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Chapter

Introductory Chapter: Liposome - A Versatile Tool for Drug Delivery in Nanobiomedicine

Prakriti Sharma, Mili Mehta, Nikunj Tandel and Rajeev K. Tyagi

1. Introduction

Liposomes are lipid mono- and bilayered structures made up of cholesterol and phospholipid and entrap lipophilic or hydrophilic agents. The lipophilic agents have higher affinity towards phospholipid bilayer allowing their encapsulation within the lipid bilayer whereas the hydrophilic compounds are entrapped in the liposome central cavity [1]. Although size of liposomes ranges from nm to μm, widely used liposomes for various biomedical applications are in the range of 50 to 450 nm. Further, numerous experimental evidences confirm the role of liposomes as an emerging carrier for an effective drug delivery due to their resemblance with the cell that in turn allowed incorporation of range of drugs [2, 3].

These nanoscale drug carriers are demonstrated to be advantageous for promoting the biodistribution of drugs to target site-specific sites in the experimental animals, stabilizing therapeutic drugs, and overcome the physiological barriers. This ultimately allows these carriers to distribute the encapsulated substances to target areas efficiently and limit the systemic toxicity. The clinical translation of liposome-assisted drug delivery systems has advanced gradually over the past 5 decades, and generated wealth of information useful for the preclinical research [4]. Empirical evidences suggest that when these phospholipids are rigorously stirred in the aqueous phase, they form closed configurations and since these structures are hollow, they could easily transport drugs regardless of their nature [5]. Liposomes can restrict cargos from deteriorating in the surrounding biological environment, enhance their bio-distribution and facilitate their administration to the target cells when compared with the conventional naked administration of drugs.

Various types of liposomes exist includes conventional, fusogenic, cationic, long circulatory, pH sensitive and immuno-liposomes that are categorized as per their formulation and composition [6]. The major role of liposomes has been explored in the area of drug delivery for developing interventional approaches against the infectious diseases. Of late, different drug delivery systems comprising of liposomes are approved by WHO and many others are in the process of being approved for translational research. Here, we have summarized a fewer of them that are currently under investigation.

Park *et al*., has developed anti-HER2 (ErbB2) immuno-liposome loaded with the anticancer drugs for the targeted delivery for the over-expressing HER cancer

cells [7]. Similarly, a liposomal formulation of cytarabine (DepoCyte®) is developed to treat neoplastic meningitis. The protraction period for neurological development, improved the quality of life and efficacies response rate was observed [8]. Doxil® was the first drug approved by the FDA against cancer [9]. Further, the role of liposomes has also been explored for the parasitic infection of visceral Leishmaniasis by using the formulation of liposome entrapped amphotericin B [10]. Currently, research is ongoing to explore the role of liposomes as biolubricant on artificial joints and promising results were observed which can be enhanced by increasing the length of liposomal carbon chain [11]. Additionally, it has been used for the combinational therapy. Paclitaxal and doxorubicin loaded liposomal formulation provides better therapeutic results compare to that seen with the physical mixture of the drugs with reduced toxic effects of individual drugs [12]. Later on, arginine-glycine-aspartate (R-G-D) based liposomes formulations rendered lesser toxicity and greater tumor inhibition [13]. Furthermore, liposomal amphotericin B along with flucytosine and fluconazole have been used for the treatment of HIV mediated cryptococcal meningitis and found promising as compared to the treatment approved by WHO with fewer adverse events [14]. The detailed information about the role of liposomes in malaria infection and its role for the determining the immunogenicity of candidate antigens aiming at developing vaccines has been reviewed recently [15]. It has been also explored for the current pandemic condition of COVID-19 and its contribution in different vaccine formulations [16].

2. Engineered liposomes: better nanocarriers

Liposomes have been used as a vehicle to deliver the drugs sustainably. However, with time, the advancement in the formulation of liposomes and their engineering helped overcome the associated issues. The composition and characteristics of liposomes differ, based on the technique of formulation, and charge present on their surface. Moreover, selection of bilayer components ultimately influences the sturdiness or fluidity of the formulated vesicles [5]. The bilayer is coupled with hydrophobic compounds and lipid vesicles potentially carry hydrophobic, hydrophilic chemicals or both. The fusion of this bilayer with cell membrane allows the site-specific and targeted administration of drugs or vaccine candidates. Nevertheless, encapsulated content delivery via liposomal formulation is a complex process. The first generation of the liposomes could overcome the problems of stability. Moreover, their composition involves the neutral or negatively charged phospholipid and cholesterol. Hence, the issues associated with the conventional liposomes were addressed by the engineered liposomes using distearoyl-phosphatidylcholine cholesterol and saturated phospholipid [17].

The conventional liposomal formulation methods involve the thin film hydration, reverse phase evaporation, solvent injection and elimination of detergent. One of the most common liposomes preparation procedures is the thin-film method; it operates by forming a thin lipid coating on the inner wall of the rotary evaporator flask. The key benefit of this process is its remarkable reproducibility even when operating with small amounts of compounds. However, lower encapsulation efficiency has been a major drawback of the thin-film method [18]. Another liposomal preparation method (injection method) has many variations and liposome formulation involves the injection of organic solvent (ethanol or ether) dissolved lipids in the aqueous solution [19]. The emulsification or reverse-phase evaporation method is similar to the injection

method involving the lipids, dissolved in the organic solvent and combined with both organic and water phase. The main advantage of the emulsification approach is that it offers the higher encapsulation efficiency than that with the injection methods [17]. The use of liposome is for the drug delivery which needs encapsulation of drug. Active and passive methods have been used for the drug encapsulation [20].

3. Liposomes mediated drug delivery

People are heavily dependent upon the use of antibiotics but the antibiotic resistance forced us finding other alternatives. Therefore, liposomes that closely mirror the cell membrane of the host, target bacterial toxins are explored. Moreover, these delivery vehicles have been used in the clinical settings to transport drugs and candidate antigens for their targeted and sustained release [21]. Recently, the ability of liposomes laden with immune stimulatory molecules to enhance the efficacy of cancer immunotherapy has been investigated [22, 23]. Using an antibody-based strategy, immunoliposomes have formulated which are specific to the cancer cells or endothelial cells of the tumor vasculature [24]. The research carried out by Zhang *et al.* showed that usage of PEGylated-immunoliposomes in murine melanoma model has shown the comparable immune-stimulatory activity to the free and with no systemic toxicity [25].

Since, one of the crucial components of the effective cancer immunotherapy is the efficient and selective transport of these stimulating chemicals to the cells of interest. Therefore, utilizing liposomes in immunostimulatory therapy can produce significant anti-tumor effects without causing systemic side effects and hence suggestive of the therapeutic application of liposome loaded drugs.

Enzyme-responsive liposomes are another method for the administration of anticancer drugs for several extracellular enzymes such as secreted phospholipase A2 (sPLA2), matrix metalloproteinases (MMPs), and intracellular enzymes (cathepsin) [26]. According to recent studies, polymeric and PEGylated liposomal nanoparticles (PLNs) can suppress antitumor immunity and promote tumor growth in murine models by preventing PLN-induced tumor growth and improved progression-free survival [27]. Recently, delivery of Bortezomib, a protease inhibitor was used for the treatment of multiple myeloma (MM) when delivered through liposomes in the humanized mouse model for MM has shown the complete tumor regression [28]. It suggests the therapeutic role of drugs mediated by the liposomes in cancer therapy. Besides, humanized mouse developed for the chronic myelomonocytic leukemia (CMML) using the patient-derived induced pluripotent stem cells (iPSCs) has confirmed the role of clodronate drug when used the liposome formulation in CMML therapy [29].

4. Limitations of liposomes as a delivery vehicle

The major hurdle of the liposome is to deal with stability, uptake by liver, spleen and lungs and the short half-life in blood. Liposomes, like any exogenous particle that enters our body, are challenged with several defensive systems, for instance- the reticuloendothelial system (RES), opsonization, and immunogenicity, which are designed to recognize, neutralize and eliminate the invading substances.

Following systemic delivery, RES is the primary location for liposome accumulation followed by liver, spleen, kidney, lungs, bone marrow, and lymph nodes

associated with RES. Plasma proteins and liposomal drug delivery systems tend to interact and their degree of interaction is crucial for defining the toxicity, efficacy and bio-distribution. Hence, plasma proteins are significant for RES-mediated opsonization and vesicular instability. Highly charged liposomes are more prone to get eliminated by the liver in minutes and the spleen within an hour.

Our intricate immune system can get triggered by the liposomal systems causing activation of the complement system that leads to the acute hypersensitivity syndrome known as complement activation-related pseudoallergy (CARPA) originating as a multitude of immunological and inflammatory processes. The complement system can be triggered by the varieties of liposomes; however, some specific liposomal characteristics elevate the tendency for complement activation, which include surface charge, absence of liposomal homogeneity, expanding size, endotoxin contamination, presence of ≥70% cholesterol in the bilayer membrane. Thus, neutral compact unimellar vesicles are found to be the poorest reactogenic species of these liposomal systems [4].

Individual variances in the EPR effect, the accelerated blood clearance (ABC) phenomena of PEGylated liposomes, scale-up, reproducibility/consistency among different batches and manufacturing sites, and excipients management is the key challenges throughout the development and commercialization of liposomes [30]. Assessing the pharmacokinetics, pharmacodynamics, and toxicity of a formulation after injection becomes increasingly challenging as the number of physicochemical variables in a nano-formulation preparation rises.

Across several biomedical fields, the application of liposomes to facilitate drug delivery has already had a massive effect. Prospective research will improve the existing liposomal platforms and help understand the current regulatory constraints by gaining a better understanding of the breakthroughs in liposomal technology as well as overcome the impediments.

5. Controlling residual innate immune responses for the sizeable grafting of human cells/tissues

The early development of knock-in/out mice to understand the host-pathogen interaction has paved the way forward. This in turn results into the higher efficacy of vaccine development. However, usage of surrogate models often resulting in the failure of clinical trials for numerous vaccine candidates. To address this issue, the concept of transplanting human cells into immunodeficient mouse which mimics the human-system has emerged and known as 'humanized mouse". Later on, advancement in the technology aided in the generation of mouse-human chimera for various biomedical applications. These mouse human chimeras have responded pretty well to understand the pathogens and their interaction with the host.

Normal or malignant human hematopoietic stem cells (huHSC) were transplanted into immunodeficient mice to develop the humanized mouse model. The success of mouse humanization depends upon the susceptibility of the host immune system towards acceptance of the graft. Therefore, different approaches have been adopted. The use of the liposome loaded with clodronate (clo-lip) drug showed the depletion of the cells of monocyte/macrophages lineage (**Figure 1**). This showed the successful engraftment of huHSC in SCID mice [31]. Further, Hu *et al*., developed the humanized mouse model (in NOD/SCID or NOD/SCID/γc−/−) having the matured CD71⁻CD235a⁺ human red blood cells (huRBCs) however their poor efficiency as well as meager

number of RBCs makes it difficult to use these mice to study various hematological disorders. Moreover, cobra venom factor (CVF) when combined with clo-lip has shown the extended survival of huRBCs in immunodeficient mice. It could help studying the function of RBCs and human erythropoiesis [32]. Since macrophages have been the major stumbling block resulting in the poor reconstitution of human platelets in human CD34+ cells-grafted mice, clo-lip treatment showed the higher level of human platelet in the periphery of chimeric mice [33]. This chimeric mouse has opened a door to study the step-wise development of human thrombopoiesis and function of platelets (**Figure 1**).

Poor understanding of the host-pathogen interaction is the major issue for the successful development of an asexual blood stage vaccine for malaria as well as for developing an understanding of the liver-stage (LS) infection of human malaria. It has been evident that treatment with clo-lip to transgenic/immunodeficient mice (TK/NOG) helped the successful transplantation of human hepatocytes (huHep) that allows the development of exoerythrocytic stages of malaria in murine models [34, 35]. The clo-lip formulation induces the apoptosis and depletes the monocytes-macrophages lineage allowing the sizeable engraftment of huHep in mice liver to develop human live chimeric mouse inevitably required to study LS infection of *P. falciparum*. Further advancements have allowed studying the asexual blood stage and transition from LS to asexual blood stage infection of *P. falciparum* in one host [36]. Similarly, another humanized mouse has developed (HIS-HEry mice) using clo-lip formulation to study of asexual blood stage infection of *Plasmodium vivax* that exhibits erythropoiesis following hematopoietic stem and progenitor cells (HSPCs) transplantation [37] (**Figure 1**).

Having confirmed the role of clo-lip in developing the humanized mouse models, Youssef and colleagues have explored the role of clo-lip in to reduce the skin allograft rejection. Data has shown that intraperitoneal injection of clo-lip markedly reduced the macrophage-lineage and hence conferred the extended survival of skin allograft in CD8 knockout mice as compared to that seen with control [38]. Similarly, clo-lip was seen to treat macrophages activation syndrome (MAS) or haemophagocytic lymphoistiocytosis, a life-threatening condition that leads to the multiple-organ failure (**Figure 1**) [39]. Very recently, role of clo-lip has also been explored in the insect system to study the innate immune response wherein depletion of phagocytic immune cells takes place in *Drosophila melanogaster* and *Aedes aegypti* mosquito [40].
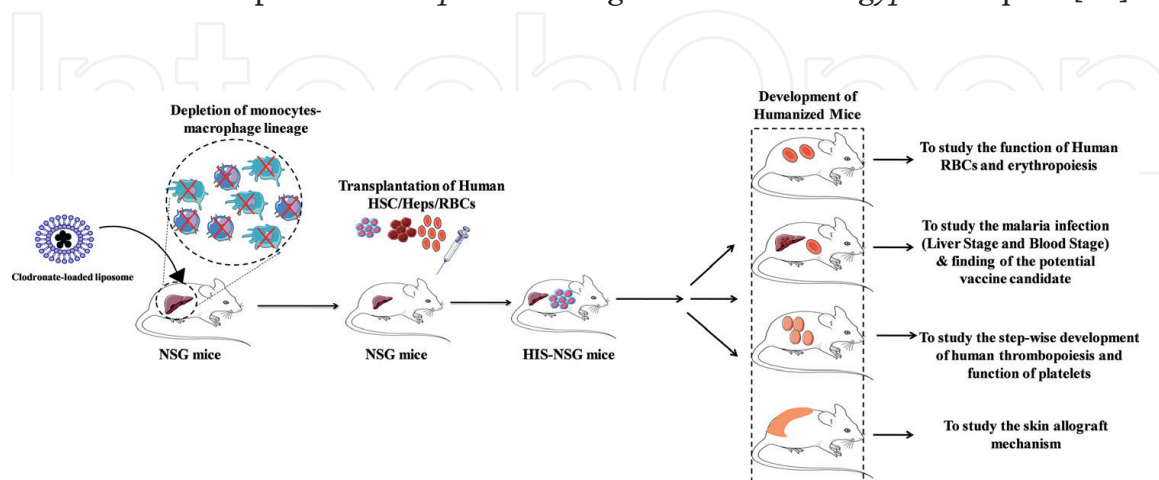

Figure 1.
Controlling residual innate immune response by the clodronate-loaded (clo-lip) liposome for the development of humanized mice. Clo-lip depletes the cells of monocyte-macrophage lineage in the immunodeficient mice (left panel). The human hematopoietic stem cells, hepatocytes or red blood cells were transplanted in these mice and generate the human-immune system mice (HIS-NSG) (middle panel). These HIS-NSG mice are used in the translational biomedical research and vaccine development (right panel).

6. Conclusions

The conventional delivery systems have earned popularity due to their economic, simple and user-friendly approach. However, recently developed specific drug delivery systems such as liposomes attracted the researchers for their target specificity, effectiveness and minimum adverse effects. The effectiveness of treatment is associated with the ability of drug to target and affect the biological functions of ailing cells and rendering minimum damage to the healthy tissues. Liposomes may be composed of one or more lipid bilayer. With the length of phospholipid and the liposome component ratio decide the liposome stability, efficiency and stability which further aid in designing the drug-delivery system for site and target-specific delivery. Further, work on usage of liposomes in the development of humanized mouse model(s) and determination of immunogenic potential of candidate antigens [41–43] has opened vistas to explore their role in translational biomedical research.

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Conflict of interest

The authors declare no conflict of interest.

Abbreviations

Clo-lip   Liposome loaded with clodronate
CMML   Chronic myelomonocytic leukemia
HSPCs   Hematopoietic stem and progenitor cells
huHSC   Human hematopoietic stem cells
huRBCs   Human red blood cells
iPSC   induced pluripotent stem cells
LS   Liver-stage
MM   Multiple myeloma
MMPs   Matrix metalloproteinases
PLNs   PEGylated liposomal nanoparticles
RES   Reticuloendothelial system

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References

[1] Xing H et al. Recent developments of liposomes as Nanocarriers for Theranostic applications. Theranostics. 2016;**6**(9):1336-1352

[2] Bozzuto G, Molinari A. Liposomes as nanomedical devices. International Journal of Nanomedicine. 2015;**10**:975-999

[3] Kim EM, Jeong HJ. Liposomes: Biomedical applications. Chonnam Medical Journal. 2021;**57**(1):27-35

[4] Sercombe L et al. Advances and challenges of liposome assisted drug delivery. Frontiers in Pharmacology. 2015;**6**:286

[5] Wagner A, Vorauer-Uhl K. Liposome technology for industrial purposes. Journal of Drug Delivery. 2011;**2011**:591325

[6] Nakhaei P et al. Liposomes: Structure, biomedical applications, and stability parameters with emphasis on cholesterol. Frontiers in Bioengineering and Biotechnology. 2021;**9**:705886

[7] Park JW et al. Tumor targeting using anti-her2 immunoliposomes. Journal of Controlled Release. 2001;**74**(1-3):95-113

[8] Rueda Domínguez A et al. Liposomal cytarabine (DepoCyte) for the treatment of neoplastic meningitis. Clinical & Translational Oncology. 2005;**7**(6):232-238

[9] Barenholz Y. Doxil®--the first FDA-approved nano-drug: Lessons learned. Journal of Controlled Release. 2012;**160**(2):117-134

[10] Bern C et al. Liposomal amphotericin B for the treatment of visceral leishmaniasis. Clinical Infectious Diseases. 2006;**43**(7):917-924

[11] Wang Z et al. Investigation of the lubrication properties and synergistic interaction of biocompatible liposome-polymer complexes applicable to artificial joints. Colloids and Surfaces. B, Biointerfaces. 2019;**178**:469-478

[12] Yu J et al. Remote loading paclitaxel-doxorubicin prodrug into liposomes for cancer combination therapy. Acta Pharmaceutica Sinica B. 2020;**10**(9):1730-1740

[13] Fu S et al. Integrin $\alpha(v)\beta(3)$-targeted liposomal drug delivery system for enhanced lung cancer therapy. Colloids and Surfaces. B, Biointerfaces. 2021;**201**:111623

[14] Jarvis JN et al. Single-dose liposomal amphotericin B treatment for Cryptococcal meningitis. The New England Journal of Medicine. 2022;**386**(12):1109-1120

[15] Memvanga PB, Nkanga CI. Liposomes for malaria management: The evolution from 1980 to 2020. Malaria Journal. 2021;**20**(1):327

[16] Attia MA et al. Brief on recent application of liposomal vaccines for lower respiratory tract viral infections: From influenza to COVID-19 vaccines. Pharmaceuticals (Basel). 2021;**14**(11):1173

[17] Nsairat H et al. Liposomes: Structure, composition, types, and clinical applications. Heliyon. 2022;**8**(5):e09394

[18] Šturm L, Poklar Ulrih N. Basic methods for preparation of liposomes and studying their interactions with

different compounds, with the emphasis on polyphenols. International Journal of Molecular Sciences. 2021;**22**(12):6547

[19] William B et al. Supercritical fluid methods: An alternative to conventional methods to prepare liposomes. Chemical Engineering Journal. 2020;**383**:123106

[20] Pattni BS et al. New developments in liposomal drug delivery. Chemical Reviews. 2015;**115**(19):10938-10966

[21] Henry BD et al. Engineered liposomes sequester bacterial exotoxins and protect from severe invasive infections in mice. Nature Biotechnology. 2015;**33**(1):81-88

[22] Rueda F et al. Effect of TLR ligands co-encapsulated with multiepitopic antigen in nanoliposomes targeted to human DCs via fc receptor for cancer vaccines. Immunobiology. 2017;**222**(11):989-997

[23] Cruz LJ et al. Liposomes containing NY-ESO-1/tetanus toxoid and adjuvant peptides targeted to human dendritic cells via the fc receptor for cancer vaccines. Nanomedicine (London, England). 2014;**9**(4):435-449

[24] Kunjachan S et al. Noninvasive imaging of nanomedicines and Nanotheranostics: Principles, Progress, and prospects. Chemical Reviews. 2015;**115**(19):10907-10937

[25] Zhang Y et al. Nanoparticle anchoring targets immune agonists to tumors enabling anti-cancer immunity without systemic toxicity. Nature Communications. 2018;**9**(1):6

[26] Olusanya TOB et al. Liposomal drug delivery systems and anticancer drugs. Molecules. 2018;**23**(4):907

[27] Rajan R et al. Liposome-induced immunosuppression and tumor growth is mediated by macrophages and mitigated by liposome-encapsulated alendronate. Journal of Controlled Release. 2018;**271**:139-148

[28] Deshantri AK et al. Complete tumor regression by liposomal Bortezomib in a humanized mouse model of multiple myeloma. Hema. 2020;**4**(5):e463

[29] Taoka K et al. Using patient-derived iPSCs to develop humanized mouse models for chronic myelomonocytic leukemia and therapeutic drug identification, including liposomal clodronate. Scientific Reports. 2018;**8**(1):15855

[30] Liu P et al. A review of liposomes as a drug delivery system: Current status of approved products, regulatory environments, and future perspectives. Molecules. 2022;**27**(4):1372

[31] Terpstra W et al. Facilitated engraftment of human hematopoietic cells in severe combined immunodeficient mice following a single injection of Cl2MDP liposomes. Leukemia. 1997;**11**(7):1049-1054

[32] Chen B et al. Complement depletion improves human red blood cell reconstitution in Immunodeficient mice. Stem Cell Reports. 2017;**9**(4):1034-1042

[33] Hu Z, Yang YG. Full reconstitution of human platelets in humanized mice after macrophage depletion. Blood. 2012;**120**(8):1713-1716

[34] Arnold L et al. Further improvements of the P. falciparum humanized mouse model. PLoS One. 2011;**6**(3):e18045

[35] Arnold L et al. Analysis of innate defences against plasmodium falciparum in immunodeficient mice. Malaria Journal. 2010;**9**:197

[36] Tyagi RK et al. Humanized mice are instrumental to the study of plasmodium falciparum infection. Frontiers in Immunology. 2018;**9**:2550

[37] Luiza-Batista C et al. Humanized mice for investigating sustained plasmodium vivax blood-stage infections and transmission. Nature Communications. 2022;**13**(1):4123

[38] Youssef A-R. The effect of in vivo macrophage depletion on skin allograft rejection in wild-type and CD8 knockout mice. Turkish Journal of Immunology. 2020;**8**(2):57-64

[39] Nishiwaki S et al. In vivo tracking of transplanted macrophages with near infrared fluorescent dye reveals temporal distribution and specific homing in the liver that can be perturbed by clodronate liposomes. PLoS One. 2020;**15**(12):e0242488

[40] Ramesh Kumar J et al. Use of Clodronate liposomes to deplete phagocytic immune cells in Drosophila melanogaster and Aedes aegypti. Frontiers in Cell and Development Biology. 2021;**9**:627976

[41] Marepally S et al. Editorial: Nanomedicine in infectious diseases: Drug delivery and vaccines. Frontiers in Pharmacology. 2022;**13**:928572

[42] Chaudhari R et al. Transdermal immunization of elastic liposome-laden recombinant chimeric fusion protein of P. falciparum (PfMSP-Fu(24)) mounts protective immune response. Nanomaterials (Basel). 2021;**11**(2):406

[43] Tyagi RK et al. Elastic liposome-mediated transdermal immunization enhanced the immunogenicity of P. falciparum surface antigen, MSP-1₁₉. Vaccine. 2015;**33**(36):4630-4638