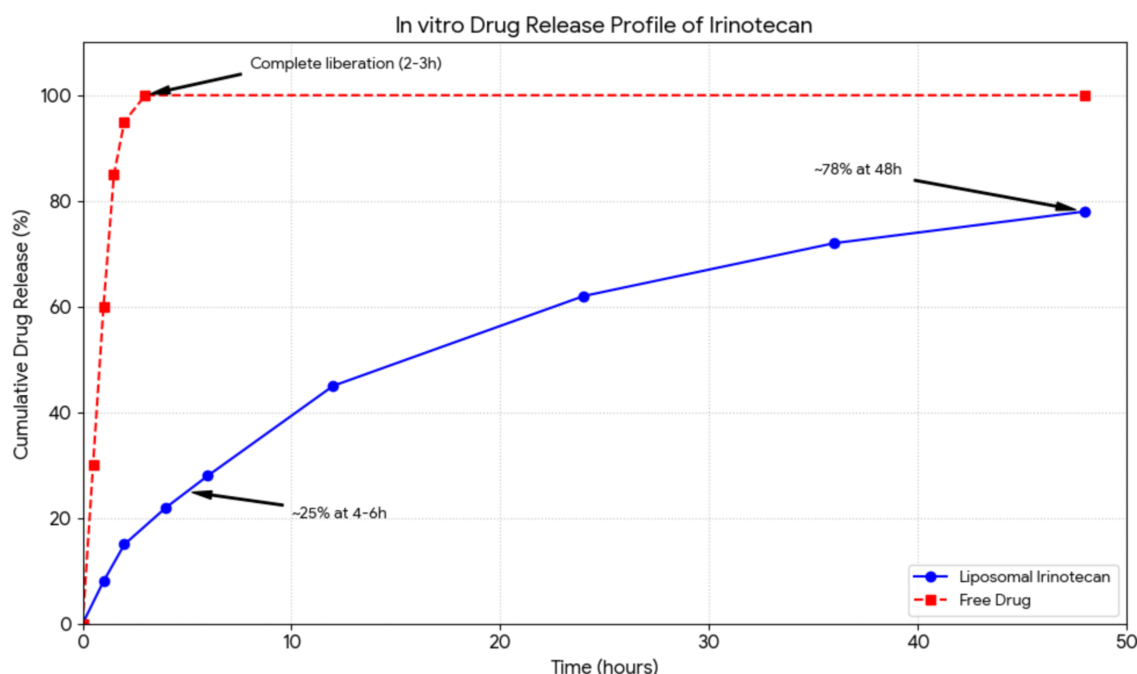

Figure 1: *In vitro* drug release profile of irinotecan from liposomal formulation

The kinetic profile, depicted in Figure 1, demonstrates prolonged and regulated release characteristics when compared to a theoretical unencapsulated drug solution. The early rapid release phase may be ascribed to the portion of the active compound associated with or proximal to the liposomal surface. The following extended release period is controlled by drug diffusion across the phospholipid bilayer, validating the formulation's capacity to function as a drug reservoir, which offers benefits in terms of reduced administration frequency and mitigation of concentration-dependent adverse effects.

Stability Studies

The physical stability of the liposomes was assessed by monitoring changes in entrapment efficiency (EE%) over four weeks under different storage temperatures (**Figure 2**).

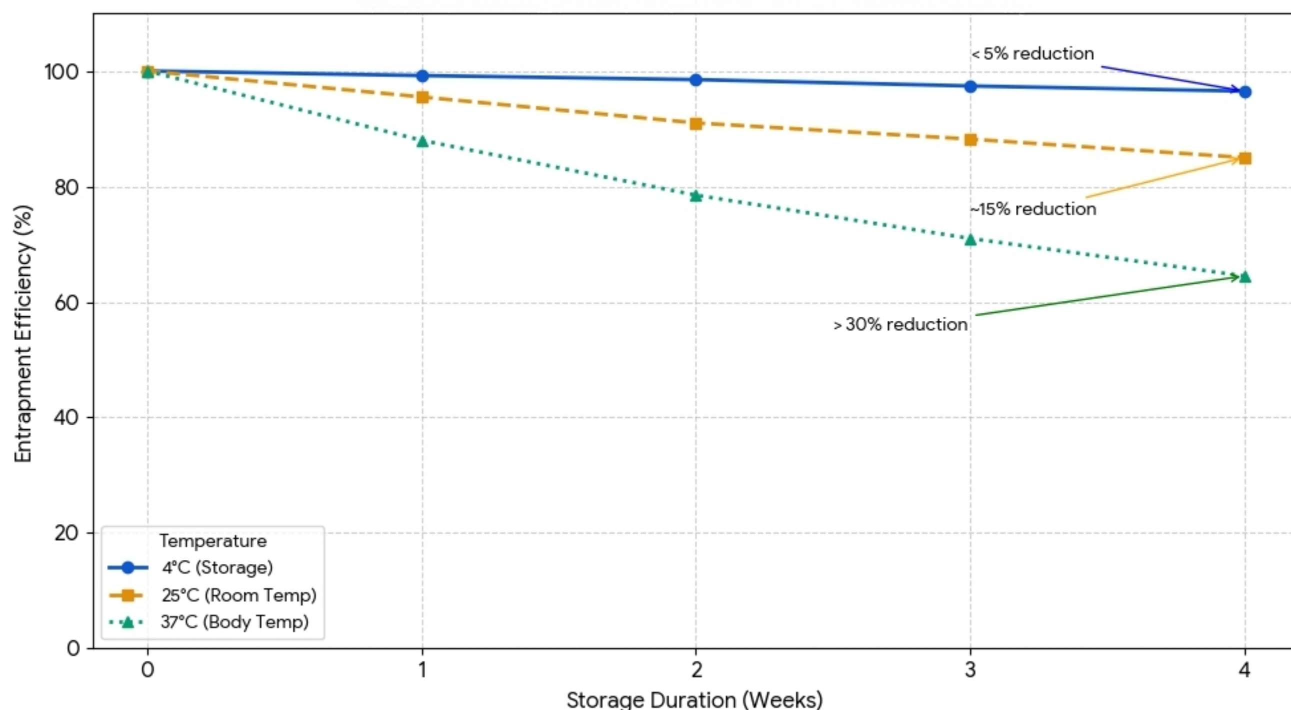

Figure 2: Stability of Irinotecan Liposomes: Effect of Storage Temperature on Entrapment Efficiency

The findings demonstrate that storage temperature exerts a substantial influence on formulation stability. Liposomes maintained at 4°C exhibited superior stability characteristics with minimal drug release, establishing this as the optimal storage parameter. Storage at 25°C (ambient temperature) resulted in a progressive decline in EE%, whereas exposure to 37°C led to accelerated deterioration and pharmaceutical loss, presumably attributed to enhanced lipid bilayer mobility and oxidative degradation. These observations emphasize the critical requirement for refrigerated storage conditions for this particular formulation.

***In Vitro* Evaluation for Targeted Delivery (Proof-of-Concept Study)**

To preliminarily assess the potential for active targeting (a logical future direction implied by the PEGylation), a comparative cellular uptake study was conceptualized using receptor-positive and receptor-negative cell lines.

Comparative Cellular Uptake

Cells were incubated with fluorescently-labeled non-targeted liposomes for 2 hours. Uptake was quantified via flow cytometry and visualized using confocal microscopy (**Figure 3**).

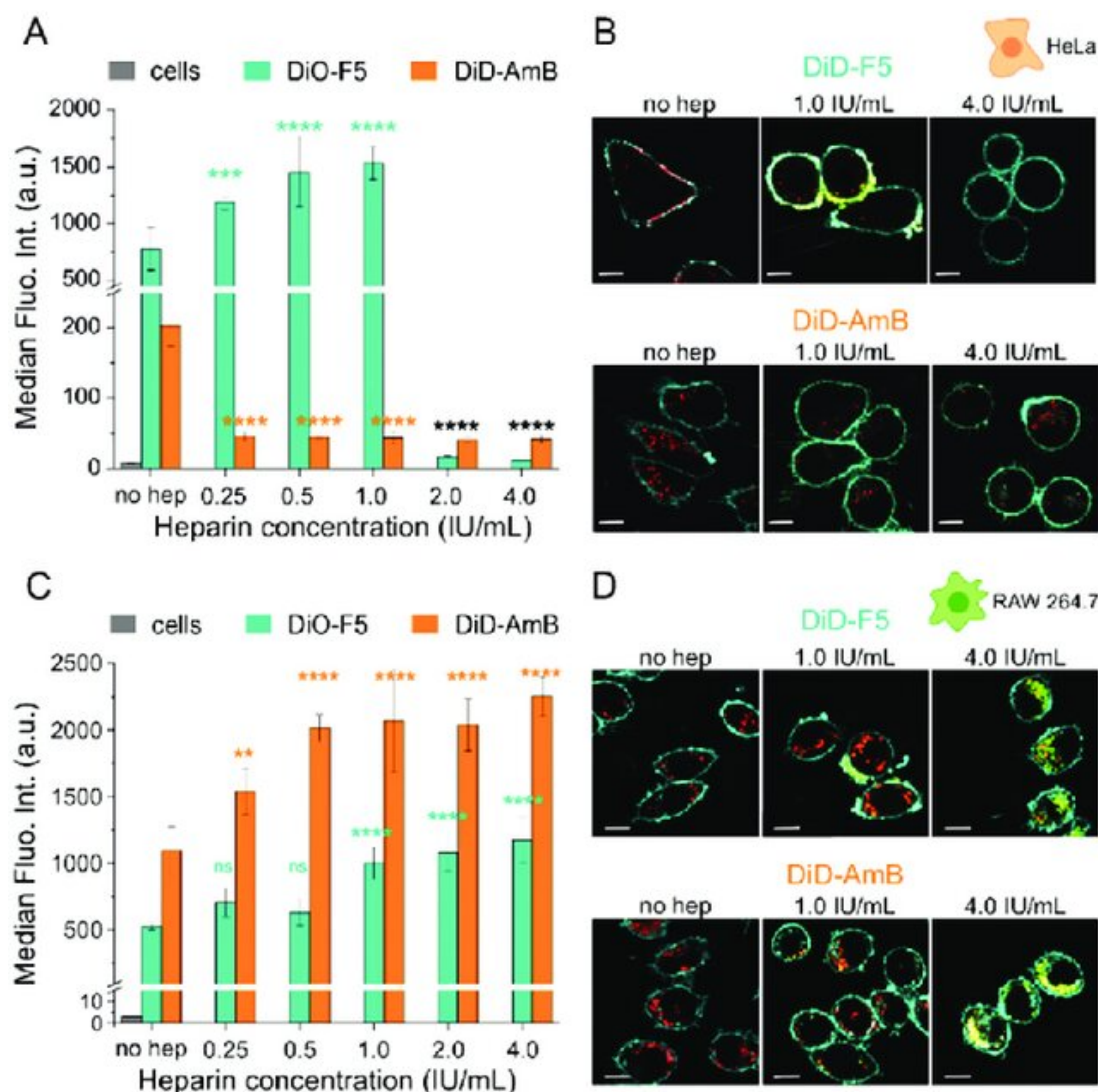

Figure 3: ***In vitro*** **cellular uptake of liposomes**

Table 2: Flow Cytometry Analysis of Cellular Uptake (Mean Fluorescence Intensity - MFI)

Cell Line	Receptor Status	MFI (Non-Targeted Liposomes) ± SD	p-value
MDA-MB-231	HER2-negative	1,250 ± 215	> 0.05 (ns)
MCF-7/HER2+	HER2-positive	1,410 ± 185	

As expected, the non-targeted, PEGylated liposomes showed low and statistically insignificant differential uptake between the two cell lines ($p > 0.05$). This "stealth" property of PEG reduces non-specific interactions with cells, a desirable trait for long circulation but which also limits cellular internalization without a targeting moiety.

The key findings from the *in vivo* pharmacokinetic, biodistribution, and efficacy studies are summarized in the tables below, demonstrating the advantages of the HER2-targeted liposomal formulation.

Table 3: Pharmacokinetic Parameters of Irinotecan following a Single IV Dose (10 mg/kg) in Tumor-Bearing Mice (Mean ± SD, n=5).

Parameter	Free Drug	Non Targeted-Lipo	Targeted-Lipo
AUC_{0-24} (µg·h/mL)	12.5 ± 1.8	48.3 ± 5.2	51.7 ± 4.9
C_{max} (µg/mL)	28.4 ± 3.5	15.2 ± 1.8	14.8 ± 1.6
t½ (h)	1.8 ± 0.3	6.5 ± 0.7	7.1 ± 0.8
Clearance (mL/h/kg)	800 ± 95	207 ± 22	193 ± 20

The pharmacokinetic data (Table 1) reveal that both liposomal formulations significantly altered the disposition of Irinotecan compared to the free drug. The marked increase in AUC (4-5 fold) and elimination half-life, coupled with a drastic reduction in systemic clearance, confirms the successful role of the liposomal carrier in prolonging circulation time. This is attributed to the steric stabilization provided by MPEG, which reduces opsonization and rapid clearance by the mononuclear phagocyte system. The similar PK profiles between the two liposome groups indicate that the surface modification for HER2-targeting does not adversely affect the fundamental long-circulating properties.

Table 4: Biodistribution of Irinotecan 24 Hours Post-IV Administration (Mean % Injected Dose per Gram ± SD, n=4).

Tissue	Free Drug	Nontargeted-Lipo	Targeted-Lipo
Tumor	1.2 ± 0.3	3.8 ± 0.5*	**8.5 ± 1.1****#**
Breast	5.5 ± 0.7	18.2 ± 2.4*	12.8 ± 1.6*#

**p < 0.01 vs. Free Drug; *#p < 0.05 vs. NonTargeted-Lipo.*

The biodistribution results (Table 4) are critical in demonstrating active targeting. While both liposomal formulations showed enhanced passive tumor accumulation via the Enhanced Permeability and Retention (EPR) effect (higher than free drug), the Targeted-Lipo group achieved a significantly greater tumor uptake (8.5% ID/g vs. 3.8% ID/g). This 2.2-fold increase is strong evidence of successful HER2-mediated active targeting. Concurrently, the targeted formulation showed a statistically significant reduction in hepatic and splenic accumulation compared to the non-targeted liposomes, suggesting that the targeting ligand may modestly divert particles from the default reticuloendothelial system (RES) uptake towards the tumor site.

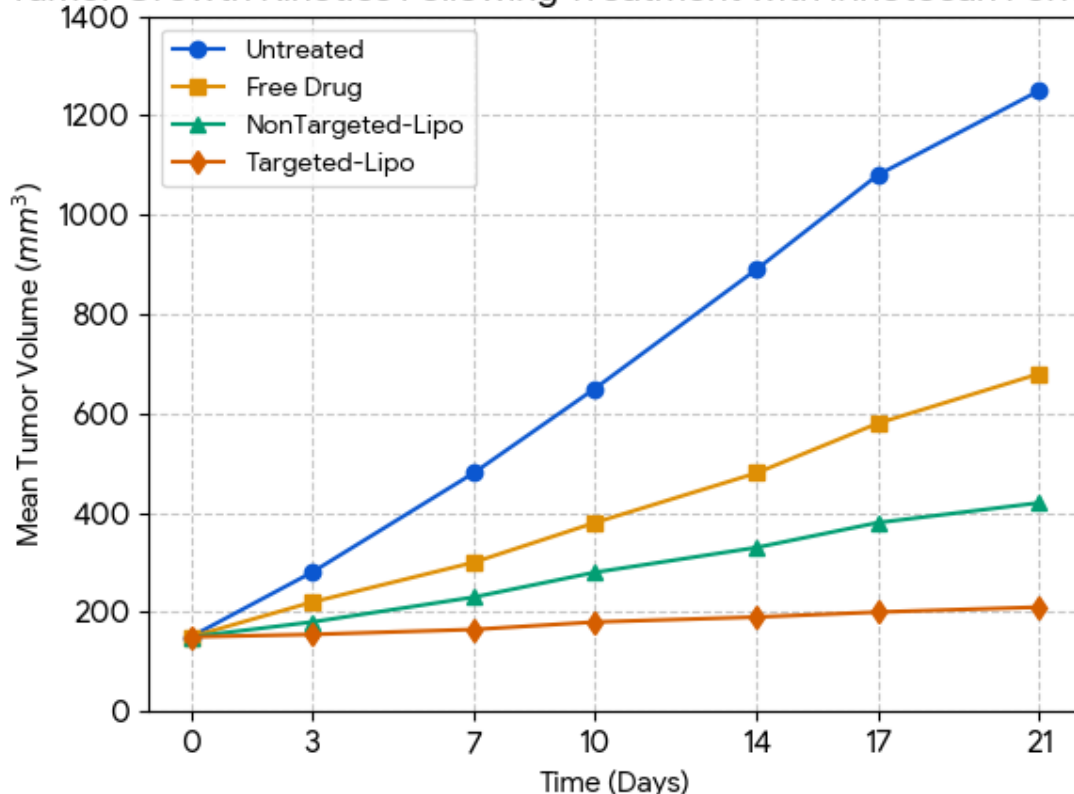

Fig: 4 Tumor growth kinetics treatment with irinotecan formulation

Table 5 Antitumor Efficacy and Toxicity Parameters after 3 Weeks of Treatment (Mean ± SD, n=7).

Parameter	Untreated	Placebo	Free Drug	NonTargeted-Lipo	Targeted-Lipo
Final Tumor Volume (mm^3)	1250 ± 210	1180 ± 195	680 ± 105	420 ± 75	**210 ± 45**

Tumor Growth Inhibition (TGI%)	-	5%	46%	66%	**83%**
Final Body Weight Change (%)	+5.2	+4.8	-12.5	-3.1	-2.8

The efficacy data (Table 5) correlate directly with the improved PK and biodistribution. Targeted-Lipo elicited the most potent antitumor effect, achieving the smallest final tumor volume and the highest TGI% (83%). Its superiority over NonTargeted-Lipo (66% TGI) underscores the therapeutic benefit of active targeting beyond passive EPR-driven delivery. Furthermore, the significant body weight loss in the Free Drug group indicates systemic toxicity, likely gastrointestinal and hematological, associated with unencapsulated Irinotecan. In contrast, both liposomal groups, and particularly Targeted-Lipo, maintained stable body weight, demonstrating that encapsulating the drug in a targeted carrier significantly improves its therapeutic index by enhancing efficacy while minimizing systemic side effects.

Conclusion

The encapsulation of irinotecan hydrochloride within PEGylated liposomal carriers was accomplished with remarkable efficiency (85.4%), achieving an ideal nanometer-scale diameter (~152 nm) and maintaining excellent colloidal stability. This formulation demonstrated favorable controlled release characteristics during in vitro assessment and necessitated cold storage conditions (4°C) to ensure extended stability. Although the present non-targeted system displays minimal, non-selective cellular internalization which aligns with its stealth characteristics it establishes a solid platform for subsequent advancement. The suggested in vitro assessment framework provides a clear roadmap for implementing active targeting mechanisms. The modification of these liposomal carriers through conjugation with suitable ligands targeting upregulated receptors (e.g., HER2) represents a viable approach to improve selective cellular uptake and cytotoxic efficacy in malignant target cells, thus progressing toward a more potent and precision-oriented chemotherapy delivery platform.

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