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ROLE OF LIPOSOME IN CANCER THERAPY

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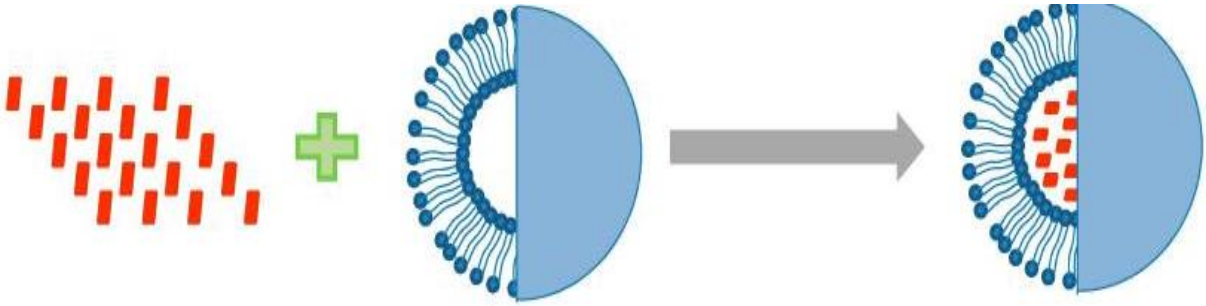
ABSTRACT
The use of liposomes for drug delivery began early in the history of nanocarriers. These are small artificial vesicle colloidal, vesiculer structures composed of one or more lipid bilayers surrounding an equal numbers of aqueous compartments. Liposomes are small artificial vesicles of spherical shape that can be created from natural phospholipids and cholesterol, has both hydrophilic and hydrophobic character. A phospholipids bilayer vesicular system is being used for drug delivery application in cancer therapy. The reasons behind the advantages that liposomes offer, improves bioavailability of anticancer drugs and versatility of encapsulating both hydrophilic as well as hydrophobic drugs, liposomes with targeted ligand this can leads to actively targeted site specific therapy of solid tumor. The liposomal formulations for cancer treatments are already in clinics many are in active research. This review explains about the basic liposomes which includes structural's, components, method of preparation, mainly focuses on recent advancements of liposomes such as various kind of surface modification currently use liposomes with active or targeted drug delivery along with its advantages and mechanism and their application in the treatments of cancer.

KEYWORDS: Liposomes, structural component of liposome, Targeted drug delivery, Cancer therapy.

1. INRODUCTION
Liposomes discovered in 1960's by Alec D. Bengham and co-workers. Liposome are small artificial vesicle of spherical shape that can be created from natural non-toxic phospholipids and cholesterol. Liposome valued for their biological and technological advantages and are the most successful drug carrier system known to date. Liposome have the capability of entrapping both lipophilic and hydrophobic agents, respectively in lipid membrane and in the aqueous core. The first formulations were composed only of natural lipids, they can include of natural or synthetics lipid surfactants.[1] Liposomes are promising system for drug delivery delivery. Generally, liposomes are spherical vesicles with a particles size ranging from 30 nm to several micrometers. They consist of lipids bilayers surrounding aqueous unit, where the polar head group are oriented in the pathway of the interior and the exterior aqueous phases.[2]. Cancer is a leading cause of disease in many countries around the globe. However, the application of liposomes has led to the development of nanosized drug delivery system commonly known as nanoparticles.[3] Nano sized structures based pharmaceutical formulation e.g liposome have shown better therapy for the APIs.[4] Also, due to the complexity of solid tumor, an effective penetration of anti-cancer agents encapsulated within a nanocarrier is the main challenge in cancer therapy.[5] Liposomes are the most commonly investigated nanostructures used in advanced drug delivery, these serve as DDs due to their versatile structructure, biocompatibility and the fact they are naturally biodegradable, non-immunogenic and nontoxic.[6] Liposome providing protection against drug degradation, improving the therapeutic index of the entrapped drug and prevent the API against their surrounding environments.[7] Liposomes providing the targeted drug delivery, reducing the toxic effect of the drug, these have several advantages contributing to drug delivery (figure 1). They have a role of enhancing drug solubility, serving as sustain release system.[8] Liposomes facilitate intracellular delivery of anticancer drugs and prolong the retention time of encapsulated payload in cancer cells.[9] Lipososmes play a vital role in resolving the issues like off-target effect of anticancer drugs by improving the pharmacokinetic-profile and pharmacological properties.[10]



Drug with toxicity and efficacy **Liposome** **Liposomal drug with reduced toxicity**

Fig 1: Advantages of formulating drug in liposome.

2. CANCER

Cancer is a life threatening disease contributing two - three millions deaths worldwide[11] and in the united state (US)[12], Worldwide, in 2013, cancer killed over 8 million; it has moved from the third leading cause of death in 1990 to the second leading cause of death in 2013 following heart disease.[13] In USA, was about 611 105 following by cancer 584 881.[14] A progress has been made since the 1990s in the USA and in Europe with regards to diagnosis, prevention and treatments of cancers.[15]

3. LIPOSOME IN CANCER THERAPY

The conventional chemotherapy is injecting a free drug either in suspension or solution form and the policy has described good clinical efficiency till today. Moreover, due to non-specific biodistribution, the anti-cancer drugs produce severe side effects, resulting into a limitation of dose or termination of therapy. Therefore, encapsulation of anti-cancer drugs in liposomes like nano-carrier can reduce its toxicity.[16] Liposomes are bilayer in nature in which an aqueous core is entirely enclosed by a membrane lipid bilayer which consist of synthetic or natural phospholipids which can encapsulate both the lipophilic and hydrophilic drugs that have become popular as drug delivery systems owing to their efficiency, biocompatibility, non-immunogenicity and enhanced bioavailability of chemotherapeutic agents. Many techniques have tried to enhance the efficiency of liposomes such as site-specific drug delivery in tumor[17], long circulation, improved loading and triggered release for delivery of anti-cancer agent to the tumor site. Amongst various modifications of liposomes, active targeting and passive targeting are technique employed till date. The most recent approach for cancer therapy using liposome is active targeting along with other approaches, e.g., stimuli sensitivity. For active targeting of liposomes, they are decorated with various targeting ligands such as antibodies, and their fragments, aptamer, peptides etc. (figure 2). Surface functionalization and modification of liposomes helps delivery of therapeutic drug specifically to solid tumors where the targeted liposomes can recognize tumor cells, can lead to endocytosis and therapeutic response after binding to targeting receptor.

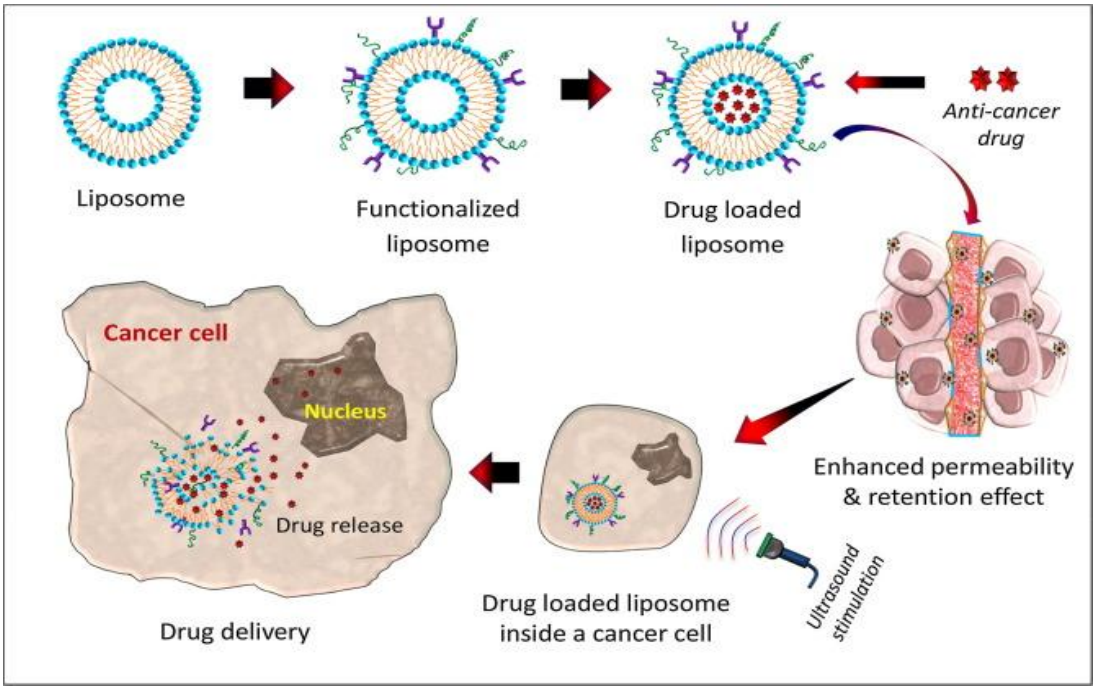


Fig. 2: Liposomal drug delivery systems in cancer therapy.

4. COMPONENTS OF LIPOSOMES

The main components of liposome is Phospholipids and Cholesterol. Phospholipids can be classified as natural and synthetic. Phospatidylocholine is mostly used phospholipid in formulation of liposomes which has amphipathic in nature. The other component, cholesterol has no ability to form a bilayer membrane however it acts as fluidity buffer. It can change the freedom of motion of carbon molecules in the acyl chain after inserting with phospholipids and thus increases separation between choline head groups in the membrane which reduces the normal hydrogen bonding and electrostatic interaction.[18]

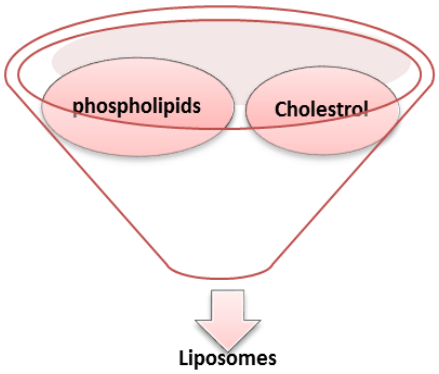


Fig 3: Composition of liposome

ADVANTAGES OF LIPOSOME

- Can load hydrophilic as well as hydrophobic drug.
- Reduction in toxicity of encapsulated agent.
- Increase stability of the encapsulated drug.
- Site avoidance effect (avoid non-target tissues).
- Improve pharmacokinetic effects (reduce elimination, increased circulation life times specifically for PEGylated liposomes).

DISADVANTAGES OF LIPOSOME

- Long term un-stability.
- High production cost.
- Low aqueous drug loading.
- Lipid may undergo oxidation.
- Sensitivity to temperature change.

5. CLASSIFICATION OF LIPOSOMES

Liposomes are classified based on their structure parameters such as vesicle size, based on the composition and application.[19] Table 1 and 2 describes about classification of liposomes.

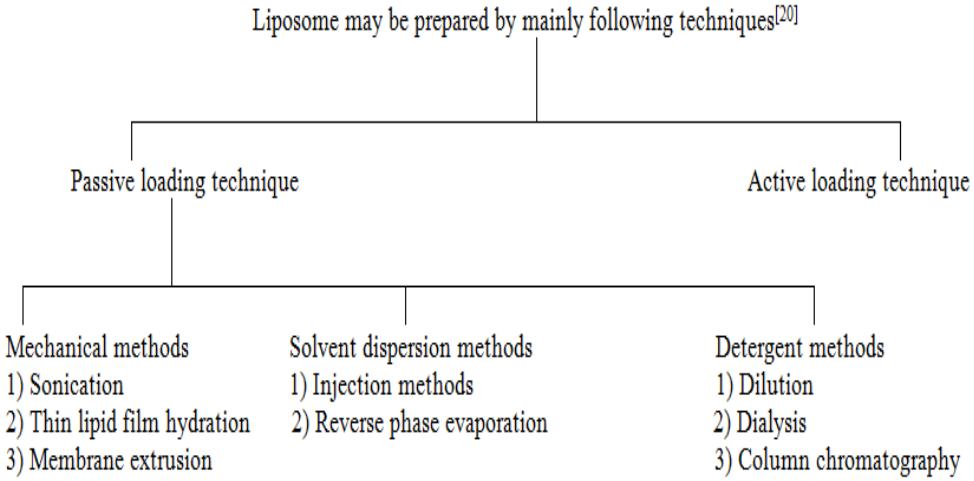
Table 1: Classification of liposome based on their sizes.

CLASS	SIZE RANGE
MLV (Multi lamaller vesicles)	(>0.5 um)
OLV (Oligo lamaller vesicles)	(0.1-1um)
ULV (Uni lamaller vesicles)	All size
SUV (Small uni lamaller vesicles)	(20-100 nm)
LUV (Large uni lamaller vesicles)	(>100 nm)
GUV (Giant uni lamaller vesicles)	(>1 um)
MVV (Multi vesicular vesicles)	(Usually >1 um)

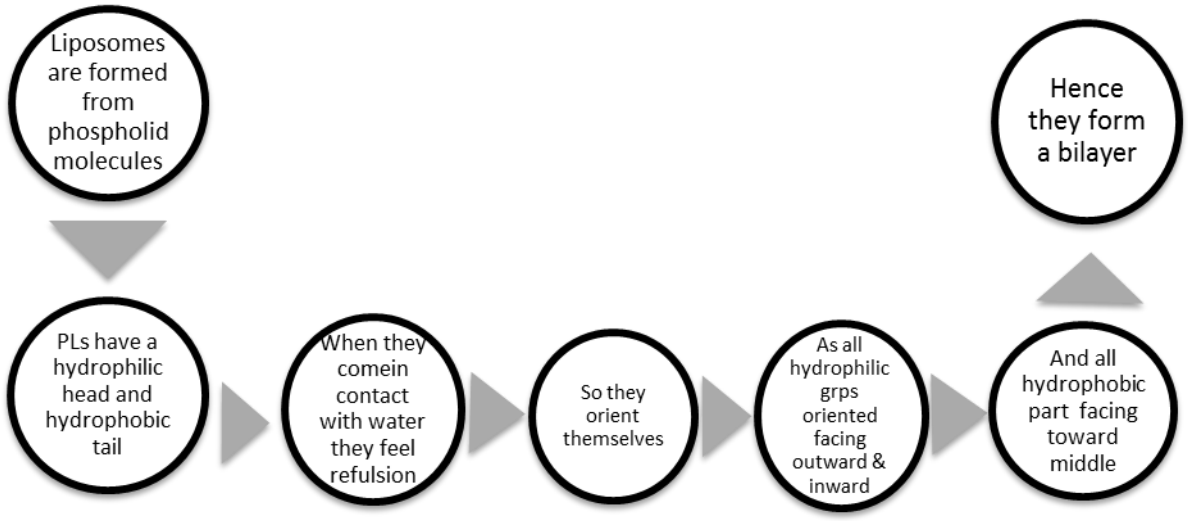
Table 2: Classification of liposome based on composition and application.

Class of liposome	Specification
PEGylated Long circulatory	Imparts long circulation and EPR effect
pH sensitive liposome	Made up of phospholipids like Phosphatidyl ethanolamine
Conventional	Made up of neutral or anionic phospholipids hence mostly is neutral or negatively charged formulation
Immunoliposome	Site specific active targeted liposome
Cationic	Made up of cationic lipid e.g. DOTAP

6. METHOD OF PREPARATION OF LIPOSOME



Concepts of Liposome Formulation



7. APPLICATIONS OF LIPOSOMES IN ANTICANCER TREATMENTS

The development of nanoscale liposomal formulations has been shown to help the selective transportation of the drug to the tumour cells.[21] This consequently avoids the off-target toxicity due to the EPR effect.[22] Some liposomal drug delivery system were approved by the food drug administration with potent anticancer activity. Table 3: summarises some of the liposomal formulations used as cancer treatments[23]

Table 3: Approval liposomal formulation

Drug	Product name	Type of drug	Lipid composition	Route of administration	Approved treatment
Doxorubicin	Myocet	Liposome	EPC and cholestrol	Intravenous	Metastatic breast cancer
	Doxin	Pegylated liposome	HSPC,cholesterol and DSPE-PEG 200	Intravenous	Kaposi's sarcoma, ovarian and breast cancer
	Lipo-dox	Pegylated liposome	HSPC,cholesterol and DSPE-PEG 200	Intravenous	Kaposi's sarcoma, ovarian and breast cancer
Amphotericin B	Ambisome	Liposome	HSPC,DSPG and cholestrol	Intravenous	Sever fungal infection
Daunorubicin	Daunoxome	Liposome	DSPC and cholestrol	Intravenous	Blood cancer
Verteporfin	Visudyne	Liposome	EPG and DMPG	Intravenous	Age related molecular degeneration
Cytarabin	Depocyt	Liposome	DOPC,DPPG cholesterol and triolein	Spinal	Neoplastic meningitis and lymphomateous meningitis
Morphine sulphate	Depodur	Liposome	DOPC, DPPG cholesterol and triolein	Epidural	Pain
Vincristine sulphate	Marqibo	Liposome	Egg spingomyelin and cholestrol	Intravenous	Acute lymphoblastic leukemia

8. SURFACE FUNCTIONALIZATION AND MODIFICATION OF LIPOSOME

1.1 PEGylated liposomes

This technique is covalently attaching polyethylene glycol (PEG) to liposomes is known as "PEGylated liposomes". Also known as Long Circulatory Liposomes OR Stealth Liposomes and is now a well-recognised method in the field of targeted drug delivery systems.

Mechanism of PEGylated liposomes

PEGylated liposomes can passively accumulate inside other tissues or organs called as passive targeting, which is especially in solid tumors undergoing angiogenesis (the presence of a discontinuous endothelial lining in the tumor vasculature). During angiogenesis, PEGylated liposomes facilitates extravasation of liposomal formulations into the interstitial space, where they accumulate due to the lack of efficient lymphatic

drainage of the tumor and functions as a sustained drug-release system which causes the preferential accumulation of liposomes in the tumor region (a process known as enhanced permeation and retention effect or EPR). Liposomal formulations do not cause extravasation from the bloodstream into normal tissues that have tight junctions between capillary endothelial cells. This mechanism appears to be responsible for the improved therapeutic effects of liposomal anticancer drugs *vs* free drugs. However, the processes involved in delivery of this carrier and release of anti cancer agent, the variability of such processes, and the degree to which the active agent is released into the tumor's extracellular fluid or into tumor cells, are still unknown. Doxil® is a formulation containing Doxorubicin hydrochloride which is the first FDA- approved nano drug delivery system based on PEGylated liposome technology. Originally Doxil® was developed in 1995 by Sequus Pharmaceuticals, USA as an i.v. injection for the management of advanced ovarian cancer, multiple myeloma and HIV-associated Kaposi's sarcoma.[24]

1.2 pH sensitive liposome

pH-sensitive copolymers can be incorporated in the liposomes to provide shielding. Diortho esters, vinyl esters, cysteine-cleavable lipopolymers, double esters and hydrazones are a few examples of pH-sensitive bonds which are quite stable at pH 7.5, but are hydrolyzed relatively rapidly at pH 6 and below. The pH-sensitive liposomal carrier releases the entrapped payloads in tissues with a low pH, such as tumors, the cell cytoplasm or endosome. Liposomes made of pH-sensitive components fuse with the endovascular membrane after endocytosis and subsequently release its contents into the cytoplasm under the action of low endosomal pH.

1.3 Immunoliposomes

The concept of targeting liposomes to cells by attaching targeting ligands such as antibodies, fragment antigen binding (Fab'), single-chain fragment variable (scFv) fragments, or peptides to the liposomal surface is known as Immunoliposomes (ILs) for targeted drug delivery in cancer therapy.

Need for Active Targeting

The high stability of liposomes which leads to poor and slow release of drug and might be associated with the low cellular uptake efficiency of liposomes. To overcome this obstacle, "Active Targeting" or ligand-based targeting has been emerged as one of the most promising strategies to improve internalization of specific drug. In this approach, targeted liposomes have ability to directly bind to cancer cells. For this, the surface of the liposomal formulations has to be attached with different types of ligands, which recognizes and bind those specific molecules expressed or over-expressed on the surface of cancer cells.

Method of preparation

Immunoliposomes (ILs) can be consider as a new strategy that has been commonly investigated in preclinical cancer models with promising results, although few have reached the step of clinical trials. ILs allows the association of different targeting ligands such as mAbs or antibody derivatives like monovalent variable fragments of Fab', to the polymers covering the surface of liposomes. The combination of this targeting strategy together along with drug encapsulation in a single formulation may contribute to enhance the efficacy of these associated agents by minimizing their toxicities. Hence, the main strategy that has been adopted for formulation of targeted liposomes is the use of an end-functionalized pegylated lipid. This lipid derivative is able to form a covalent bond with the ligands sch as mAbs, which normally requires previous activation or modification. These coupling methods (eg, using thiolated antibodies coupled to maleimide (Mal)-PEG lipids or using modified amino-reactive PEG lipids) in random fashion risk antibody inactivation and liposome aggregation by cross- linking which results into whole antibodies possess several disadvantages for the generation of ILs. These disadvantages can be avoided by the use of antibody fragments such as fragment antigen binding (Fab') or single-chain fragment variable (scFv) fragments.

Fab' fragments

Fab' fragments have been broadly investigated for drug delivery with sterically stabilized ILs (SILs). A direct comparison of Fab'-SILs and IgG-SILs results into reduced immunogenicity and also improve pharmacokinetics, as shown in comparative animal study of doxorubicin-loaded sterically stabilized anti-GD2 ILs for the treatment of metastatic neuroblastoma which shows clearance of Fab'- SILs was similar to that of PEGylated liposomes, while IgG- SILs showed approximately 3-fold faster elimination. These Fab'-SILs also proven to exhibit potent antitumor activity.

scFv fragments

scFv fragments are the smallest fragments that contains the entire antigen-binding site of an antibody. Human scFv fragments have been employed for the production of various ILs and to deliver drugs to target cells. For e.g., Using anti-HER-2 scFv-SILs, maximal binding was observed with 30 to 40 scFv molecules attached. Doxorubicin-loaded anti-HER-2 scFv-SILs based on this formulation are currently produced under a GMP-compliant for preclinical process. Binding of these ILs to cells is a highly efficient process which requires only a few scFv molecules coupled to the liposomes One or more additional cysteine residues are attached to the C-terminus of scFv fragments in order to achieve coupling to liposomes which allows site-directed conjugation, with the reactive sulfhydryl group(s) located opposite the antigen-binding site. Thus, conjugation of this scFv fragments does not interfere with target cell recognition in comparison to coupling of Fab' fragments. Therefore,

scFv molecules are well established that have been used by several research groups for the generation of targeted liposomes.[25,26,27,28]

1.5 Triggered release/stimuli sensitive liposomes

Stimuli cause instability to the liposomes leading to the release of entrapped material. Therefore, the concept of stimuli sensitivity have been developed which is based on certain characteristics of the tumor microenvironment, including a lower pH, higher temperature and overexpression of several proteolytic enzymes.[29] The stimuli-sensitive liposomes have ability to maintain their structure and physical properties throughout circulation. However, they are designed to undergo rapid changes (aggregation, disruption and permeability) that trigger drug release when exposed to a particular tumor microenvironment by utilizing either internal stimuli that are characteristic for a tumor microenvironment or externally applied stimuli, such as magnetic fields, ultrasound or light, for targeting tumor tissues.[30]

9. CONCLUSION

Liposomes have revolutionized cancer therapy by their broad clinical applications. Liposomes overcome the limitations of conventional chemotherapy by improving the bioavailability and stability of the drug molecules and minimizing side effects by site-specific targeted delivery of the drugs. Liposomes were the first nanotechnology-based drug delivery systems approved for the clinical applications because of their biocompatibility and biodegradability like features. Some liposome-based drug delivery systems are already in the market and many more are undergoing research and clinical trials. So far, liposomes have established themselves in nanocarriers-based drug delivery systems as evident by the successful clinical applications of liposomal formulations in anti-cancer therapy.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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