International Journal of Applied Pharmaceutics

ISSN- 0975-7058 Vol 16, Issue 3, 2024

Review Article

AN UPDATED REVIEW OF STEALTH LIPOSOMES AND ITS ABILITY TO EVADE THE IMMUNE SYSTEM: A NEW FRONTIER IN CANCER CHEMOTHERAPY

DURGARAMANI SIVADASAN*

Department of Pharmaceutics, College of Pharmacy, Jazan University, Jazan, Saudi Arabia
***Corresponding author: Durgaramani Sivadasan; *Email: dsivadasa@jazanu.edu.sa**

Received: 13 Feb 2024, Revised and Accepted: 21 Mar 2024

ABSTRACT

Liposomes have been the delivery of choice for the cancer targeting therapy for the last few decades. Since the 1990s, the development of sterically stabilized (stealth) liposomes has garnered interest for their long circulating half-life. PEGylated (Polyethylene Glycol) liposomes are most extensively studied for delivering cancer therapeutics in a sustained manner. Stealth liposomes are having a less intrinsic toxicity with higher efficacy in cancer treatment. There are numerous clinical trials on the liposomes in tackling cancer is evident for the better outcome of the delivery system. Stealth liposomes are extensively studied for their improved circulation time and better pharmacokinetic profile in cancer treatment. The steric hindrance of the stealth liposomes bypasses the reticuloendothelial system clearance. Further the ligands conjugation in the surface of the liposomes able to achieve better target to the cancer cells. The vascularization nature of the cancerous cells is readily making the liposomal delivery of the cancer drugs accumulate in the cancerous cells rather than healthy cells. There is an utmost need to understand the possible mechanism of stealth liposomes and the basic science behind the development of liposomal delivery system in advancing the cancer treatment with less toxicity. The present review addresses the various modalities of the liposomal development, liposome characterization, mechanism of PEGylated liposomes, the advancements and results of the liposomes in the treatment of various diseases, and the clinical trials and regulatory considerations of liposomal drug delivery system.

Keywords: Liposomes, Stealth liposomes, Cancer, PEGylation, Targeted delivery, Enhanced permeation and retention effect

DOI: https://dx.doi.org/10.22159/ijap.2024v16i3.50601 Journal homepage: https://innovareacademics.in/journals/index.php/ijap

INTRODUCTION

There are various drug delivery techniques adopted for delivering cancer therapeutics for effective treatment outcomes. In this line, various drugs, vaccines, enzymes, proteins, and genetic materials are delivered in sustained action forms using conventional liposomes, which are microscopic in nature. The liposomes contain the molecular "payload," which eventually degrades spontaneously and releases its contents into the bloodstream or tissues by diffusing via capillary walls. When conventional liposomes are used for treatment, several drawbacks have been documented, including blood clearance, limited specificity, phagocytosis, opsonization low binding site, low bioavailability, low retention, and low effectiveness. The rapid Mononuclear Phagocyte System (MPS) absorption of typical liposomes upon systemic injection is a significant disadvantage. Therefore, new liposome generations known as stealth liposomes were developed to overcome the demerits of conventional liposomal delivery systems.

Stealth liposomes and immuno-liposomes are avoid being detected by the immune system. Conventional liposomes, particularly ones that are injected into the body, are not stable for extended periods of time. Stealth liposomes are sterically stabilized, gained attention for its long circulating half-life. PEGylated liposomes are more extensively studied for its potential effect in delivering cancer therapeutics [1-4].

Conventional and stealth liposomes

The conventional liposomes are the first generation of liposomes created from phospholipids, either natural or synthetic, with or without cholesterol [5]. Cholesterol was incorporated to enhance liposome fluidity, which reduces the rigidity of the liposomal membrane bilayer and the stability of the liposomes composed of Distearoylphosphatidylcholine Ethanolamine-Polyethylene Glycol (DSPE-PEG_{2000}) and Hydrogenated Soybean Phospholipids (HSPC) [6, 7]. Moreover, rigid liposomes exhibit better tumor penetration and enhanced anti-tumor efficacy [8]. Dipalmitoyl phosphatidylcholine (DPPC) liposome membrane permeability and fluidity are both influenced by cholesterol levels. Elevated cholesterol levels led to a rise in the size of typical liposomes and a change in their morphology from amorphous to nanosized, regular, and spherical vesicles. Further, cholesterol affects the release of hydrophilic molecules from lipid vesicles and reduced the fluidity of the bilayer [9].

Reports clarified that a stable liposome has to include the appropriate amount of membrane fluidity and cholesterol (about 50 mol %) [10]. As a result, cholesterol is essential for the fluidity and rigidity of the liposome bilayer. Nevertheless, these properties are impacted by the cholesterol to phospholipid molar ratio, the specific phospholipids utilized, and the type of drug being encapsulated. Upon administration, conventional liposomes go to the site of action via systemic circulation. Owing to their tiny size in microns, they passively diffuse through the blood vessel's permeable walls and reach the tumour region. Conventional liposomes can pass through cancer cells yet lack the appropriate retention action since the outer surface does not have a binding mechanism.

Conventional liposomes have been shown to be ineffective in treating tumour cells due to their short blood circulation time and its inability to bind to the tumor sites, the mononuclear phagocyte system quickly eliminates them and they rapidly accumulate up in the liver and spleen [11]. Hence, MPS prevents conventional liposomes from reaching the target area and limits their dissemination to other tissues and reticuloendothelial system (RES), makes them susceptible to phagocytosis induced degradation or deactivation. The conventional liposomes showed only a moderate degree of stability in an *in vitro* environment and to improve *in vivo* liposome stability and blood circulation, stealth (sterically stabilized) liposomes were developed [12].

Over the past few decades, various hypothesis have been proposed to understand the pharmacokinetic properties of two nanoscale systems for drug delivery, polymeric nanoparticles and liposomes. In conventional liposome, opsonization facilitates the phagocytosis process by utilizing Vander Waals, electrostatic, ionic and hydrophobic/hydrophilic forces to interact with nanoparticles in the circulation. Therefore, the nano carriers surface characteristics are crucial to the opsonization process. Hydrophobic and charged particles are more opsonized than hydrophilic and neutrally charged particles. Surfaces can be coated with hydrophilic polymers to produce stealth systems to prevent the opsonization process.

The early 1990s witnessed the culmination of this liposome engineering process with the identification that encapsulating liposomes with PEG, a synthetic hydrophilic polymer. The most effective technique for creating "stealth" masks was liposomal surface modification utilizing polyacrylamide, polyvinyl alcohol, polycarbohydrates, and PEG and its co-polymers [13-15]. PEGylation is the process of using PEG as a polymer. Stealth liposomes drug delivery system are spherical, phospholipid bilayer membrane coated vesicles utilized to carry genetic material or drugs into cells and able to escape the detection by the mononuclear phagocyte system [16]. PEG exists on the surface of liposomes, which limits MPS absorption, reduces renal clearance rate and prolongs circulation time by enhancing bioavailability and preventing MPS from binding to opsonins. Strong inter bilayer repulsion produced by the PEG coated liposomes enables them to resist attractive van der Waals forces, preventing aggregation and stabilizing liposomes [17, 18].

Sterically stabilized or stealth liposomes are other identities for these PEG coated liposomes. The several blood components interact hydrophobically and electrostatically with highly concentrated regional groups at the liposome surface, which causes the PEG stabilizing action. It has been shown that stealth liposomes differ from conventional liposomes in their dose independent pharmacokinetics [19, 20]. The highly effective intravenous injectable drug Doxil®, used as a remedy to treat advanced ovarian cancer, multiple myeloma, and human immunodeficiency virus (HIV) associated Kaposi's sarcoma, was developed using this technique [21]. The stealth liposome technology has made considerable progress, and several important formulations for the treatment of different diseases are now available commercially or in advanced clinical trials (table 1).

Publications from various sources (pubmed, ScienceDirect, google scholar; years of use: 2000-2024 and the key words used are liposomes, stealth liposomes, cancer, PEGylation, targeted delivery) are noted with emphasis on methods of preparation, characterization and recent advances of stealth liposomes. A detailed analysis is also given to commercially available conventional and stealth liposomal compositions and their applications. Further, ongoing research on clinical trials and patents sanctioned in recent past is well detailed. To support this topic, the most pertinent literature from recent studies has been compiled. In order to pave the way for liposomes from manufacture to clinical use, this review article can provide up-to-date knowledge on a variety of uses along with an idea on current research in clinical trials and patents.

Table 1: List of stealth liposomes under clinical trials

Trail/identifier number	Study	Status	Phase	Study completion date	References
NCT03983096	PEGylated liposomal doxorubicin or epirubicin in neoadjuvant or adjuvant chemotherapy for breast cancer	Completed	Not applicable	May 1, 2020	[22-30]
NCT03033316	An evaluation of the safety of oncocort IV PEGylated liposomal dexamethasone phosphate in patients with progressive multiple myeloma (AMETHYST)	Terminated (Patient enrolment issues)	Phase 1/Phase 2	August 2020	[22-30]
NCT01379989	Inovatyon study-international, randomized study in patients with ovarian cancer (INOVATYON)	Completed	Phase 3	December 17, 2020	[22-30]
NCT01837251	Evaluation of optimal treatment with bevacizumab in patients with platinum sensitive recurrent ovarian cancer	Completed	Phase 3	January 2021	[22-30]
NCT02606305	Study of mirvetuximab soravtansine in combination with bevacizumab, carboplatin, PEGylated liposomal doxorubicin, pembrolizumab, or bevacizumab+carboplatin in participants with folate receptor alpha (FRα) positive advanced epithelial ovarian cancer, primary peritoneal, or fallopian tube cancer	Completed	Phase 1/Phase 2	March 12, 2021	[22-30]
NCT03719326	A study to evaluate safety/tolerability of immunotherapy combinations in participants with triple-negative breast cancer or gynecologic malignancies	Completed	Phase 1	July 2, 2021	[22-30]
NCT02865811	Pembrolizumab combined with PEGylated liposomal doxorubicin (PLD) for recurrent platinum resistant ovarian, fallopian tube or peritoneal cancer	Completed	Phase 2	March 2022	[22-30]
NCT04592692	A pharmacokinetic and clotting activity study of FVIII-PEGylated liposomes	Recruiting	Phase 2	May 31, 2022	[22-30]
NCT03470805	Olaparib after response to trabectedin PEGylated liposomal doxorubicin in recurrent ovarian carcinoma	Completed	Phase 2	July 27, 2022	[22-30]
NCT01210768	A study of PEGylated liposomal doxorubicin and cyclophosphamide in human epidermal growth factor receptor 2 (Her2) negative stage I and II breast cancer patients	Active, not recruiting	Phase 2	October 31, 2022	[22-30]
NCT03071926	Metronomic PLD in patients with primary endocrine resistant advanced breast cancer	Recruiting	Phase 2	December 1, 2022	[22-30]
NCT03161132	Resistant ovarian cancer, olaparib and liposomal doxorubicin (ROLANDO)	Completed	Phase 2	December 31, 2022	[22-30]
NCT01990352	Correlate BRCA1 protein expression with response to DNA damaging chemotherapy	Completed	Phase 2	December 31, 2022	[22-30]
NCT03952572	Efficacy and safety of CDOP (PEGylated liposomal doxorubicin, vincristine and prednisone) vs CHOP (cyclophosphamide-hydroxy daunorubicin-oncovin-prednisone) for newly diagnosed peripheral T-cell lymphoma	Recruiting	Phase 3	April 30, 2023	[22-30]
NCT03596281	Pembrolizumab in combination with bevacizumab and PEGylated liposomal doxorubicin in patients with ovarian cancer (PEMBOV)	Active, not recruiting	Phase 1	April 2023	[22-30]
NCT02456857	Liposomal doxorubicin, bevacizumab, and everolimus	Active, not	Phase 2	May 24, 2023	[22-30]

Trail/identifier number	Study	Status	Phase	Study completion date	References
	in patients with locally advanced triple negative breast cancer (TNBC)with tumors predicted insensitive to standard chemotherapy; a moonshot initiative	recruiting			
NCT03907475	Locally advanced malignant solid neoplasm metastatic malignant solid neoplasm unresectable malignant solid neoplasm	Recruiting	Phase 2	June 1, 2023	[22-30]
NCT00657878	Efficacy study of chemotherapy to treat ovarian cancer recurrence by prolonging the platinum free interval (MITO-8)	Active, not recruiting	Phase 3	July 2023	[22-30]
NCT02312245	Recurrent fallopian tube carcinoma recurrent ovarian carcinoma recurrent primary peritoneal carcinoma	Recruiting	Phase 2	July 15, 2023	[22-30]
NCT04092270	A study combining the peposertib (M3814) pill with standard chemotherapy in patients with ovarian cancer with an expansion in high grade serous ovarian cancer and low grade serous ovarian cancer	Recruiting	Phase 1	September 1, 2023	[22-30]
NCT04092270	A study combining the peposertib (M3814) pill with standard chemotherapy in patients with ovarian cancer with an expansion in high grade serous ovarian cancer and low grade serous ovarian cancer	Recruiting	Phase 1	September 1, 2023	[22-30]
NCT02446600	Testing the use of a single drug (olaparib) or the combination of two drugs (cediranib and olaparib) compared to the usual chemotherapy for women with platinum sensitive ovarian, fallopian tube, or primary peritoneal cancer	Active, not recruiting	Phase 3	December 30, 2023	[22-30]
NCT05346107	PLD-cyclophosphamide-Nab-P continuously combined with dual HER2 blockage for HER2-positive breast cancer	Recruiting	Phase 2	December 31, 2023	[22-30]
NCT04274426	Mirvetuximab soravtansine (IMGN853), in folate receptor alpha (FRα) high recurrent ovarian cancer (MIROVA)	Recruiting	Phase 2	December 2023	[22-30]
NCT01970722	Surgery and chemotherapy with or without chemotherapy after surgery in treating patients with ovarian, fallopian tube, uterine, or peritoneal cancer	Active, not recruiting	Phase 1	December 22, 2023	[22-30]
NCT01802749	Bevacizumab beyond progression in platinum sensitive ovarian cancer (MITO16MANGO2b)	Active, not recruiting	Phase 3	December 2023	[22-30]
NCT04209855	A study of mirvetuximab soravtansine vs. investigator's choice of chemotherapy in platinum-resistant, advanced high-grade epithelial ovarian, primary peritoneal, or fallopian tube cancers with high folate receptor-alpha expression (MIRASOL)	Active, not recruiting	Phase 3	April 2024	[22-30]
NCT02839707	PEGylated liposomal doxorubicin hydrochloride with atezolizumab and/or bevacizumab in treating patients with recurrent ovarian, fallopian tube, or primary peritoneal cancer	Active, not recruiting	Phase 2/Phase 3	June 30, 2024	[22-30]
NCT04877275	ATG-010 (selinexor) in combination with chemotherapy in relapsed/refractory multiple myeloma (RRMM)	Recruiting	Phase 2	December 30, 2024	[22-30]
NCT04729387	Alpelisib plus olaparib in platinum resistant/refractory, high grade serous ovarian cancer, with no germline breast cancer gene (BRCA) mutation detected	Recruiting	Phase 3	January 31, 2025	[22-30]
NCT04244552	A phase 1b trial of anti-ribonucleoprotein antibody (ATRC-101) in adults with advanced solid malignancies	Recruiting	Phase 1	March 2025	[22-30]
NCT05386524	Sintilimab and bevacizumab biosimilar combined with PLD in metastatic triple negative breast cancer (mTNBC)	Recruiting	Phase 2	March 15, 2025	[22-30]
NCT04814875	A study to evaluate the combination of ATX-101 and platinum based chemotherapy	Recruiting	Phase 1/Phase 2	March 31, 2025	[22-30]
NCT05483933	Phase 1b study of SL-172154 administered with combination agent(s) in subjects with ovarian cancers	Recruiting	Phase 1	April 2025	[22-30]
NCT05711615	Testing low-dose common chemotherapy (liposomal doxorubicin) in combination with an anticancer drug, peposertib, in advanced sarcoma	Not yet recruiting	Phase 1	May 3, 2025	[22-30]
NCT05656079	To evaluate the cardiac safety of PEGylated liposomal doxorubicin concurrently plus trastuzumab and pertuzumab in the adjuvant setting for early-stage HER-2-positive breast cancer: a multicenter, randomized controlled clinical study	Recruiting	Not applicable	September 2025	[22-30]
NCT05388487	Tolerability and safety of HF1K16 (investigational PEGylated liposome formulation of All-Trans Retinoic Acid) injection in patients with refractory solid tumors	Recruiting	Phase 1	November 2025	[22-30]
NCT05261490	Study of maplirpacept (PF-07901801) in combination with PLD in patients with platinum resistant ovarian cancer	Recruiting	Phase 2	December 4, 2025	[22-30]
NCT05684731	Safety and efficacy of KM1 (injection of polyphase liposome-encapsulated cisplatin) in subjects with	Not yet recruiting	Phase 1	June 1, 2026	[22-30]

Trail/identifier number	Study	Status	Phase	Study completion date	References
	recurrent or refractory ovarian cancer (K19017-004)				
NCT03409198	Phase IIb study evaluating immunogenic chemotherapy combined with ipilimumab and nivolumab in breast cancer (ICON)	Active, not recruiting	Phase 2	May 11, 2027	[22-30]
NCT05536102	The effectiveness and safety of oxaliplatin and capecitabine (XELOX) and tislelizumab+PLD for resectable gastric cancer (Liding study)	Recruiting	Phase 2	September 30, 2027	[22-30]
NCT05467670	Safety and efficacy of anti-CD47, ALX148 (CD47 myeloid checkpoint inhibitor) in combination with liposomal doxorubicin and pembrolizumab in recurrent platinum-resistant ovarian cancer	Recruiting	Phase 2	December 2027	[22-30]
NCT05257018	R-CDOP combined with intrathecal methotrexate for diffuse large B cell lymphoma (DLBCL)patients with high risk of CNS relapse	Recruiting	Phase 2	January 30, 2028	[22-30]
NCT05159193	Neo-adjuvant treatment PEGylated liposomal doxorubicin plus cyclophosphamide sequential docetaxel plus trastuzumab and pertuzumab versus docetaxel plus carboplatin combined with trastuzumab and pertuzumab in HER-2 positive breast cancer	Recruiting	Phase 3	January 31, 2028	[22-30]
NCT02364713	MV-NIS or investigator's choice chemotherapy in treating patients with ovarian, fallopian, or peritoneal cancer	Recruiting	Phase 2	February 28, 2028	[22-30]
NCT03164993	Atezolizumab combined with immunogenic chemotherapy in patients with metastatic triple-negative breast cancer (ALICE)	Active, not recruiting	Phase 2	December 31, 2028	[22-30]
NCT02315196	Estrogen receptor negative breast cancer HER2-negative breast cancer, etc.	Active, not recruiting	Phase 2	July 2041	[22-30]

Characteristics of stealth liposomes

The structure and composition of the stealth liposome are identical to those of the host cell and consist of cholesterol and phospholipids like phosphotidylcholine or diacetylphosphate [31]. The phospholipid bilayer is made up of a hydrophilic head portion, a hydrophobic tail and an outer coat made of PEG or another type of polymer. In nature, stealth liposomes are stable. A stealth liposome's size and form might vary depending on the substance and material utilized. They delay the release of drugs, as the endoplasmic reticulum cannot absorb them. They range from 50 to 200 nm in size. Phospholipids and spingolipids, glycolipids, and sterols are the most frequently used lipids.

The uniform, colloidal stealth liposomes have a colloidal structure (fig. 1). These spheres, also known as stealth liposomes, are tiny deposits that can be designed to retain a gene for gene therapy, an antibiotic, an allergen, an antigen, or both. The stealth liposome is uniform and colloidal in structure. Drugs that are both hydrophilic and lipophilic can be administered via the liposome without any chemical modification.

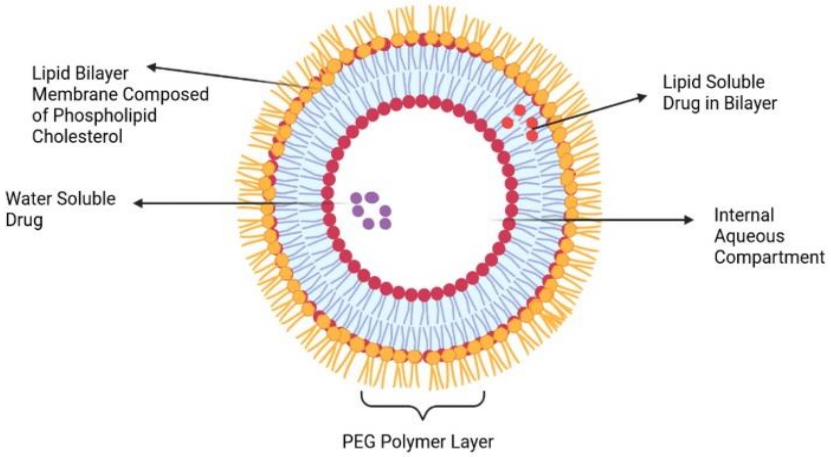

Fig. 1: Structure of a stealth liposome (Created by BioRender. com)

Structure of a stealth liposome

PEG has been extensively utilized as the polymeric stearic stabilizer over the numerous polymers assessed to accelerate the blood circulation period of liposomes. Although there are other ways to incorporate it into the liposomal surface, the most popular technique now is to anchor the polymer in the liposomal membrane using a cross-linked lipid, such as PEG distearoyl phosphatidylethanolamine [DSPE] (fig. 2).

Formulation modalities

There are various preparations methods established for the liposomal formulation development [32-38]. The same techniques are utilized to produce stealth liposomes. However, PEGylation is a crucial step that must be included in these techniques for the design of stealth liposomes. The process of enabling polymer to encapsulate a liposome is known as PEGylation (fig. 3).

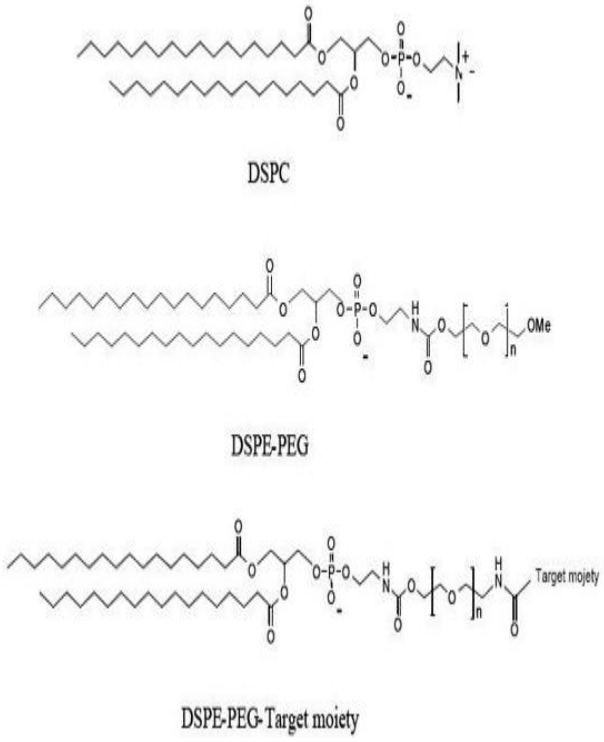

Fig. 2: Structure of various cross-linked polymers

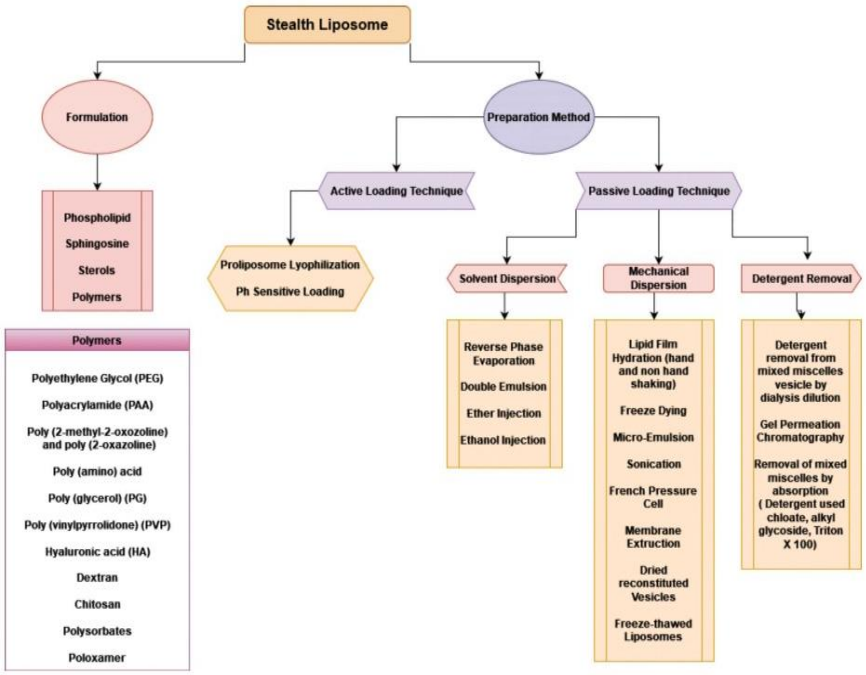

Fig. 3: Formulation of liposomes (Created by BioRender.com)

Mechanism-stealth liposome transportation

Numerous mechanisms exist for liposomes to interact with cells viz., phagocytic reticuloendothelial system cells like neutrophils, macrophages and endocytose. Weaker electrostatic or hydrophobic forces that are generally present and/or particular interactions with cell surface components may induce adsorption on the cell surface. By embedding the liposome's lipid bilayer into the plasma membrane and simultaneously releasing its contents into the cytoplasm, the liposome is able to fuse with the plasma cell membrane. Without any direct association to the liposomes contents, lipids from liposomes are transferred to cellular or subcellular membranes or vice versa. When multiple mechanisms are involved, it can be challenging to distinguish which one is in use [39].

PEGylation

There are two techniques for PEGylation: the pre-insertion technique, which involves adding PEG-lipids to the lipid composition and the post-insertion technique, which combines PEG-lipids with liposomal

dispersion before the formation of the liposomes. The efficiency of liposomal PEGylation depends on the length and density of PEG. The extremely long PEG chains significantly reduce the transfection activity and the very small PEG molecules neither restrict protein absorption nor increase blood circulation. Typically, medium-length PEG molecules are employed to modify liposomes. The covering density increases as the molar PEG-lipid/lipid composition ratio increases. PEG concentrations of 5% or less develop a mushroom-like shape, 5%-15% PEG forms a mushroom or brush like shape with 100% coverage and>15% PEG gives a brush like shape with 100% coverage (fig. 4).

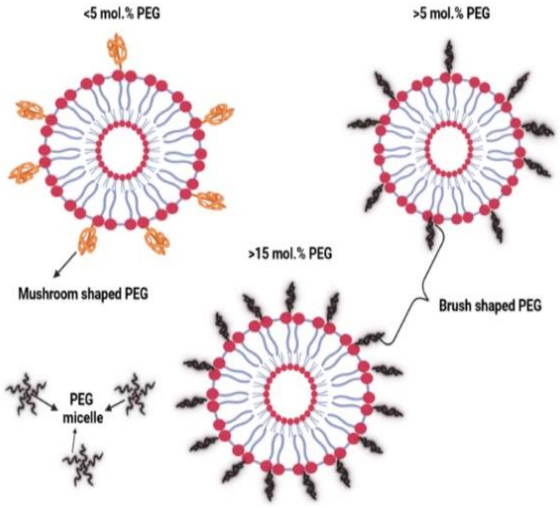

Fig. 4: PEGylation density (Created by BioRender.com)

By inhibiting MPS absorption, or the interaction between liposomes and macrophages, PEGylation prolongs the duration of circulation [40, 41]. Additionally, PEG liposomes are known to accumulate in tumour foci. The steric barrier and blood protein binding mediate the interaction between PEG liposomes and macrophages. Prolonged circulation periods and more stable steric barriers are frequently found in liposomes with a brush-like covering (fig. 5) [42].

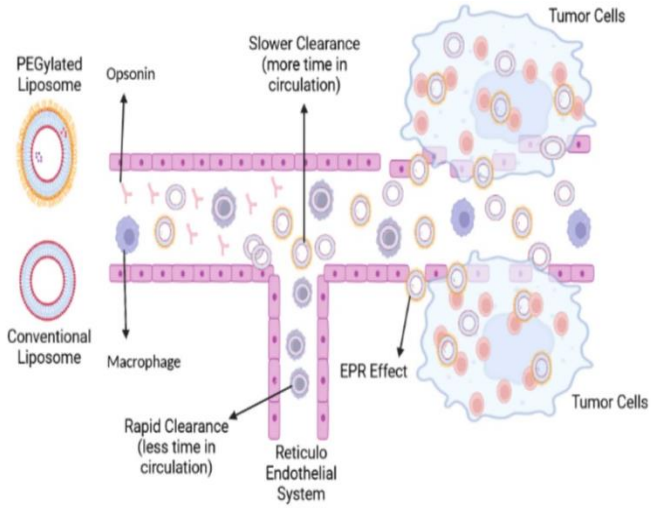

Fig. 5: Enhanced permeation and retention effect of sterically stabilized liposomes (Created by BioRender.com)

Pre-insertion PEGylation

Traditionally, the pre-insertion approach is used to develop PEGylated liposomes. Sonication is used to hydrate thin lipid films, resulting in the formation of liposomal particles [43]. Lipids bearing PEGs at their head groups are mixed with cationic, anionic, or neutral lipids on the thin film stage [44-46]. For liposomal PEGylation, the most prevalent phosphatidylethanolamines (PE) are DSPE-mPEG,35-37, DPPE-mPEG,38, and DOPE-mPEG, which vary in the hydrophobic domain, PEG chain length and fatty acid type [47-49].

The most effective PEG compounds for stabilizing liposomal structures in the circulation were found to have a 2000 Da molecular mass. *In vivo* tests revealed that PEG2000 modified liposomes are four times as efficient at targeting tumour tissue than conventional liposomes [50]. Utilizing defined sequence PEGs is now feasible due to the latest advances in polymer synthesis (liquid phase iterative synthesis coupled with selective molecular sieving). Excellent biocompatibility and purity are found in PEGs with precisely defined length and oxyethylene unit count. PEG chain length has an impact on PEGylated liposome transfection effectiveness, but so does the lipid hydrophobic domain's nature on cellular uptake.

Rapidly diffusing short aliphatic derivatives from the liposomal surface lessens the prolonging impact. Furthermore, bases for PEGylated lipids can be used, such as steroids and lipopeptides.

When PEG containing lipids are incorporated during the formation of thin lipid films, PEG chains form on the two distinct inner and outer membranes of the lipid bilayer. The liposomal bilayer surface is the only thing that changes with the post-insertion method. The post-insertion technique only changes the outer liposomal bilayer surface [51, 52].

Post-insertion PEGylation

To achieve a significant amount of PEGylation (greater than 5% PEG), the post-insertion approach is more efficient [53]. The PEG derivative interacts with certain hydrophobic anchor or matrix lipids on the liposomal membrane's external surface, where PEGylated conjugates are mostly located [54]. Due to an adequate ligand arrangement on the outer liposomal surface and a high degree of embedding under ideal circumstances, the execution of this strategy decreases non-specific interactions between ligands and liposomes [55]. The conventional drug loading approach is unaltered by this technique. Post-insertion technique is a promising method for liposomal system mediated gene transfer to cancer cells [56, 57].

Significance of PEGylation

For more than 25 y, PEGylation is one of the most important approaches, has been used therapeutically to minimize the immunogenicity of nano-therapeutics and improve the pharmacokinetic (PK) properties of drugs [58]. Many pharmaceutical products on the market today utilize PEGylated therapeutics, and new PEGylated therapeutics are being developed for longer drug half-lives [59]. Systems for delivering PEGylated drugs are crucial in the treatment of cancer. The plasma half-life of several therapeutics, including proteins, enzymes, small molecule drugs, nanoparticles, and liposomes, can be increased by PEGylation. It could be executed by blocking opsonins from removing them from the bloodstream, increasing their therapeutic index.

The surface modification of nanocarriers using PEG with various chain lengths, shapes, densities and molecular weights demonstrated a tremendous potential for the creation of enhanced drug delivery techniques for the treatment of cancer [60]. Protein conjugates that have been PEGylated are often employed in therapeutics. Covalent bonds between poly (ethylene glycol) and most proteins that the Food and Drug Administration (FDA) has approved for conjugation (PEG). These PEGylated drugs showed longer blood circulation duration, requiring fewer doses which improve patient compliance [61]. In pharmaceutical and biological applications, PEG improves protein stability, therapeutic efficacy, and shelf life for bioactive compounds; nevertheless, the optimal position of PEG attachment into proteins is still not clearly revealed. More research is needed for clinical validation since the researchers found that PEGylation efficiency, protein stability and protein function vary depending on the PEGylation location [62, 63].

Characterization parameters of liposomes

Liposomes are generally possessing low intrinsic toxicity and there are various characterization studies for the liposomes are as follows: (fig. 6).

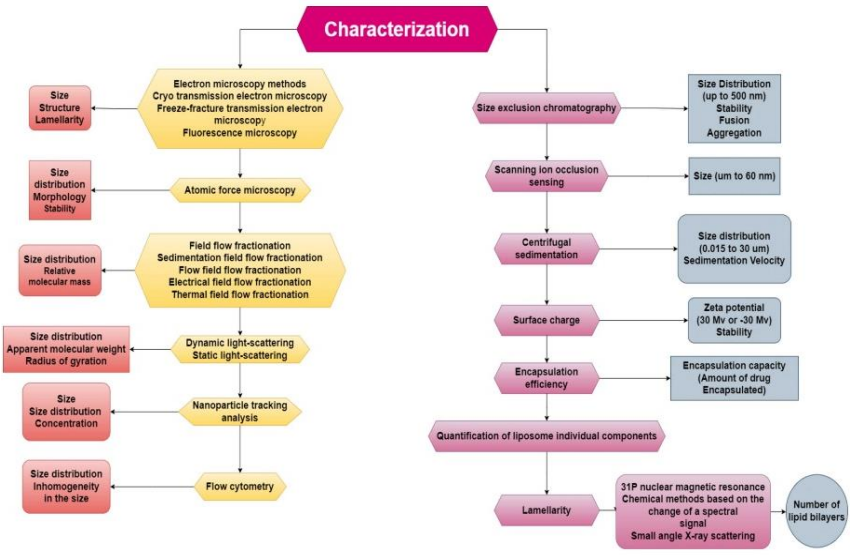

Fig. 6: Characterization of liposomes (Created by BioRender.com)

Size distribution

Photon correlation spectroscopy was used to determine the liposome size distribution which ensures the stealth liposomes identical size (mean liposome size of 100 nm) [64, 65].

Zeta potential

Electrophoretic mobility measured at a 90° angle was used to determine the Zeta potential. The 3000Hs zetasizer equipment was used to do the measurements in triplicate and the samples are diluted with suitable solvent system [66].

Lipid quantification and chemical stability

The amount, purity of cholesterol and the concentration of phospholipids were determined using High Performance Liquid Chromatography (HPLC) or evaluated using cholesterol oxidase enzymatically, and. the purity of phospholipids as raw materials and the degree of their hydrolysis throughout various liposome synthesis were evaluated by Thin Layer Chromatography (TLC).

Drug quantification

Drug concentration of the liposomes is usually estimated using a spectrophotometric technique. The drug amount is validated by HPLC throughout the development and storage of the liposomes, the drug's purity and level of degradation is evaluated using a combination of HPLC.

Liposome stability

Stealth liposomes stability is studied by dilution of the formulation with 10 times either with mouse plasma pH 7.4 or 0.9% NaCl, pH 6.5, and incubated for 30 min at 37 °C. The drug release from the liposome is separated and evaluated by ultracentrifugation at 150,000g for 60 min at 10 °C, followed by atomic absorption spectrometry in a graphite furnace [67].

Drug release determination

Using a dialysis method, *in vitro* drug release assessments of drugs from both naked liposomes and stealth liposomes can be performed. To remove any free drugs, bare liposomal dispersion is first pre-dialyzed in buffered saline using dialysis tubing in a dialysis tube resembling a blank marine plasma tube, 2 ml of stealth liposomal solution and 2 ml of blank marine plasma is mixed. Two beakers, each holding 50 ml of HBS are used to hold naked and stealth liposome loaded dialysis tubing. A water bath is used to incubate the beakers at 37 °C. Aliquots of the samples removed carefully out of the beakers at time intervals of 0 min, 5 min, 15 min, 30 min, 1 hour, 4 h, 6 h, 10 h, 24 h, and 48 h and replaced with an equal amount of HBS. Both the fluoro-spectrophotometry method and the HPLC method can be used to determine the drug concentrations based on the loaded drug. The percentage release rate can be calculated using the formula below. The amount of medication released, or the release medium was measured as Wn (g), while the total amount of drug added to the liposomes is measured as W (g). The formula for the drug's release rate is (Wn/W) x100% [68].

Level of free drug

The two techniques used for this study are small gel exclusion chromatography and selective drug adsorption on Dowex cation exchangers, which is carried out in either small glass columns or polycarbonate pipette tips (0.1-1.0 ml).

Applications of pegylated liposomes in cancer and other diseases

To prolong circulation periods and prevent interactions with other components as well as avoid reticuloendothelial system clearance stealth liposomal formulations are widely used for *in vivo* administration of chemotherapy drugs [69, 70]. To facilitate drug transport to the targeted site, the surface of nanoparticles is conjugated by ligands for site specific delivery. Because malignant cells are more vascularized than healthy cells, stealth liposomes have the capacity to gather in cancer tissue. However, it has been discovered that functionalizing or conjugating Stealth liposomes with different ligands improves active targeting of cancers.

A study was conducted to find out whether quercetin could be delivered to HeLa and cervical cancer cells using stealth liposomes [71] and it demonstrated improved particle stability and 44 fold increased flavonoid absorption. To prolong the drug's biological half-life and enhance systemic circulation, trans-resveratrol loaded DSPE-PEG 2000 was tested on glioma cells. It was demonstrated that the developed stealth formulations increased the drug's plasma half-life by 18 times and improved cerebral distribution by 9 times when compared to drug solution [72]. In a different investigation, cholesteryl liposomes with folate were specifically developed to target HeLa cells and release doxorubicin [73]. Intravenous drug loaded liposomes improved cell interaction and cytotoxicity in cancer cells without having a deleterious impact on the heart or kidneys.

Liposomes functionalization with transferrin (Tf)

Most of the cancers have high levels of transferrin receptors (Tf) and using modified liposomes can enhance the absorption of nanoparticles by the cancer cells that form tumours. The results point to functionalizing Tf for site specific anticancer drug delivery via Stealth liposomes, carbon quantum dots, or polymeric surfaces. The transferrin receptor induces endocytosis due to the serum glycoprotein Tf, which transports ferric ions when internalized. After adhering to the cell surface, Tf internalizes complexes quickly via covered pits as a ligand receptor, entering to the cellular compartment where the anticancer drug delivery occurs [74].

Stealth liposomes were combined with Tf for the active targeting of sirolimus (SRM) in the treatment of breast cancer. When sirolimus loaded Tf-DPPC stealth liposomes was compared to Tf-DSPC-stealth, the later demonstrated greater cell cytotoxicity and less cell viability, demonstrating an antiproliferative effect. Compared to free drug and non-conjugated stealth nanoparticles, sirolimus loaded liposomes had a greater anticancer impact for Tf stealth nanoparticles and significantly suppressed tumor formation. The results demonstrate the potential of sirolimus encapsulated Tf stealth liposomes as a drug delivery system for the treatment of breast cancer and tumour active targeting.

Liposomes as theranostics and cancer therapeutics

Liposomes that are loaded with chemotherapy drugs are now an effective delivery system in treatment of cancer. Cancer Multidrug Resistance (MDR), a condition where the disease becomes resistant to anticancer drugs, is the greatest barrier to obtaining a potential effective cure. The chemotherapeutic drugs encapsulated in nanocarriers like liposomes are able to bypass many MDR causing pathways, enhancing the treatment efficacy against multidrug resistant malignancies. PEGylated Liposomes can also be utilized to deliver cytotoxic drugs to cancerous cells. USA and Europe authorized the PEGylated Liposomal Doxorubicin (PLD) (DOXIL/Caelyx) which is the first and only stealth liposome for treating Kaposi's sarcoma and recurrent ovarian cancer. Presently, multiple myeloma, breast cancer, and recurrent high grade glioma are among the cancers that can be treated with DOXIL/Caelyx. Currently being investigated (paclitaxel or docetaxel) are the anticancer effects of PLD in conjunction with vinorelbine, taxanes, and temozolomide (Temodal® Schering-Plough, Kenilworth, NJ, USA). In a molar ratio of 55:40:5, HSPC, CHOL, and mPEG-DSPE (molecular weight 2000) make up the rigid bilayer of PLD. Doxorubicin may be integrated into liposomes with a mean diameter of 85 nm at a dose of 2 mg/ml. In contrast, the plasma half-lives of the free drug are 0.2 h, those of myocet and daunoxome are 2-3 h, and those of the free drug are 5 h, and the pharmacokinetics is rather sluggish. Half-lives for plasma elimination range between 1.5 and 45 h and follow a bi-exponential curve (median values).

The distribution volume is small, and the plasma clearance is clearly slow (0.1L/hour), with almost all of the drug identified in the plasma after PLD injection being liposome encapsulated when compared to a similar dose of traditional doxorubicin, PLD's pharmacokinetic includes considerably lower cardiotoxicity, myelosuppression, alopecia, and nausea S-CKD602 (Alza Corporation), a PEGylated stealth liposomes loaded with CKD-602, a semisynthetic analogue of camptothecin, shown low toxicity and exceptional efficacy. At doses of 0.5 mg/m2, S-CKD602 had a plasma AUC 50 times greater than non-liposomal CKD-602. In addition to soyPC, CHOL, mPEG2000-DSPE, dipalmitoylphosphatidyl glycerol (DPPG), and soy phosphatidyl choline, lipoplatin is a unique liposomal cisplatin formulation. Depending on the dosage, it's stated half-life ranges from 60 to 117 h. According to the study, lipoplatin exhibits none of the adverse effects that cisplatin does up to a dosage of 125 mg/m2 every 14 d [75].

The Doxil®, which comprises 100 nm doxorubicin loaded PEGylated Liposomes with mPEG2000 as a surface coating, numerous cancers, including ovarian, breast, and AIDS related tumours linked to Kaposi's sarcoma, have been effectively treated with it [76]. Rhenium-186 (186Re) labelled Doxil is developed from Doxil by remotely adding 186Re-BMEDA [N,N-bis (2-mercaptoethyl)-N,N-diethyl ethylene diamine] through the already-present ammonium sulphate gradient. BMEDA is a weak base that may actively load into the Doxil core and chelate 186Re. The 90-hour half-life of the dual emitting radionuclide 186Re makes it appropriate for single photon emission computed tomography imaging as well as cancer treatment (SPECT). Relabeled Doxil outperformed Doxil by itself in a xenograft model of head and neck squamous cell cancer. These novel theranostic stealth liposomes allowed for 120 hour real time and quantitative drug delivery tracking, which showed significant tumour development from 4 to 120 h [1-2% injected dosage (ID)/g tissue] and delayed blood clearance with a half-life of 24 h.

By active loading technique, 186Re could be maintained inside the aqueous core of Doxil resulting in greater labelling stability than the traditional method, which only tagged 186Re at the liposomal surface via a lipid containing a metal chelating group. Recent developments in the treatment of brain cancer include the formulations (2B3-101) and (G-Technology®) that are based on glutathione PEGylated liposomal doxorubicin. Based on Doxil®, it has a glutathione layer added to the surface to improve the

distribution of drug across the blood brain barrier (BBB) without compromising safety. Two separate experiments utilizing 2B3-101 liposomes revealed a substantial reduction in brain tumour growth factor as indicated by the level of bioluminescence [77]. When treated once a week at a dose of 5 mg/kg, 2B3-101 showed a more effective anticancer effect than saline and PEGylated liposomal doxorubicin. Only 2 out of 9 rats receiving 2B3-101 indicated complete cancer remission, hence there was a minimal tumour decrease observed.

The growth of brain tumour was significantly inhibited by 2B3-101 injections given twice weekly at a dose of 5 mg/kg, in contrast to doxorubicin based PEGylated liposomes and saline, and a complete suppression was observed in just one rat given the drug. Additionally, 2B3-101 given twice weekly significantly extended average survival time by 38.5% and 16.1%, respectively, over saline and PEGylated liposomal doxorubicin [78]. The findings demonstrated that glutathione PEGylated liposomal doxorubicin enhanced the drug's effectiveness in transporting the targeting ligands directly to brain tissue, potentially resulting in the treatment of brain cancer. The FDA has approved LipoDox®, a different PEGylated liposomal version of DOX produced by Sun Pharma in 2012 [79]. Onivyde®, a different irinotecan containing PEGylated liposome, shows an extended anticancer impact [80].

The FDA authorized OnivydeTM, which also contains the anticancer drug irinotecan, to treat metastatic adenocarcinoma Distearoyl phosphatidylcholine, cholesterol, and distearoylphosphatidyl ethanolamine are all present in OnivydeTM in the amounts of 3:2:0.015. The co-delivery of molecules of ribonucleic acid interference (RNAi) towards thymidylate synthase complexed to PEGylated based liposomes [PEGylated thymidylate synthase complexed with cationic liposomes(TS shRNA-lipoplex)] and oxaliplatin (1-OHP) containing liposomes for the treatment of solid tumors is a well-researched example of combination therapy. This combined therapy is a potential way to improve the therapeutic efficacy of 1-OHP and lower the immunogenic response caused by the RNAi molecule by precisely delivering TS-shRNA to cancer tissues. In contrast to single therapies using l-OHP-based liposomes or TS-shRNA alone, TS-shRNA significantly inhibits tumour development by fundamentally preventing cell proliferation through gene silencing [81].

Other clinical uses for PEGylated liposomes include the clinical diagnostic application of positron emission tomography (PET) imaging and the use of internal cancer radiotherapy with PEGylated 177Lu loaded liposomes [82]. Patients with brain metastases are the primary target for the PEGylated Liposomal DOX formulation 2B3-101 [83]. The blood brain barrier (BBB), which prevents the entry of xenobiotics and endogenous substances, makes it difficult to target brain cancers. G-technology, based on the biology of human safe receptors, was the area of research that made 2B3-101 possible. It is the most adaptable technique for encasing different molecules, including drugs of both high and low molecular weights, hydrophilic and lipophilic chemicals, and other substances [84].

The transport of the encapsulated moiety across the blood brain barrier is facilitated by glutathione PEGylation, was employed in the formulation of this product S-CKD602, a potent topoisomerase I inhibitor (CKD-602) containing PEGylated liposomal formulation manufactured by the Alza Company [85]. The formulation's extended circulation property is associated with increase in area under curve (AUC). In comparison to unencapsulated form, the AUC of the semi synthetic camptothecin analogue CKD-602 was 50 times greater in liposomes [86]. The mPEG linked phospholipids on the outside of the lipid bilayer that composes the camptothecin analogue CKD-602 stealth liposomes. mPEG lipids provide liposomes the advantages of extended plasma circulation period and enhanced administration of drugs into tumours over conventional liposomes [87].

In preclinical investigations using these liposomes, there is a 3-10 fold improvement in therapeutic index over the non liposomal formulation. When compared to muscle tissue, fat tissue exhibits a distinctive characteristic of enhanced dispersion that vary depending on the patient's body composition, according to the phase II trial study on S-CKD602 [88]. In the aqueous core of sterically stabilized liposomes, cisplatin is present in the stealth liposome formulation SPI-0770 (totally hydrogenated soy HSPC, cholesterol, and DSPE-PEG). These compounds behave stealthily because of their apparent half-lives of 60 to 100 h.

According to investigations using zoledronic acid (ZOL) encapsulating PEGylated liposomes (LipoZOL) on a laboratory animal model of neuropathic pain, two intravenous doses of 10 g of ZOL administered at days 2 and 4 upon an injury, either free or combined with liposomes, significantly decreased mechanical hypersensitivity at days 3 and 7 after nerve injury. Free ZOL, however, made no noticeable difference to the mechanical barrier. Seven days after spared nerve injury (SNI), glial fibrillary acidic protein (GFAP)-labeled astrocytes revealed as hypertrophic, cells that were activated in the ipsilateral dorsal horn, according to an immunohistochemical investigation of the spinal cord.

LipoZOL caused a protective phenotype, which significantly changed astrocyte shape without changing the total number of cells. Interleukin 10 was also found within the spinal cord astrocytes of the LipoZOL treated rats. By monitoring the biodistribution of fluorescently labelled liposomes, ZOL's transit to the Central Nervous System (CNS) was exhibited. However, a fluorescence spike in the brain and spinal cord only emerged in neuropathic mice at the 30 min and 1 hour period. Notably, the liver and kidney of both the normal and neuropathic animal groups accumulated liposomes. The results indicate that ZOL, if administered with a delivery mechanism that can pass through the altered BBB, may provide a unique pathway for the treatment of neuropathic pain [89].

Palbociclib (PAB) was encapsulated in stealth liposomes (LPS) with a vesicle size of less than 100 nm to target cyclin-dependent kinase (CDK4 and CDK6) in triple negative breast cancer. First order, biphasic release kinetics can be observed in LPS-PAB. LPS-PAB also exhibits a lower half-maximal inhibitory concentration (IC50) value (1.99 M) compared to PAB alone (3.24 M). The proposed nanoliposomes were dyed with fluorescein isothiocyanate (FITC) to enable fast cellular uptake. Notably, stealth LPS-PAB has shown a 1.75 fold reduced hemolytic potential than PAB plain medicine at a dose of 100 g/ml. The PK results revealed 2.5 fold higher C_{max}, 1.45 fold higher AUC_{tot}, 1.8 fold higher half-life, and 1.3 fold higher MRT when compared to PAB suspension given orally [90]. The stealth liposomes developed with PEG5000 possessed a greater encapsulation efficiency (83 0.4%) and a delayed rate of drug release (32.2% in 9 h) when compared to stealth liposomes developed with PEG2000 (79.0 0.4% and 45.3%, respectively) and conventional liposomes (64.8 0.8% and 52.4%) respectively [91].

Liposomal delivery for vaccines

It has been proved that genetic vaccinations with antigens from bacteria, viruses, and cancer have the potential to maintain cellular and humoral immunity. Clinical trials have shown the effectiveness of liposome based vaccines, and additional human trials are currently underway. Hepatitis A virus that has been rendered inactive and is anchored to a phospholipid bilayer can be discovered in Epaxal, a liposomal based hepatitis A vaccine [92, 93]. In healthy human volunteers, the liposome based malaria vaccine has been shown to increase levels of anti malarial antibody [94]. It is being studied whether the liposomal mycobacterium tuberculosis vaccine enhances T cell mediated immunity in human subjects [95].

Liposomes in transfection

Stealth liposome formulation makes for the ideal cationic transfecting vectors. By enclosing the gene encoding a therapeutic protein in liposomal vesicles, allowing DNA plasmid to condense into a highly organized structure, the gene can be protected against DNA breakdown. The structure of the gene delivery system must bypass the biological membranes and permit endosomal exit to prevent DNA degradation in the liposomal compartment. For the delivery of genes, numerous cationic liposomal formulations have been tested.

The therapeutic application of cationic liposomes is subject to several limitations, such as rapid clearance, large particle size,

immunostimulation, and complement activation. To skirt these restrictions, PEGylated cationic liposomes are used to increase *in vivo* circulation time, reduce immunostimulation, and activate complement less. Commercially available cationic liposomes for gene transfection include Lofectamine 2000. Curcumin liposomes containing signal transducer and activator of transcription 3 small interfering RNA (STAT3 si-RNA) are produced using the Bangham method to treat skin cancer. Liposomes inhibit the development of B16F10 melanoma cells better than free STAT3 si-RNA and free curcumin. For the treatment of many tumors and genetic disorders, the clustered regularly interspaced short palindromic repeats (CRISPR or Cas9 gene) can be delivered via liposomes [96].

Stealth liposomes in diagnostic imaging

In contrast methods like gamma scintillation, magnetic resonance imaging (MRI), computed tomography imaging (CTG), and sonography, stealth liposomes are used as vesicles to carry different types of compounds in bilayer or in the aqueous compartment. The ability of stealth liposomes to integrate several contrast moieties, deliver the agent accurately to the target area, and increase the contrast signal to the diagnostic agent are only a few advantages of employing stealth liposomes in diagnostic imaging (Tc, Mn, Gd). A soluble chelating compound that will be enclosed in the aqueous core of vesical may be utilized for complex metals. Gadolinium containing PEGylated liposomes that have been sterically stabilized serve as incredibly potent contrast agents in magnetic resonance imaging [92, 97].

Stealth liposomes in targeted drug delivery

Targeted stealth liposomes offer several advantages over individual drugs targeted by polymers or antibodies. One of the most recent developments is the dramatic increase in the amount of drug that can be delivered to the target. Additional ligand molecules exposed on the liposome surface may also increase ligand avidity and absorption. Due to the drug molecules tendency to disseminate into nearby tumour cells, immune liposomes also have a "bystander killing effect" [92]. The first human monoclonal antibody for metastatic breast cancer was the anti-HER2 antibody known as trastuzumab [98]. To specifically target doxorubicin, daunorubicin, and cisplatin to the cancer cells, folic acid containing liposomes are used [99]. Liposomes with a transferrin ligand attached are used to deliver anticancer medications, proteins, and genes to cancer cells [100]. Stealth liposomes containing haloperidol are being used to target specific genes in breast cancer cells [101]. Target delivery is accomplished using stealth liposomes with 13 L-peptides [102].

PEGylated liposomes-enhanced permeability and retention (EPR) effect

The reticuloendothelial system (RES) interacts significantly with conventional approaches in cancer therapy. The development of a delivery system or nanocarrier that can evade the body's phagocytosis defense mechanisms. PEGylated liposome is one of the methods to prolong the duration of blood circulation. Cancer cells have increased leakiness or permeability. This strategy is called passive targeting. Moreover, macromolecule buildup is also driven on by restricted lymphatic outflow. This phenomenon is known as the enhanced permeability and retention (EPR) effect [103, 104]. To provide appropriate oxygen and nutrients supply that sustain tumour development, neovascularization is crucial for tumour progression.

A tumor's abnormal vascular network is formed by an unbalanced angiogenic regulatory system and is marked by dilated, leaky blood vessels, poor lymphatic drainage, and other characteristics. A long circulation period for nano sized drug carriers is made possible by the distinct pathophysiologic characteristics of tumour vasculature. Rather than normal tissues, PEGylated liposomes were specifically retained and released into malignant tissues. Hence, it is anticipated that utilizing PEGylated liposomes containing anticancer drugs will result in less drug toxicity [105, 106].

Maeda and his coworkers gave the initial description of EPR [107]. The primary mechanism for the selective deposition of nanomedicines in tumour site is recognized to be the EPR effect [108]. Using the EPR effect, PEGylated Liposomes, a form of nanocarrier treatment, are used as a drug delivery method to target malignant cells. It has been demonstrated that PEG surface modification decreases liposome absorption by MPS substantially, increases the concentration of PEGylated liposomes in tumours, and increases the half-life of liposomes. PEGylated liposome upon passive accumulation in solid tumours doesn't disperse evenly due to the wide range of EPR effects found in tumour tissues [109].

On the contrary, extravasation and tumour accumulation of PEGylated liposomes are significantly influenced by tumour factors such as tumour size, type of malignancy, and quantity of tumour vasculature [110]. Doxil® PEGylated Doxorubicin Liposomes, which exhibited stealth characteristics were proven to enhance the serum half-life of their loaded active ingredient, are a perfect example. Since glutaminolysis, a specific metabolic pathway for tumour cells, induces a significantly greater quantity of ammonia in tumour tissues, it was claimed that doxorubicin was exclusively released in the interstitial fluid of the tumour. After the doxorubicin has been released from the liposomes, the cancer cells absorb it and get damaged.

Pharmacokinetic considerations of PEGylated liposomes

The phagocytosis Reticuloendothelial System (RES) components often eliminate the nanocarrier based drugs from the blood, such as liposomes (both PEGylated and Non-PEGylated Liposomes). RES is mostly found in the spleen, bone marrow, and liver (hepatic kupffer cells), which also contain monocytes, macrophages, and dendritic cells. Hence, elements that affect RES activity might alter the rate of clearance, toxicity, and responsiveness of PEGylated liposomes [111]. With the intervention of the immune system, particularly RES macrophages, nanomedicine and liposomes are eliminated from the circulation system after systemic delivery [112]. Hence, the distinctive pharmacokinetics of nanocarrier systems affect the drug delivery. As a result, the unique pharmacokinetics of nanocarrier systems has an impact on medication delivery.

PEGylated liposomes enhanced tumour delivery by modifying the pharmacokinetic and distribution properties of the encapsulated drugs upon intravenous administration. Chemotherapy is identified as having increased anticancer efficacy and reduced toxicity as compared to conventional cytotoxic drugs because of its stagnant blood vessel leakage and tendency for accumulation in tumour tissues. Nanocarrier pharmaceuticals should be developed with the ability to withstand these clearance pathways and inhibit complement activation in order to prolong the half-life of anticancer drugs in the blood stream [113, 114].

Regulatory considerations and clinical trials

The Food and Drug Administration (FDA) and European Medicines Agency (EMA) approved narrowly 100 nanomedicines while numerous nanomedicines are currently undergoing clinical trials, during the past several decades [115-117]. The FDA, EMA, Ministry of Health, Labour, Welfare Japan (MHLW), and Chinese National Medical Products Administration (NMPA) released several guidelines about nanomaterials and nanoproducts to share the regulatory network's experience in the scientific evaluation of nanomedicines in response to the tremendous growth in nanomedicine applications. These recommendations involve several different nanoparticles delivery systems viz., liposomes, nanocolloidal products (iron based), block copolymer micelles, and nano products containing small interfering RNA (SiRNA). Owing to the very extensive dosage form and the relatively high number of liposomes approved in market and clinical studies, all four regulatory agencies collaborated to develop the liposome regulatory guidance. The overview of the guidelines is depicted in (fig. 7).

Based on these guidelines, we emphasize the significance of developing a broad knowledge base to better comprehend any risk to a product's physicochemical and biological characteristics that is encountered during manufacturing, analysis, and material control. Early pharmaceutical research and development can provide knowledge that can be updated over time by future production and associated control strategies. Lower risk results emerge from an understanding of the connections between Key Material Attribution (CMA), Critical Process Parameters (CPP), physicochemical characteristics, and *in vivo* performance of liposomes [118]. The

additives, particularly those used in lipids, are crucial to the effectiveness of liposomal products. A slight modification to the lipid components might cause a change in the drug's pharmacokinetics or pharmacodynamics, which could result in significant toxicity. The FDA regulation insists on guidelines regarding the requirements for lipid supply (extraction or synthesis), lipid characteristic, specification, and stability are necessary for lipid control. Sterilization is regarded as a challenging process in the manufacture of liposomes since most liposomal substances are designed to be administered via parenteral routes.

Fig. 7: Nanomedicine guidelines (Created by BioRender.com)

Sterile filtering using a 0.22 m membrane is extensively used in the pharmaceutical industrial sector. Nevertheless, issues such as membrane blockage, impaired liposome integrity, and insufficient retention of tiny bacteria might emerge [119]. Hence, for batch consistency and sterility assurance of liposome products, the promising sterilization method and the process validation are essential. While manufacturing liposome preparations, the rate at which the nanoparticle payload leaks may be significantly quicker than anticipated and the *in vivo* destiny of liposomal carriers should be taken into consideration [120]. The Center for Drug Evaluation (CDE), in a recently published guideline for nonclinical pharmacokinetics of nanomedicine known as NMPA, recommends *in vivo* research on vehicles other than cargos [121]. The most useful technique for tracking the movement of vehicles in real time is fluorescent labelling.

Thus, it is crucial to distinguish fluorescent chemicals released from nanoparticles in free form apart from intact carriers [122]. Aggregation Caused Quenching (ACQ) is a promising method for the removal of free probe interference due to the environment responsiveness properties, even if the phenomenon was negative in bio imaging [123-125]. Due to "π-π stacking," the near infrared fluorescence that the ACQ probes exhibit when placed in carrier matrix (usually molecularly dispersed) completely disappears as soon as they are released into the aqueous environment. Hence, the undamaged automobiles are identified by the fluorescence. With the ACQ probes, several nanoparticles' *in vivo* fates have been studied, including those administered via various routes (such as oral, intravenous, transdermal, nasal, and ocular), micelles, nanoemulsions, and nanocrystals [126-130].

CONCLUSIONS

Stealth liposomes that actively target tumour cells have long become the topic of extensive study. Even so, due to the steric stability provided by the mPEG group, the liposome-encapsulated DNA seems to be challenging for cells to ingest. Possible remedies and several proposals have addressed the delivery conundrum. One such technology that employs mPEG groups that may be removed from the liposome surface during extravasations and penetration into malignancies is the polymer modified liposomes (PolyVERSE liposome system). The idea of using stealth liposomes to deliver anti-inflammatory medications to inflamed areas seems appealing. Clinical research demonstrates that stealth liposomes bearing radioactive tracers passively target infection and inflammatory areas in a way like how Doxil targets to tumour site. To date, liposomal delivery systems with modifications have been researched for many clinical dimensions. Liposomes are one of the most preferred delivery approaches in cancer and other diseases. More studies are ongoing in understanding the pharmacokinetics of the PEGylated stealth liposomes, which is one of the promising delivery systems in combating various types of cancer.

FUNDING

Nil

AUTHORS CONTRIBUTIONS

All authors have contributed equally

CONFLICT OF INTERESTS

Declared none

REFERENCES

1. Liu P, Chen G, Zhang J. A review of liposomes as a drug delivery system: current status of approved products, regulatory environments, and future perspectives. Molecules. 2022 Feb 17;27(4):1372. doi: 10.3390/molecules27041372, PMID 35209162.

2. Cattel L, Ceruti M, Dosio F. From conventional to stealth liposomes: a new frontier in cancer chemotherapy. J Chemother. 2004 Nov 1;16 Suppl 4:94-7. doi: 10.1179/joc.2004.16.Supplement-1.94[Suppl], PMID 15688621.
3. Sivadasan D, Sultan MH, Madkhali OA, Alsabei SH, Alessa AA. Stealth liposomes (pegylated) containing an anticancer drug camptothecin: *in vitro* characterization and *in vivo* pharmacokinetic and tissue distribution study. Molecules. 2022 Feb 6;27(3):1086. doi: 10.3390/molecules27031086, PMID 35164350.
4. Srivastava A, Yadav K, Verma NK. Liposomes for the drug delivery: a review. EAS J Pharm Pharmacol. 2021;3(5):117-32. doi: 10.36349/easjpp.2021.v03i05.003
5. Nakhaei P, Margiana R, Bokov DO, Abdelbasset WK, JadidiKouhbanani MA, Varma RS, Be-Heshtkhoo N. Liposomes: structure, biomedical applications, and stability parameters with emphasis on cholesterol. Front Bioeng Biotechnol. 2021;748:9. doi: 10.3389/fbioe.2021.705886
6. Wu H, Yu M, Miao Y, He S, Dai Z, Song W. Cholesterol-tuned liposomal membrane rigidity directs tumor penetration and anti-tumor effect. Acta Pharm Sin B. 2019 Jul 1;9(4):858-70. doi: 10.1016/j.apsb.2019.02.010, PMID 31384544.
7. Bulbake U, Doppalapudi S, Kommineni N, Khan W. Liposomal formulations in clinical use: an updated review. Pharmaceutics. 2017 Mar 27;9(2):12. doi: 10.3390/pharmaceutics9020012, PMID 28346375.
8. Wu H, Yu M, Miao Y, He S, Dai Z, Song W. Cholesterol-tuned liposomal membrane rigidity directs tumor penetration and anti-tumor effect. Acta Pharm Sin B. 2019 Jul 1;9(4):858-70. doi: 10.1016/j.apsb.2019.02.010. PMID 31384544.
9. Kaddah S, Khreich N, Kaddah F, Charcosset C, Greige Gerges H. Cholesterol modulates the liposome membrane fluidity and permeability for a hydrophilic molecule. Food Chem Toxicol. 2018 Mar 1;113:40-8. doi: 10.1016/j.fct.2018.01.017, PMID 29337230.
10. Kasagi N, Doi I, Nakabayashi J, Saito K, Tadakuma A, Muraki N. Optimization of dihydrosphingomyelin/cholesterol mol ratio in topotecan-loaded liposomes to enhance drug retention and plasma half-life by understanding physicochemical and thermodynamic properties of the lipid membrane. J Mol Struct. 2023 Jul 5;1283:135333. doi: 10.1016/j.molstruc.2023.135333.
11. Van der Koog L, Gandek TB, Nagelkerke A. Liposomes and extracellular vesicles as drug delivery systems: a comparison of composition, pharmacokinetics, and functionalization. Adv Healthc Mater. 2022 Mar;11(5):e2100639. doi: 10.1002/adhm.202100639, PMID 34165909.
12. Nsairat H, Khater D, Sayed U, Odeh F, Al Bawab A, Alshaer W. Liposomes: structure, composition, types, and clinical applications. Heliyon. 2022 May 1;8(5):e09394. doi: 10.1016/j.heliyon.2022.e09394, PMID 35600452.
13. Owens III DE, Peppas NA. Opsonization, biodistribution, and pharmacokinetics of polymeric nanoparticles. Int J Pharm. 2006 Jan 3;307(1):93-102. doi: 10.1016/j.ijpharm.2005.10.010, PMID 16303268.
14. Lombardo D, Kiselev MA. Methods of liposomes preparation: formation and control factors of versatile nanocarriers for biomedical and nanomedicine application. Pharmaceutics. 2022 Feb 28;14(3):543. doi: 10.3390/pharmaceutics14030543, PMID 35335920.
15. Rai R, Alwani S, Badea I. Polymeric nanoparticles in gene therapy: new avenues of design and optimization for delivery applications. Polymers (Basel). 2019 Apr 25;11(4):745. doi: 10.3390/polym11040745, PMID 31027272.
16. Chen BM, Cheng TL, Roffler SR. Polyethylene glycol immunogenicity: theoretical, clinical, and practical aspects of anti-polyethylene glycol antibodies. ACS Nano. 2021 Sep 1;15(9):14022-48. doi: 10.1021/acsnano.1c05922, PMID 34469112.
17. Kirpotin DB, Drummond DC, Shao Y, Shalaby MR, Hong K, Nielsen UB. Antibody targeting of long-circulating lipidic nanoparticles does not increase tumor localization but does increase internalization in animal models. Cancer Res. 2006;66(13):6732-40. doi: 10.1158/0008-5472.CAN-05-4199, PMID 16818648.
18. Suk JS, Xu Q, Kim N, Hanes J, Ensign LM. Pegylation as a strategy for improving nanoparticle-based drug and gene delivery. Adv Drug Deliv Rev. 2016;99(A):28-51. doi: 10.1016/j.addr.2015.09.012, PMID 26456916.
19. Tang Y, Wang X, Li J, Nie Y, Liao G, Yu Y. Overcoming the reticuloendothelial system barrier to drug delivery with a "don't-eat-us" strategy. ACS Nano. 2019 Nov 5;13(11):13015-26. doi: 10.1021/acsnano.9b05679, PMID 31689086.
20. Weber C, Voigt M, Simon J, Danner AK, Frey H, Mailander V. Functionalization of liposomes with hydrophilic polymers results in macrophage uptake independent of the protein corona. Biomacromolecules. 2019 Jul 3;20(8):2989-99. doi: 10.1021/acs.biomac.9b00539, PMID 31268685.
21. Bulbake U, Doppalapudi S, Kommineni N, Khan W. Liposomal formulations in clinical use: an updated review. Pharmaceutics. 2017 Mar 27;9(2):12. doi: 10.3390/pharmaceutics9020012, PMID 28346375.
22. Agarwal K. Liposome assisted drug delivery-an updated review. Indian J Pharm Sci. 2022 Jul 14;84(4):797-811. doi: 10.36468/pharmaceutical-sciences.975.
23. Molinaro R, Wolfram J, Federico C, Cilurzo F, Di Marzio L, Ventura CA. Polyethylenimine and chitosan carriers for the delivery of RNA interference effectors. Expert Opin Drug Deliv. 2013 Dec 1;10(12):1653-68. doi: 10.1517/17425247.2013.840286, PMID 24090239.
24. Celia C, Trapasso E, Locatelli M, Navarra M, Ventura CA, Wolfram J. Anticancer activity of liposomal bergamot essential oil (BEO) on human neuroblastoma cells. Colloids Surf B Biointerfaces. 2013 Dec 1;112:548-53. doi: 10.1016/j.colsurfb.2013.09.017, PMID 24099646.
25. Wang Y, Wang J, Sun M, Zhang J, Bi Y. Coating liposomes with ringlike PEG: the synthesis and stealth effect of cho-lesterol–PEG–cholesterol. Materials Advances. 2022;3(5):2417-24.
26. Akbarzadeh A, Rezaei Sadabady R, Davaran S, Joo SW, Zarghami N, Hanifehpour Y. Liposome: classification, preparation, and applications. Nanoscale Res Lett. 2013 Dec;8(1):102. doi: 10.1186/1556-276X-8-102, PMID 23432972.
27. Wagner A, Vorauer Uhl K. Liposome technology for industrial purposes. J Drug Deliv. 2011;2011:591325. doi: 10.1155/2011/591325, PMID 21490754.
28. Van der Koog L, Gandek TB, Nagelkerke A. Liposomes and extracellular vesicles as drug delivery systems: a comparison of composition, pharmacokinetics, and functionalization. Adv Healthc Mater. 2022 Mar;11(5):e2100639. doi: 10.1002/adhm.202100639, PMID 34165909.
29. Molinaro R, Wolfram J, Federico C, Cilurzo F, Di Marzio L, Ventura CA. Polyethylenimine and chitosan carriers for the delivery of RNA interference effectors. Expert Opin Drug Deliv. 2013 Dec 1;10(12):1653-68. doi: 10.1517/17425247.2013.840286, PMID 24090239.
30. Shi L, Zhang J, Zhao M, Tang S, Cheng X, Zhang W. Effects of polyethylene glycol on the surface of nanoparticles for targeted drug delivery. Nanoscale. 2021;13(24):10748-64. doi: 10.1039/d1nr02065j, PMID 34132312.
31. Mitchell MJ, Billingsley MM, Haley RM, Wechsler ME, Peppas NA, Langer R. Engineering precision nanoparticles for drug delivery. Nat Rev Drug Discov. 2021 Feb;20(2):101-24. doi: 10.1038/s41573-020-0090-8, PMID 33277608.
32. Liu P, Chen G, Zhang J. A review of liposomes as a drug delivery system: current status of approved products, regulatory environments, and future perspectives. Molecules. 2022 Feb 17;27(4):1372. doi: 10.3390/molecules27041372, PMID 35209162.
33. Sturm L, Poklar Ulrih N. Basic methods for preparation of liposomes and studying their interactions with different compounds, with the emphasis on polyphenols. Int J Mol Sci. 2021 Jun 18;22(12):6547. doi: 10.3390/ijms22126547, PMID 34207189.
34. Kapoor M, Burgess DJ. Efficient and safe delivery of siRNA using anionic lipids: formulation optimization studies. International Journal of Pharmaceutics. 2012 Aug 1;432(1-2):80-90.
35. Akinc A, Zumbuehl A, Goldberg M, Leshchiner ES, Busini V, Hossain N. A combinatorial library of lipid-like materials for delivery of RNAi therapeutics. Nat Biotechnol. 2008 May;26(5):561-9. doi: 10.1038/nbt1402, PMID 18438401.

36. Llamas S, Guzman E, Ortega F, Rubio RG. Adsorption of mixtures of a pegylated lipid with anionic and zwitterionic surfactants at solid/liquid. Colloids Interfaces. 2020 Oct 29;4(4):47. doi: 10.3390/colloids4040047.
37. Saw PE, Park J, Lee E, Ahn S, Lee J, Kim H. Effect of PEG pairing on the efficiency of cancer-targeting liposomes. Theranostics. 2015;5(7):746-54. doi: 10.7150/thno.10732, PMID 25897339.
38. Nakamura H, Abu Lila AS, Nishio M, Tanaka M, Ando H, Kiwada H. Intra-tumor distribution of pegylated liposome upon repeated injection: no possession by prior dose. J Control Release. 2015 Dec 28;220(A):406-13. doi: 10.1016/j.jconrel.2015.11.002, PMID 26548975.
39. Nakhaei P, Margiana R, Bokov DO, Abdelbasset WK, Jadidi Kouhbanani MA, Varma RS, Be-heshtkhoo N. Liposomes: structure, biomedical applications, and stability parameters with emphasis on cholesterol. Front Bioeng Biotechnol. 2021;9:748.
40. Majzoub RN, Chan CL, Ewert KK, Silva BF, Liang KS, Jacovetty EL. Uptake and transfection efficiency of pegylated cationic liposome–DNA complexes with and without RGD-tagging. Biomaterials. 2014 Jun 1;35(18):4996-5005. doi: 10.1016/j.biomaterials.2014.03.007, PMID 24661552.
41. Pozzi D, Colapicchioni V, Caracciolo G, Piovesana S, Capriotti AL, Palchetti S. Effect of polyethyleneglycol (PEG) chain length on the bio-nano-interactions between PEGylated lipid nanoparticles and biological fluids: from nanostructure to uptake in cancer cells. Nanoscale. 2014;6(5):2782-92. doi: 10.1039/c3nr05559k, PMID 24463404.
42. Tang J, Kuai R, Yuan W, Drake L, Moon JJ, Schwendeman A. Effect of size and pegylation of liposomes and peptide-based synthetic lipoproteins on tumor targeting. Nanomedicine. 2017 Aug 1;13(6):1869-78. doi: 10.1016/j.nano.2017.04.009, PMID 28434931.
43. Nosova AS, Koloskova OO, Nikonova AA, Simonova VA, Smirnov VV, Kudlay D. Diversity of pegylation methods of liposomes and their influence on RNA delivery. Med Chem Comm. 2019 Feb 12;10(3):369-77. doi: 10.1039/c8md00515j, PMID 31015904.
44. Nosova AS, Koloskova OO, Nikonova AA, Simonova VA, Smirnov VV, Kudlay D. Diversity of pegylation methods of liposomes and their influence on RNA delivery. Med Chem Comm. 2019;10(3):369-77. doi: 10.1039/c8md00515j, PMID 31015904.
45. Van der Koog L, Gandek TB, Nagelkerke A. Liposomes and extracellular vesicles as drug delivery systems: a comparison of composition, pharmacokinetics, and functionalization. Adv Healthc Mater. 2022 Mar;11(5):e2100639. doi: 10.1002/adhm.202100639, PMID 34165909.
46. Golkar N, Tamaddon AM, Samani SM. Effect of lipid composition on incorporation of trastuzumab-PEG-lipid into nanoliposomes by post-insertion method: physicochemical and cellular characterization. J Liposome Res. 2016 Apr 2;26(2):113-25. doi: 10.3109/08982104.2015.1048692, PMID 26023889.
47. Belletti D, Tosi G, Forni F, Lagreca I, Barozzi P, Pederzoli F. Pegylated siRNA lipoplexes for silencing of BLIMP-1 in primary effusion lymphoma: *in vitro* evidences of antitumoral activity. Eur J Pharm Biopharm. 2016 Feb 1;99:7-17. doi: 10.1016/j.ejpb.2015.11.007, PMID 26625717.
48. Li C, Wang J, Wang Y, Gao H, Wei G, Huang Y. Recent progress in drug delivery. Acta Pharm Sin B. 2019 Nov 1;9(6):1145-62. doi: 10.1016/j.apsb.2019.08.003, PMID 31867161.
49. Gupta V, Bhavanasi S, Quadir M, Singh K, Ghosh G, Vasamreddy K. Protein pegylation for cancer therapy: bench to bedside. J Cell Commun Signal. 2019 Sep;13(3):319-30. doi: 10.1007/s12079-018-0492-0, PMID 30499020.
50. Mishra P, Nayak B, Dey RK. Pegylation in anti-cancer therapy: an overview. Asian J Pharm Sci. 2016 Jun 1;11(3):337-48. doi: 10.1016/j.ajps.2015.08.011.
51. Pelegri-O'Day EM, Lin EW, Maynard HD. Therapeutic protein-polymer conjugates: advancing beyond pegylation. J Am Chem Soc. 2014 Oct 15;136(41):14323-32. doi: 10.1021/ja504390x, PMID 25216406.
52. Wilding KM, Smith AK, Wilkerson JW, Bush DB, Knotts IV TA, Bundy BC. The locational impact of site-specific PEGylation: streamlined screening with cell-free protein expression and coarse-grain simulation. ACS Synth Biol. 2018 Feb 16;7(2):510-21. doi: 10.1021/acssynbio.7b00316, PMID 29295615.
53. Webber MJ, Appel EA, Vinciguerra B, Cortinas AB, Thapa LS, Jhunjhunwala S. Supramolecular PEGylation of biopharmaceuticals. Proc Natl Acad Sci USA. 2016 Dec 13;113(50):14189-94. doi: 10.1073/pnas.1616639113, PMID 27911829.
54. Nkanga CI, Bapolisi AM, Okafor NI, Krause RW. General perception of liposomes: formation, manufacturing and applications. Liposomes Adv Perspect; 2019 Mar 26.
55. Gregoridis G. Entrapment of drug and other material in to liposome "liposome technology". 3rd ed, Vol-ll; 2007. p. 56-7.
56. Caponigro F, Comella P, Budillon A, Bryce J, Avallone A, De Rosa V. Phase I study of Caelyx (doxorubicin HCL, pegylated liposomal) in recurrent or metastatic head and neck cancer. Ann Oncol. 2000 Mar 1;11(3):339-42. doi: 10.1023/a:1008319618638, PMID 10811502.
57. Du J, Lu WL, Ying X, Liu Y, Du P, Tian W. Dual-targeting topotecan liposomes modified with tamoxifen and wheat germ agglutinin significantly improve drug transport across the blood–brain barrier and survival of brain tumor-bearing animals. Mol Pharm. 2009 Jun 1;6(3):905-17. doi: 10.1021/mp800218q, PMID 19344115.
58. Kanásova M, Nesmerak K. Systematic review of liposomes' characterization methods. Monatsh Chem. 2017 Sep;148(9):1581-93. doi: 10.1007/s00706-017-1994-9.
59. Saraf S, Jain A, Tiwari A, Verma A, Panda PK, Jain SK. Advances in liposomal drug delivery to cancer: an overview. J Drug Deliv Sci Technol. 2020 Apr 1;56:101549. doi: 10.1016/j.jddst.2020.101549.
60. Silindir Gunay M, Karpuz M, Ozturk N, Yekta Ozer AY, Erdogan S, Tuncel M. Radiolabeled, folate-conjugated liposomes as tumor imaging agents: formulation and *in vitro* evaluation. J Drug Deliv Sci Technol. 2019 Apr 1;50:321-8. doi: 10.1016/j.jddst.2019.02.003.
61. Saraswat AL, Maher TJ. Development and optimization of stealth liposomal system for enhanced *in vitro* cytotoxic effect of quercetin. J Drug Deliv Sci Technol. 2020 Feb 1;55:101477. doi: 10.1016/j.jddst.2019.101477.
62. Vijayakumar MR, Kosuru R, Vuddanda PR, Singh SK, Singh S. Trans resveratrol loaded DSPE PEG 2000 coated liposomes: an evidence for prolonged systemic circulation and passive brain targeting. J Drug Deliv Sci Technol. 2016 Jun 1;33:125-35. doi: 10.1016/j.jddst.2016.02.009.
63. Choi JS, Park JW, Seu YB, Doh KO. Enhanced efficacy of folate-incorporated cholesteryl doxorubicin liposome in folate receptor abundant cancer cell. J Drug Deliv Sci Technol. 2021 Apr 1;62:102385. doi: 10.1016/j.jddst.2021.102385.
64. Nandi U, Onyesom I, Douroumis D. Transferrin conjugated stealth liposomes for sirolimus active targeting in breast cancer. J Drug Deliv Sci Technol. 2021 Dec 1;66:102900. doi: 10.1016/j.jddst.2021.102900.
65. Immordino ML, Dosio F, Cattel L. Stealth liposomes: review of the basic science, rationale, and clinical applications, existing and potential. Int J Nanomedicine. 2006;1(3):297-315. PMID 17717971.
66. Kibria G, Hatakeyama H, Sato Y, Harashima H. Anti-tumor effect via passive anti-angiogenesis of pegylated liposomes encapsulating doxorubicin in drug resistant tumors. Int J Pharm. 2016 Jul 25;509(1-2):178-87. doi: 10.1016/j.ijpharm.2016.05.047, PMID 27234700.
67. Rip J, Chen L, Hartman R, van den Heuvel A, Reijerkerk A, van Kregten J. Glutathione pegylated liposomes: pharmacokinetics and delivery of cargo across the blood–brain barrier in rats. J Drug Target. 2014 Jun 1;22(5):460-7. doi: 10.3109/1061186X.2014.888070, PMID 24524555.
68. Gaillard PJ, Appeldoorn CC, Dorland R, Van Kregten J, Manca F, Vugts DJ. Pharmacokinetics, brain delivery, and efficacy in brain tumor-bearing mice of glutathione pegylated liposomal doxorubicin (2B3-101). PLOS ONE. 2014 Jan 8;9(1):e82331. doi: 10.1371/journal.pone.0082331, PMID 24416140.
69. Chou H, Lin H, Liu JM. A tale of the two pegylated liposomal doxorubicins. Onco Targets Ther. 2015 Jul 13;8:1719-20. doi: 10.2147/OTT.S79089, PMID 26203262.

70. Passero Jr FC, Grapsa D, Syrigos KN, Saif MW. The safety and efficacy of Onivyde (irinotecan liposome injection) for the treatment of metastatic pancreatic cancer following gemcitabine-based therapy. Expert Rev Anticancer Ther. 2016 Jul 2;16(7):697-703. doi: 10.1080/14737140.2016.1192471, PMID 27219482.
71. Alaaeldin E, Abu Lila AS, Ando H, Fukushima M, Huang CL, Wada H. Co-administration of liposomal l-OHP and pegylated TS shRNA-lipoplex: a novel approach to enhance anti-tumor efficacy and reduce the immunogenic response to RNAi molecules. J Control Release. 2017 Jun 10;255:210-7. doi: 10.1016/j.jconrel.2017.04.040, PMID 28461099.
72. Petersen AL, Henriksen JR, Binderup T, Elema DR, Rasmussen PH, Hag AM. *In vivo* evaluation of PEGylated ^{64}Cu-liposomes with theranostic and radiotherapeutic potential using micro PET/CT. Eur J Nucl Med Mol Imaging. 2016 May;43(5):941-52. doi: 10.1007/s00259-015-3272-6, PMID 26646780.
73. Gaillard PJ, Appeldoorn CC, Dorland R, Van Kregten J, Manca F, Vugts DJ. Pharmacokinetics, brain delivery, and efficacy in brain tumor-bearing mice of glutathione pegylated liposomal doxorubicin (2B3-101). PLOS ONE. 2014 Jan 8;9(1):e82331. doi: 10.1371/journal.pone.0082331, PMID 24416140.
74. Mohamed M, Alaaeldin E, Hussein A, Sarhan H. Liposomes and PEGylated liposomes as drug delivery system. Journal of Advanced Biomedical and Pharmaceutical Sciences. 2020;14:51. doi: 10.21608/jabps.2020.22937.1068.
75. Birngruber T, Raml R, Gladdines W, Gatschelhofer C, Gander E, Ghosh A. Enhanced doxorubicin delivery to the brain administered through glutathione PEGylated liposomal doxorubicin (2B3-101) as compared with generic Caelyx,(®)/Doxil(®)-a cerebral open flow microperfusion pilot study. J Pharm Sci. 2014 Jul 1;103(7):1945-8. doi: 10.1002/jps.23994, PMID 24801480.
76. Bulbake U, Doppalapudi S, Kommineni N, Khan W. Liposomal formulations in clinical use: an updated review. Pharmaceutics. 2017 Mar 27;9(2):12. doi: 10.3390/pharmaceutics9020012, PMID 28346375.
77. Dragovich T, Mendelson D, Hoos A, Lewis J, Kurtin S, Richardson K. 268 A phase II trial of aroplatin (L-NDDP), a liposomal DACH platinum, in patients with metastatic colorectal cancer (CRC)-a preliminary report. European Journal of Cancer Supplements. 2003;1(5):S82-3. doi: 10.1016/S1359-6349(03)90301-6.
78. Zamboni WC, Strychor S, Joseph E, Walsh DR, Zamboni BA, Parise RA. Plasma, tumor, and tissue disposition of STEALTH liposomal CKD-602 (S-CKD602) and nonliposomal CKD-602 in mice bearing A375 human melanoma xenografts. Clin Cancer Res. 2007 Dec 1;13(23):7217-23. doi: 10.1158/1078-0432.CCR-07-1035, PMID 18056203.
79. Caraglia M, Luongo L, Salzano G, Zappavigna S, Marra M, Guida F. Stealth liposomes encapsulating zoledronic acid: a new opportunity to treat neuropathic pain. Mol Pharm. 2013 Mar 4;10(3):1111-8. doi: 10.1021/mp3006215, PMID 23327778.
80. Kommineni N, Paul D, Saka R, Khan W, Nanjappan S. Stealth liposomal chemotherapeutic agent for triple negative breast cancer with improved pharmacokinetics. Nanotheranostics. 2022 Aug 21;6(4):424-35. doi: 10.7150/ntno.76370, PMID 36051857.
81. Sivadasan D, Sultan MH, Madkhali OA, Alsabei SH, Alessa AA. Stealth liposomes (pegylated) containing an anticancer drug camptothecin: *in vitro* characterization and *in vivo* pharmacokinetic and tissue distribution study. Molecules. 2022 Feb 6;27(3):1086. doi: 10.3390/molecules27031086, PMID 35164350.
82. Tenchov R, Bird R, Curtze AE, Zhou Q. Lipid nanoparticles-from liposomes to mRNA vaccine delivery, a landscape of research diversity and advancement. ACS Nano. 2021 Jun 28;15(11):16982-7015. doi: 10.1021/acsnano.1c04996, PMID 34181394.
83. Copland MJ, Rades T, Davies NM, Baird MA. Lipid based particulate formulations for the delivery of antigen. Immunol Cell Biol. 2005 Apr;83(2):97-105. doi: 10.1111/j.1440-1711.2005.01315.x, PMID 15748206.
84. Kester KE, Cummings JF, Ofori Anyinam O, Ockenhouse CF, Krzych U, Moris P. Randomized, double-blind, phase 2a trial of falciparum malaria vaccines RTS,S/AS01B and RTS,S/AS02A in malaria-naive adults: safety, efficacy, and immunologic associates of protection. J Infect Dis. 2009 Aug 1;200(3):337-46. doi: 10.1086/600120, PMID 19569965.
85. van Dissel JT, Joosten SA, Hoff ST, Soonawala D, Prins C, Hokey DA. A novel liposomal adjuvant system, CAF01, promotes long-lived mycobacterium tuberculosis-specific T-cell responses in human. Vaccine. 2014 Dec 12;32(52):7098-107. doi: 10.1016/j.vaccine.2014.10.036, PMID 25454872.
86. Li L, Hu S, Chen X. Non-viral delivery systems for CRISPR/Cas9-based genome editing: challenges and opportunities. Biomaterials. 2018 Jul 1;171:207-18. doi: 10.1016/j.biomaterials.2018.04.031, PMID 29704747.
87. Lee W, Jung KH, Park JA, Kim JY, Lee YJ, Chang Y. *In vivo* evaluation of pegylated-liposome encapsulating gadolinium complexes for gadolinium neutron capture therapy. Biochem Biophys Res Commun. 2021 Sep 3;568:23-9. doi: 10.1016/j.bbrc.2021.06.045, PMID 34174538.
88. Goodwin PJ. Flashback foreword: anti-HER2 monoclonal antibody as a single agent. J Clin Oncol. 2023 Mar 10;41(8):1499-500. doi: 10.1200/JCO.22.02679, PMID 36881997.
89. Low PS, Henne WA, Doorneweerd DD. Discovery and development of folic-acid-based receptor targeting for imaging and therapy of cancer and inflammatory diseases. Acc Chem Res. 2008 Jan 15;41(1):120-9. doi: 10.1021/ar7000815, PMID 17655275.
90. Li X, Ding L, Xu Y, Wang Y, Ping Q. Targeted delivery of doxorubicin using stealth liposomes modified with transferrin. Int J Pharm. 2009 May 21;373(1-2):116-23. doi: 10.1016/j.ijpharm.2009.01.023, PMID 19429296.
91. Mukherjee A, Prasad TK, Rao NM, Banerjee R. Haloperidol-associated stealth liposomes: a potent carrier for delivering genes to human breast cancer cells. J Biol Chem. 2005 Apr 22;280(16):15619-27. doi: 10.1074/jbc.M409723200, PMID 15695518.
92. Lee TY, Wu HC, Tseng YL, Lin CT. A novel peptide specifically binding to nasopharyngeal carcinoma for targeted drug delivery. Cancer Res. 2004 Nov 1;64(21):8002-8. doi: 10.1158/0008-5472.CAN-04-1948, PMID 15520208.
93. Zylberberg C, Matosevic S. Pharmaceutical liposomal drug delivery: a review of new delivery systems and a look at the regulatory landscape. Drug Deliv. 2016 Nov 21;23(9):3319-29. doi: 10.1080/10717544.2016.1177136, PMID 27145899.
94. Barenholz YC. Doxil®-the first FDA-approved nano-drug: lessons learned. J Control Release. 2012 Jun 10;160(2):117-34. doi: 10.1016/j.jconrel.2012.03.020, PMID 22484195.
95. Andriyanov AV, Portnoy E, Koren E, Inesa S, Eyal S, Goldberg SN. Therapeutic efficacy of combined pegylated liposomal doxorubicin and radiofrequency ablation: comparing single and combined therapy in young and old mice. J Control Release. 2017 Jul 10;257:2-9. doi: 10.1016/j.jconrel.2017.02.018, PMID 28215670.
96. Buyens K, De Smedt SC, Braeckmans K, Demeester J, Peeters L, van Grunsven LA. Liposome based systems for systemic SiRNA delivery: stability in blood sets the requirements for optimal carrier design. J Control Release. 2012 Mar 28;158(3):362-70. doi: 10.1016/j.jconrel.2011.10.009, PMID 22023849.
97. Blanco E, Shen H, Ferrari M. Principles of nanoparticle design for overcoming biological barriers to drug delivery. Nat Biotechnol. 2015 Sep;33(9):941-51. doi: 10.1038/nbt.3330, PMID 26348965.
98. La-Beck NM, Gabizon AA. Nanoparticle interactions with the immune system: clinical implications for liposome-based cancer chemotherapy. Front Immunol. 2017 Apr 6;8:416. doi: 10.3389/fimmu.2017.00416, PMID 28428790.
99. Nakamura H, Doi Y, Abu Lila AS, Nagao A, Ishida T, Kiwada H. Sequential treatment of oxaliplatin-containing PEGylated liposome together with S-1 improves intratumor distribution of subsequent doses of oxaliplatin-containing PEGylated liposome. Eur J Pharm Biopharm. 2014 May 1;87(1):142-51. doi: 10.1016/j.ejpb.2013.12.007, PMID 24361534.

100. Petersen AL, Henriksen JR, Binderup T, Elema DR, Rasmussen PH, Hag AM. *In vivo* evaluation of PEGylated ^{64}Cu-liposomes with theranostic and radiotherapeutic potential using micro PET/CT. Eur J Nucl Med Mol Imaging. 2016;43(5):941-52. doi: 10.1007/s00259-015-3272-6, PMID 26646780.
101. La-Beck NM, Zamboni BA, Gabizon A, Schmeeda H, Amantea M, Gehrig PA. Factors affecting the pharmacokinetics of pegylated liposomal doxorubicin in patients. Cancer Chemother Pharmacol. 2012 Jan;69(1):43-50. doi: 10.1007/s00280-011-1664-2, PMID 21590446.
102. Moghimi SM, Szebeni J. Stealth liposomes and long circulating nanoparticles: critical issues in pharmacokinetics, opsonization and protein-binding properties. Prog Lipid Res. 2003 Nov 1;42(6):463-78. doi: 10.1016/s0163-7827(03)00033-x, PMID 14559067.
103. Kobayashi H, Watanabe R, Choyke PL. Improving conventional enhanced permeability and retention (EPR) effects; what is the appropriate target? Theranostics. 2013;4(1):81-9. doi: 10.7150/thno.7193, PMID 24396516.
104. Nakamura H, Abu Lila AS, Nishio M, Tanaka M, Ando H, Kiwada H. Intra-tumor distribution of pegylated liposome upon repeated injection: no possession by prior dose. J Control Release. 2015 Dec 28;220(A):406-13. doi: 10.1016/j.jconrel.2015.11.002, PMID 26548975.
105. Poornima K, Puri A, Gupta A. Understanding the stealth properties of PEGylated lipids: a mini-review. IJL. 2020;1(2):1-20. doi: 10.14302/issn.2835-513X.ijl-20-3457.
106. Bulbake U, Doppalapudi S, Kommineni N, Khan W. Liposomal formulations in clinical use: an updated review. Pharmaceutics. 2017 Mar 27;9(2):12. doi: 10.3390/pharmaceutics9020012, PMID 28346375.
107. Gadekar V, Borade Y, Kannaujia S, Rajpoot K, Anup N, Tambe V. Nanomedicines accessible in the market for clinical interventions. J Control Release. 2021 Feb 10;330:372-97. doi: 10.1016/j.jconrel.2020.12.034, PMID 33370576.
108. Q8(R2) Pharmaceutical Development. Available from: https://www.ich.org/page/quality-guidelines. [Last accessed on 20 Jun 2021]
109. Delma KL, Lechanteur A, Evrard B, Semde R, Piel G. Sterilization methods of liposomes: drawbacks of conventional methods and perspectives. Int J Pharm. 2021 Mar 15;597:120271. doi: 10.1016/j.ijpharm.2021.120271, PMID 33548365.
110. He H, Jiang S, Xie Y, Lu Y, Qi J, Dong X. Reassessment of long circulation via monitoring of integral polymeric nanoparticles justifies a more accurate understanding. Nanoscale Horiz. 2018;3(4):397-407. doi: 10.1039/c8nh00010g, PMID 32254127.
111. Guideline for the nonclinical pharmacokinetics of nanomedicines. Available from: http://www.cde.org.cn/zdyz.do?method=largePageandid=1be98aec2c8f7a72. [Last accessed on 25 Jun 2021]
112. Hu X, Dong X, Lu Y, Qi J, Zhao W, Wu W. Bioimaging of nanoparticles: the crucial role of discriminating nanoparticles from free probes. Drug Discov Today. 2017 Feb 1;22(2):382-7. doi: 10.1016/j.drudis.2016.10.002, PMID 27742534.
113. Qi J, Hu X, Dong X, Lu Y, Lu H, Zhao W. Towards more accurate bioimaging of drug nanocarriers: turning aggregation-caused quenching into a useful tool. Adv Drug Deliv Rev. 2019 Mar 15;143:206-25. doi: 10.1016/j.addr.2019.05.009, PMID 31158405.
114. Xia F, Chen Z, Zhu Q, Qi J, Dong X, Zhao W. Gastrointestinal lipolysis and trans-epithelial transport of SMEDDS via oral route. Acta Pharm Sin B. 2021 Apr 1;11(4):1010-20. doi: 10.1016/j.apsb.2021.03.006, PMID 33996413.
115. He H, Wang L, Ma Y, Yang Y, Lv Y, Zhang Z. The biological fate of orally administered mPEG-PDLLA polymeric micelles. J Control Release. 2020 Nov 10;327:725-36. doi: 10.1016/j.jconrel.2020.09.024, PMID 32946874.
116. Li Y, Wang C, Zong S, Qi J, Dong X, Zhao W. The trigeminal pathway dominates the nose-to-brain transportation of intact polymeric nanoparticles: evidence from aggregation-caused quenching probes. J Biomed Nanotechnol. 2019 Apr 1;15(4):686-702. doi: 10.1166/jbn.2019.2724, PMID 30841963.
117. He H, Xie Y, Lv Y, Qi J, Dong X, Zhao W. Bioimaging of intact polycaprolactone nanoparticles using aggregation-caused quenching probes: size-dependent translocation via oral delivery. Adv Healthc Mater. 2018 Nov;7(22):e1800711. doi: 10.1002/adhm.201800711, PMID 30345713.
118. Kumar P, Huo P, Liu B. Formulation strategies for folate-targeted liposomes and their biomedical applications. Pharmaceutics. 2019 Aug 2;11(8). doi: 10.3390/pharmaceutics11080381, PMID 31382369:381.
119. Kumar P, Huo P, Liu B. Formulation strategies for folate-targeted liposomes and their biomedical applications. Pharmaceutics. 2019;11(8). doi: 10.3390/pharmaceutics11080381, PMID 31382369.
120. Bangale GS, Rajesh KS, Shinde GV. Stealth liposomes: a novel approach of targeted drug delivery in cancer therapy. Int J Pharm Sci Res. 2014;5:750-9.
121. Zhao Z, Ukidve A, Kim J, Mitragotri S. Targeting strategies for tissue-specific drug delivery. Cell. 2020 Apr 2;181(1):151-67. doi: 10.1016/j.cell.2020.02.001, PMID 32243788.
122. Khan YY, Suvarna V. Liposomes containing phytochemicals for cancer treatment-an update. Int J Curr Pharm Sci 2017;9(1). doi: 10.22159/ijcpr.2017v9i1.16629.
123. Iden DL, Allen TM. *In vitro* and *in vivo* comparison of immunoliposomes made by conventional coupling techniques with those made by a new post-insertion approach. Biochim Biophys Acta. 2001 Aug 6;1513(2):207-16. doi: 10.1016/s0005-2736(01)00357-1, PMID 11470092.
124. Jokerst JV, Lobovkina T, Zare RN, Gambhir SS. Nanoparticle pegylation for imaging and therapy. Nanomedicine (Lond). 2011 Jun;6(4):715-28. doi: 10.2217/nnm.11.19, PMID 21718180.
125. Fundaro A, Cavalli R, Bargoni A, Vighetto D, Zara GP, Gasco MR. Non-stealth and stealth solid lipid nanoparticles (SLN) carrying doxorubicin: pharmacokinetics and tissue distribution after i.v. administration to rats. Pharmacol Res. 2000 Oct 1;42(4):337-43. doi: 10.1006/phrs.2000.0695, PMID 10987994.
126. Man F, Gawne PJ, T M de Rosales R. Nuclear imaging of liposomal drug delivery systems: a critical review of radiolabelling methods and applications in nanomedicine. Adv Drug Deliv Rev. 2019;143:134-60. doi: 10.1016/j.addr.2019.05.012, PMID 31170428.
127. Princely S, Dhanaraju MD. Design, formulation, and characterization of liposomal-encapsulated gel for transdermal delivery of fluconazole. Asian J Pharm Clin Res. 2018 Aug 8;11:417-24. doi: 10.22159/ajpcr.2018.v11i8.25621.
128. Singh D, Pahwa S. A review on physico-chemical parameters of liposomal doxorubicin. Int J App Pharm. 2019 Dec 12;2:1-5. doi: 10.22159/ijap.2020v12i2.35330.
129. Tenchov R, Bird R, Curtze AE, Zhou Q. Lipid nanoparticles-from liposomes to mRNA vaccine delivery, a landscape of research diversity and advancement. ACS Nano. 2021 Jun 28;15(11):16982-7015. doi: 10.1021/acsnano.1c04996, PMID 34181394.
130. Kusuma P, Vinod K, Damini VKK. Somes: a review on composition, formulation methods and evaluations of different types of "somes" drug delivery system. Int J App Pharm 2020;12(6):7-18. doi: 10.22159/ijap.2020v12i6.38996.