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PERSPECTIVE

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Theranostics Based on Liposome: Looking Back and Forward

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Abstract

Liposome is one of the oldest yet most successful nanomedicine platforms. Doxil®, PEGylated liposome loaded with doxorubicin (DOX), was approved by the FDA in 1995 for the treatment of AIDS-related Kaposi's sarcoma, and it was the first approval for nanomedicine. Since then, liposome-based therapeutics were approved for the treatment of various diseases and many clinical trials are underway. The success of the liposome-based therapeutics was due to following factors: (1) ease of synthesis, (2) biocompatibility, (3) the ability to load both hydrophilic and hydrophobic agents, and (4) long circulation property after application of polyethylene glycol (PEG). Recently, more functionalities are introduced to liposome platform, which are (1) in vivo imaging probes for optical, magnetic resonance imaging (MRI), positron emission tomography (PET), and single-photon emission computed tomography (SPECT), (2) pH and temperature-sensitive lipid moiety, and (3) novel agents for photodynamic and photothermal therapies (PDT, PTT). These conventional and newly tested advantages make the liposome to be one of the most promising nanoplatforms for theranostics.

Keywords Liposome · Theranostics · Nanomedicine · Controlled drug release

Introduction

Liposomes are small artificial spherical shape vesicles with lipid bilayers which mainly consist of cholesterol and non-toxic phospholipids [1]. Synthesis of the liposome is done by three simple stages which are drying down lipids from an organic solvent, dispersing the lipid in aqueous solution, and purifying the resultant liposome [2]. Generally, small-sized liposomes (size less than 100 nm) are used for the systemic administration of liposome. Small-sized liposomes are made by mechanical dispersion methods such as sonication or extrusion methods. The liposome is amphiphilic, specifically, hydrophobic inside the lipid bilayer and hydrophilic inside the vesicle. Thus, both hydrophobic and hydrophilic drugs can be easily loaded during the dispersion stage. For example, hydrophilic doxorubicin can be loaded within the hydrophilic core of the liposomes for the production of Doxil® [3], while a hydrophobic antifungal agent, amphotericin B, is loaded inside the lipid bilayer of the liposome for production of Ambison® [4]. The liposomal drugs would show reduced toxicity and enhanced efficacy because of the improved biodistribution of the drugs [5]. With these advantages, liposome-based therapeutics were approved for the treatment of various diseases including breast cancer [6, 7], leukemia [8], fungal disease [9], hepatitis [10], and macular degeneration [11]. Also, multiple clinical trials are undergoing currently [12]. The clinically successful liposome-based therapeutics are well summarized in other recent review papers [5, 13]. In this perspective, the conventional advantages of liposome will be overviewed, and newer approaches using liposome for theranostics will be introduced (Fig. 1).

PEGylation: Advantages and Challenges

The first description of liposomes was given in 1965 and liposome has no surface modification [14]. PEGylation enhanced the circulation time and targeting efficiency of liposomes substantially. Many liposome-based therapeutics were based on high enhanced permeability and retention (EPR) effect because of the long circulation time of PEGylated liposome with adequate size [15–17]. High hydrophilicity and flexibility of PEG polymers interfere with the hydrophobic interaction between liposomes and serum proteins. Reduced

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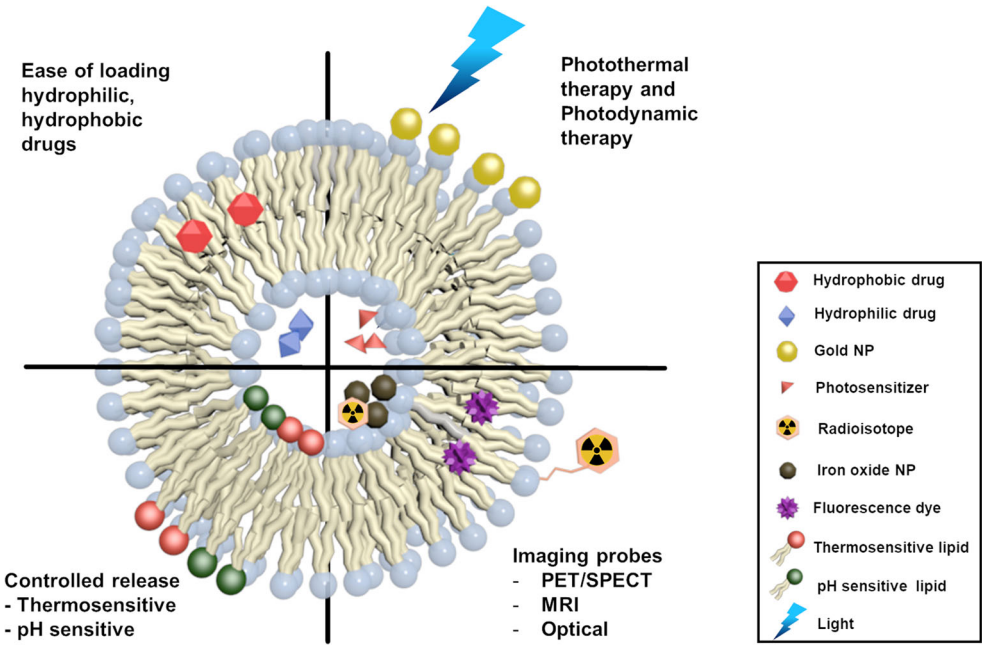

Fig. 1 Conventional and newer advantages of liposome for theranostics

adherence of serum proteins to liposomes reduces the absorption of liposomes by the reticuloendothelial system (RES), such as the liver and spleen, resulting in stealth effects. However, there is still a major challenge for the PEGylated liposome, which is called the accelerated blood clearance (ABC) phenomenon. The ABC phenomenon refers to the faster RES recognition and blood clearance of the nanoparticles (NPs) when the PEGylated NPs are re-injected to the same subject. Formation of anti-PEG IgM antibody is the main reason for the ABC phenomenon. Anti-PEG IgM antibody binds to the re-injected PEGylated NPs. After the recognition by anti-PEG IgM antibody, the NPs are further opsonized by complements and finally cleared by RES [18]. The ABC phenomenon was first noticed in the PEGylated liposomes [19]. The phenomenon was found in mice, rat, rabbits, and guinea pigs [20, 21]. Recently, also in spontaneous canine cancer model, ^{64}Cu-labeled liposomes showed ABC phenomenon by the induction of anti-IgM antibody [21]. Although the immune response to PEGylated liposome is a challenge for the translation of liposomes, the challenge would not be the shortcoming of liposome nanoplatform compared with other types of NPs because the immune response is found in other types of NPs as well. For example, the ABC phenomenon was found in PEGylated reduced-graphene oxide (rGO)-iron oxide hybrid nanoparticles and decreased passive targeting efficiency [18]. Recently, the strategies to overcome ABC have been developed. The strategies include coating the NPs with poly(N-vinyl-2-pyrrolidone) (PVP) [22], hyaluronic acid (HA) [23], or zwitterionic poly(carboxybetaine) (PCB) [24] rather than PEG.

Liposomes as an In Vivo Imaging Platform

Liposomes are utilized as imaging, and theranostic platform since various imaging functionalities can be added easily. For optical imaging, hydrophobic fluorescence dyes are incorporated into the lipid bilayers. A modified fluorescent dye DY-676-C18 was used for in vivo imaging for an edema model of the mouse [25]. Also, fluorescent nanoparticles such as quantum dots can be loaded to the hydrophilic area of the liposomes [26]. Liposome-based magnetic resonance imaging (MRI) contrast agents also developed either loading Gd-DTPA for T1 weighted imaging or iron oxide NPs for T2 weighted imaging [27, 28]. In vivo PET or SPECT imaging can be performed by radiolabeling the liposomes. The liposomes are radiolabeled by loading a chelator in the hydrophilic area of liposome and incubating with radioisotopes [29, 30]. Also, the liposomes can be radiolabeled by the incorporation of the lipid-conjugated bifunctional chelator to the liposome bilayer for further radiolabeling [31]. A glutathione-loaded liposome is used for labeling ^{99m}Tc-HMPAO as well [32]. With above-mentioned various methods, therapeutic radiometals also can be labeled to the liposome.

Theranostics Based on Liposome

Recently, there is a growing number of studies using liposome for theranostic applications. Feng et al. reported a ^{64}Cu-labeled and hypoxia-activated prodrug AQ4N and a photosensitizer, hexadecyl amine chlorin e6 (hCe6)-loaded liposome. The

liposome can be imaged by PET and has the ability for photodynamic therapy (PDT) under irradiation of 660-nm light emitting diode (LED) [33]. Also, Poly(9,9-dioctylfluorene-2,7-diylco-benzothiadiazole) (PFBT) as fluorescence probe and anticancer drugs (doxorubicin and folate)-loaded liposome showed effective imaging and therapeutic efficacy in tumor-bearing mice [34]. Zhang et al. developed liposome loaded with photosensitizer Ce6, hypoxia-activated prodrug Tirapazamine (TPZ), and gene probe for synergistic photodynamic chemotherapy [35]. Also, Rengan et al. reported the gold-coated liposome NPs for photothermal therapy (PTT) [36]. One report found that ^{177}Lu-labeled liposome showed moderate tumor uptake of 3%ID/g in the tumor-bearing mouse. Thus, ^{177}Lu-labeled liposome could be used for the theranostic platform. While ^{64}Cu-labeled liposome showed similar biodistribution and tumor uptake, thus can be used as a companion imaging agent for ^{177}Lu-labeled liposome [29]. Also, ^{64}Cu liposome (MM-Dx-929) was used for EPR effect surrogate marker of other liposome-based therapeutics. And tumor with a higher uptake of MM-Dx-929 showed better treatment effect of liposome-based therapeutics [30].

Strategies for Controlled Drug Release

The functionality of controlled drug release can be added to liposome [37]. At a certain temperature, the lipid bilayer of the liposome can be transferred from a solid gel phase to a liquid crystalline phase. The temperature is called the melting phase transition temperature, Tm. Encapsulated drugs can be released at Tm. For the synthesis of the thermosensitive liposome, 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) is used as a major component, since the Tm of DPPC is 41.4 °C [38]. Tagami et al. reported that Gd-DTPA and DOX-loaded thermosensitive liposome can be utilized as an MRI T1 contrast agent and cancer therapeutics. DOX was released 100% under mild hyperthermia (40–42 °C) [27]. External heating was applied after injection of the theranostic liposome for the treatment of tumor-bearing mouse and showed a superior effect compared with the group without external heating. However, external heating seems not to be applicable in a deeper tumor and bigger animal. The photothermal effect can be used to induce heating for a deeper tumor using near-infrared (NIR) laser coupled with dye or NPs with photothermal effect. Yu et al. developed DOX, indocyanine green (ICG)-loaded liposome, for controlled drug release by 808-nm laser because ICG elicits photothermal effect under the laser irradiation. Cumulative DOX release found to be 20% in vitro by 30 min of NIR laser irradiation, which is a relatively modest amount compared with the local heating method [39]. Meanwhile, the pH-sensitive liposome is used for controlled release of the drugs. Since the extracellular environment of cancer is acidic than of normal tissue or blood, the pH-sensitive liposome is widely utilized for cancer-specific drug release. For pH-sensitive liposome, a neutral cone-shaped lipid dioleoylphosphatidyl-ethanolamine (DOPE) and a weakly acidic amphiphile, such as cholesteryl hemisuccinate (CHEMS), are commonly used [40]. Also, dioleoylphosphatidylcholine (DOPC) [41] or *N*-succinyl-DOPE [42] is utilized for the pH-sensitive drug release. Zhao et al. reported that tumor-specific pH-responsive peptide ($H_7K(R_2)_2$)-modified liposome can release the loaded DOX over 80% at a pH of 6.5. The pH-sensitive DOX-loaded liposome showed better tumor control ability than DOX-loaded liposome without pH-sensitive peptide [43]. However, drug release triggered by low pH may not be cancer specific because endosomal and lysosomal lumens of immune cells are also acidic.

Conclusion

Thousands of novel NPs for imaging and therapy other than liposome have been developed after the success of Doxil®. Although these NPs have unique advantages for imaging and therapy, however, in many cases, the NPs suffered from toxicity, low stability in vivo, high RES recognition, and low targeting efficiency [44]. Therefore, liposome is still one of the most viable and promising nanoplatforms because of its biocompatibility, long-circulating ability, and high passive targeting efficiency. Furthermore, liposome has been tested and found to be feasible for adding functionalities including various imaging probes, therapeutics, and controlled release properties. The next goals for the successful theranostics based on liposomes would be to find effective ways to avoid ABC phenomenon on multiple injections and to develop more specific controlled drug release methods.

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Compliance with Ethical Standards

Conflict of Interest Wooseung Lee and Hyung-Jun Im declare that they have no conflict of interest.

Ethical Approval This work does not contain any studies with human participants or animals performed by any of the authors.

Informed Consent Not applicable.

References

1. Xing H, Hwang K, Lu Y. Recent developments of liposomes as nanocarriers for theranostic applications. Theranostics. 2016;6:1336–52.

2. Akbarzadeh A, Rezaei-Sadabady R, Davaran S, et al. Liposome: classification, preparation, and applications. Nanoscale Res Lett. 2013;8:102.
3. Barenholz Y. Doxil(R)–the first FDA-approved nano-drug: lessons learned. J Control Release. 2012;160:117–34.
4. Adler-moore JP, Proffitt RT. Development, characterization, efficacy and mode of action of ambisome, a unilamellar liposomal formulation of amphotericin B. J Liposome Res. 1993;3:429–50.
5. Bulbake U, Doppalapudi S, Kommineni N, Khan W. Liposomal formulations in clinical use: an updated review. Pharmaceutics. 2017;9.
6. Gardikis K, Tsimplouli C, Dimas K, Micha-Screttas M, Demetzos C. New chimeric advanced drug delivery nano systems (chiaDDnSs) as doxorubicin carriers. Int J Pharm. 2010;402:231–7.
7. Batist G, Ramakrishnan G, Rao CS, et al. Reduced cardiotoxicity and preserved antitumor efficacy of liposome-encapsulated doxorubicin and cyclophosphamide compared with conventional doxorubicin and cyclophosphamide in a randomized, multicenter trial of metastatic breast cancer. J Clin Oncol. 2001;19:1444–54.
8. Silverman JA, Deitcher SR. Marqibo(R) (vincristine sulfate liposome injection) improves the pharmacokinetics and pharmacodynamics of vincristine. Cancer Chemother Pharmacol. 2013;71:555–64.
9. Merhav H, Mieles L. Amphotericin B lipid complex in the treatment of invasive fungal infections in liver transplant patients. Transplant Proc. 1997;29:2670–4.
10. Bovier PA. Epaxal: a virosomal vaccine to prevent hepatitis A infection. Expert Rev Vaccines. 2008;7:1141–50.
11. Jonas JB. Verteporfin therapy of subfoveal choroidal neovascularization in age-related macular degeneration. Am J Ophthalmol. 2002;133:857–9.
12. Bulbake U, Doppalapudi S, Kommineni N, Khan W. Liposomal formulations in clinical use: an updated review. Pharmaceutics. 2017;9:12.
13. Pattni BS, Chupin VV, Torchilin VP. New developments in liposomal drug delivery. Chem Rev. 2015;115:10938–66.
14. Bangham AD, Horne RW. Negative staining of phospholipids and their structural modification by surface-active agents as observed in the electron microscope. J Mol Biol. 1964;8:660–8.
15. Immordino ML, Dosio F, Cattel L. Stealth liposomes: review of the basic science, rationale, and clinical applications, existing and potential. Int J Nanomedicine. 2006;1:297–315.
16. Gabizon A, Catane R, Uziely B, et al. Prolonged circulation time and enhanced accumulation in malignant exudates of doxorubicin encapsulated in polyethylene-glycol coated liposomes. Cancer Res. 1994;54:987–92.
17. Golombek SK, May J-N, Theek B, et al. Tumor targeting via EPR: strategies to enhance patient responses. Adv Drug Deliv Rev. 2018;130:17–38.
18. Im HJ, England CG, Feng L, et al. Accelerated blood clearance phenomenon reduces the passive targeting of PEGylated nanoparticles in peripheral arterial disease. ACS Appl Mater Interfaces. 2016;8:17955–63.
19. Ishida T, Harada M, Wang XY, Ichihara M, Irimura K, Kiwada H. Accelerated blood clearance of PEGylated liposomes following preceding liposome injection: effects of lipid dose and PEG surface-density and chain length of the first-dose liposomes. J Control Release. 2005;105:305–17.
20. Xu H, Ye F, Hu M, et al. Influence of phospholipid types and animal models on the accelerated blood clearance phenomenon of PEGylated liposomes upon repeated injection. Drug Deliv. 2015;22:598–607.
21. Borresen B, Henriksen JR, Clergeaud G, et al. Theranostic imaging may vaccinate against the therapeutic benefit of long circulating PEGylated liposomes and change cargo pharmacokinetics. ACS Nano. 2018;12:11386–98.
22. Ishihara T, Maeda T, Sakamoto H, et al. Evasion of the accelerated blood clearance phenomenon by coating of nanoparticles with various hydrophilic polymers. Biomacromolecules. 2010;11:2700–6.
23. Zhang Q, Deng C, Fu Y, Sun X, Gong T, Zhang Z. Repeated administration of hyaluronic acid coated liposomes with improved pharmacokinetics and reduced immune response. Mol Pharm. 2016;13:1800–8.
24. Li Y, Liu R, Shi Y, Zhang Z, Zhang X. Zwitterionic poly(carboxybetaine)-based cationic liposomes for effective delivery of small interfering RNA therapeutics without accelerated blood clearance phenomenon. Theranostics. 2015;5:583–96.
25. Deissler V, Ruger R, Frank W, Fahr A, Kaiser WA, Hilger I. Fluorescent liposomes as contrast agents for in vivo optical imaging of edemas in mice. Small. 2008;4:1240–6.
26. Mukthavaram R, Wrasidlo W, Hall D, Kesari S, Makale M. Assembly and targeting of liposomal nanoparticles encapsulating quantum dots. Bioconjug Chem. 2011;22:1638–44.
27. Tagami T, Foltz WD, Ernsting MJ, et al. MRI monitoring of intratumoral drug delivery and prediction of the therapeutic effect with a multifunctional thermosensitive liposome. Biomaterials. 2011;32:6570–8.
28. Mikhaylov G, Mikac U, Magaeva AA, et al. Ferri-liposomes as an MRI-visible drug-delivery system for targeting tumours and their microenvironment. Nat Nanotechnol. 2011;6:594.
29. Petersen AL, Henriksen JR, Binderup T, et al. In vivo evaluation of PEGylated (6)(4)Cu-liposomes with theranostic and radiotherapeutic potential using micro PET/CT. Eur J Nucl Med Mol Imaging. 2016;43:941–52.
30. Lee H, Gaddy D, Ventura M, et al. Companion diagnostic (64)Cu-liposome positron emission tomography enables characterization of drug delivery to tumors and predicts response to cancer nanomedicines. Theranostics. 2018;8:2300–12.
31. Seo JW, Zhang H, Kukis DL, Meares CF, Ferrara KW. A novel method to label preformed liposomes with 64Cu for positron emission tomography (PET) imaging. Bioconjug Chem. 2008;19:2577–84.
32. Phillips WT, Rudolph AS, Goins B, Timmons JH, Klipper R, Blumhardt R. A simple method for producing a technetium-99m-labeled liposome which is stable in vivo. Int J Rad Appl Instrum B. 1992;19:539–47.
33. Feng L, Cheng L, Dong Z, et al. Theranostic liposomes with hypoxia-activated prodrug to effectively destruct hypoxic tumors post-photodynamic therapy. ACS Nano. 2017;11:927–37.
34. Ma M, Lei M, Tan X, Tan F, Li N. Theranostic liposomes containing conjugated polymer dots and doxorubicin for bio-imaging and targeted therapeutic delivery. RSC Adv. 2016;6:1945–57.
35. Zhang K, Zhang Y, Meng X, et al. Light-triggered theranostic liposomes for tumor diagnosis and combined photodynamic and hypoxia-activated prodrug therapy. Biomaterials. 2018;185:301–9.
36. Rengan AK, Bukhari AB, Pradhan A, et al. In vivo analysis of biodegradable liposome gold nanoparticles as efficient agents for photothermal therapy of Cancer. Nano Lett. 2015;15:842–8.
37. Kneidl B, Peller M, Winter G, Lindner LH, Hossann M. Thermosensitive liposomal drug delivery systems: state of the art review. Int J Nanomedicine. 2014;9:4387–98.
38. Demel RA, De Kruyff B. The function of sterols in membranes. Biochim Biophys Acta. 1976;457:109–32.
39. Yu L, Dong A, Guo R, Yang M, Deng L, Zhang J. DOX/ICG Coencapsulated liposome-coated thermosensitive nanogels for nir-triggered simultaneous drug release and photothermal effect. ACS Biomater Sci Eng. 2018;4:2424–34.
40. Lai MZ, Duzgunes N, Szoka FC. Effects of replacement of the hydroxyl group of cholesterol and tocopherol on the thermotropic behavior of phospholipid membranes. Biochemistry. 1985;24:1646–53.
41. Webb MS, Wheeler JJ, Bally MB, Mayer LD. The cationic lipid stearylamine reduces the permeability of the cationic drugs

verapamil and prochlorperazine to lipid bilayers: implications for drug delivery. Biochim Biophys Acta. 1995;1238:147–55.
42. Nayar R, Schroit AJ. Generation of pH-sensitive liposomes: use of large unilamellar vesicles containing N-succinyldioleoylphosphatidylethanolamine. Biochemistry. 1985;24:5967–71.
43. Zhao Y, Ren W, Zhong T, et al. Tumor-specific pH-responsive peptide-modified pH-sensitive liposomes containing doxorubicin for enhancing glioma targeting and anti-tumor activity. J Control Release. 2016;222:56–66.
44. Wilhelm S, Tavares AJ, Dai Q, et al. Analysis of nanoparticle delivery to tumours. Nat Rev Mater. 2016;1:16014.