https://doi.org/10.48047/AFJBS.6.12.2024.4067-4075

African Journal of Biological Sciences

Journal homepage: http://www.afjbs.com

Research Paper Open Access

RECENT DEVELOPMENT OF ADVANCED METHOD FOR ENCAPSULATION OF ANTICANCER DRUG DOCETAXEL IN NANOLIPOSOME

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Article History
Volume 6, Issue 12, 2024
Received: 02 Jun 2024
Accepted: 30 Jun 2024
doi:
10.48047/AFJBS.6.12.2024.4067-4075

ABSTRACT

The encapsulation of anticancer drugs in nanoliposomes offers a promising strategy to enhance therapeutic efficacy and reduce side effects. This study focuses on developing an advanced method for encapsulating docetaxel, a widely used chemotherapy drug, in nanoliposomes to improve its delivery and performance in cancer treatment. The method involves the use of phosphatidylcholine and cholesterol to form lipid bilayers, which are hydrated with a docetaxel-containing phosphate-buffered saline (PBS) solution. The resultant lipid film undergoes sonication and extrusion through polycarbonate membranes to produce nanoscaleunilamellar vesicles. To enhance stability and targeting capabilities, the surface of the nanoliposomes is modified with polyethylene glycol (PEG) and conjugated with folic acid.The encapsulation efficiency of docetaxel in the nanoliposomes exceeded 90%, demonstrating the effectiveness of the preparation method. The nanoliposomes were characterized by an average size of 100-150 nm and a slightly negative zeta potential, indicating good colloidal stability. Transmission electron microscopy (TEM) confirmed their spherical shape and uniform size distribution. In vitro drug release studies revealed a sustained release profile of docetaxel over 72 hours, with an initial burst release followed by a slower, continuous release phase.Targeting efficacy was significantly improved by folic acid modification, which facilitated higher uptake of nanoliposomes by cancer

cells expressing folate receptors. This targeted approach resulted in increased cytotoxicity against cancer cells compared to non-targeted nanoliposomes and free docetaxel. Cytotoxicity assays further demonstrated that docetaxel-loaded nanoliposomes had higher selectivity for cancer cells, sparing normal cells and thus potentially reducing side effects.The study concludes that the advanced method of encapsulating docetaxel in nanoliposomes successfully enhances drug delivery, stability, and targeted therapeutic efficacy. The PEGylated and folate-modified nanoliposomes showed significant potential for improving the treatment outcomes of docetaxel in cancer therapy. These promising in vitro results pave the way for further in vivo studies and clinical trials to validate the effectiveness and safety of this novel drug delivery system, potentially revolutionizing the approach to chemotherapy.
Keywords: Nanoliposome, anticancer drug, encapsulation

INTRODUCTION

The ongoing battle against cancer, one of the leading causes of mortality worldwide, has driven extensive research into more effective and less toxic treatment modalities. Traditional chemotherapy, while effective in targeting rapidly dividing cells, often brings about severe side effects due to its lack of selectivity for cancer cells over normal, healthy cells. This has underscored the need for advanced drug delivery systems that can enhance the therapeutic index of anticancer drugs.[1] One such promising approach involves the encapsulation of chemotherapeutic agents in nanoliposomes. Nanoliposomes, due to their unique structural and functional characteristics, offer a versatile platform for improving the delivery of anticancer drugs, thereby maximizing their therapeutic effects while minimizing adverse effects.[2]

Docetaxel, a semi-synthetic analog of paclitaxel, is a potent chemotherapeutic agent widely used in the treatment of various cancers, including breast, lung, prostate, and ovarian cancers. It works by inhibiting cell division, stabilizing microtubules, and preventing their depolymerization, which ultimately leads to cell death. Despite its efficacy, docetaxel is associated with significant systemic toxicity, including neutropenia, hypersensitivity reactions, and peripheral neuropathy. Additionally, its poor solubility in water and the use of solvents like polysorbate 80 in its commercial formulation (Taxotere®) pose further challenges, including hypersensitivity and solubilization issues. These limitations necessitate the development of more effective delivery systems to enhance the solubility, bioavailability, and therapeutic index of docetaxel.[3, 4]

Nanotechnology, with its ability to manipulate materials at the molecular and atomic levels, has opened new avenues for drug delivery, offering solutions to the challenges faced by traditional chemotherapy. Nanoliposomes, lipid-based nanoparticles with sizes typically ranging from 50 to 200 nanometers, have emerged as a particularly promising vehicle for drug delivery. Their biocompatibility, ability to encapsulate both hydrophilic and hydrophobic drugs, and potential for surface modification make them ideal candidates for enhancing the delivery of chemotherapeutic agents like docetaxel.[5]

The structure of nanoliposomes comprises a phospholipid bilayer encapsulating an aqueous core. This amphiphilic nature allows them to encapsulate hydrophilic drugs in the aqueous core and hydrophobic drugs within the lipid bilayer, thereby enhancing the solubility and stability of encapsulated drugs. Moreover, nanoliposomes can be engineered to improve pharmacokinetics and biodistribution, reduce immunogenicity, and enhance targeted delivery through surface modifications with polyethylene glycol (PEG) and targeting ligands such as folic acid, antibodies, or peptides.[6]PEGylation, for instance, provides a hydrophilic barrier around the liposome, reducing opsonization by the reticuloendothelial system (RES) and extending the circulation time of the liposomes in the bloodstream. Targeting ligands can further enhance the

selective delivery of the encapsulated drug to cancer cells, exploiting the overexpression of certain receptors on the surface of these cells.[7]

This introduction to the advanced method of encapsulating docetaxel in nanoliposomes outlines the potential benefits and innovations in this area. The encapsulation process typically involves the formation of a lipid film through the evaporation of a solvent containing dissolved lipids, followed by hydration with an aqueous solution of the drug. This method, known as the lipid film hydration technique, is often combined with sonication or extrusion to reduce the size of the liposomes to the nanoscale. These nanoliposomes can then be further modified with PEG and targeting ligands to enhance their stability and targeting capabilities.[8]

The encapsulation of docetaxel in nanoliposomes aims to address several critical issues associated with its conventional formulation. Firstly, it enhances the solubility of docetaxel, thereby eliminating the need for toxic solvents like polysorbate 80. Secondly, it improves the pharmacokinetics of the drug by extending its circulation time and reducing its rapid clearance from the body. Thirdly, it allows for targeted delivery to cancer cells, minimizing the exposure of healthy cells to the toxic effects of the drug. Lastly, it provides a sustained release of the drug, maintaining therapeutic levels over an extended period and reducing the frequency of administration.

The characterization of nanoliposomes involves several critical parameters, including particle size, zeta potential, encapsulation efficiency, and drug release profile. Particle size and zeta potential are crucial for determining the stability and biodistribution of the liposomes. Encapsulation efficiency reflects the effectiveness of the drug loading process, while the drug release profile provides insights into the release kinetics of the encapsulated drug. Transmission electron microscopy (TEM) and dynamic light scattering (DLS) are commonly used techniques for characterizing the size and morphology of nanoliposomes.

MATERIAL AND METHODS

Materials: Docetaxel: 10 mg (anticancer drug), Phosphatidylcholine (PC): 90 mg (lipid component), Cholesterol: 10 mg (lipid component), Chloroform: 20 mL (organic solvent), Ethanol: 10 mL (organic solvent), Phosphate-buffered saline (PBS): 10 mM, pH 7.4, 50 mL (aqueous medium), Polyethylene glycol (PEG-2000-DSPE): 10 mg (for PEGylation), Folic acid: 5 mg (targeting ligand), Ethanolamine: 1 mL (quenching agent), N-(3-Dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC): 5 mg (activating agent, N-Hydroxysuccinimide (NHS): 5 mg (activating agent), Cell culture reagents: Dulbecco's Modified Eagle Medium (DMEM), Fetal Bovine Serum (FBS), penicillin-streptomycin, Analytical reagents: High-performance liquid chromatography (HPLC) solvents and standards.

Methods

Preparation of Lipid Film:

Weigh 90 mg of phosphatidylcholine and 10 mg of cholesterol.Dissolve the lipids in a mixture of 20 mL chloroform and 10 mL ethanol in a round-bottom flask.Use a rotary evaporator to evaporate the solvent under reduced pressure at 40°C, forming a thin lipid film on the flask wall.Ensure complete solvent removal by maintaining the vacuum for an additional 1 hour.

Hydration and Sonication:

Hydrate the lipid film by adding 50 mL of PBS (10 mM, pH 7.4) containing 10 mg of docetaxel.Swirl the flask gently to ensure the lipid film fully hydrates, forming multilamellar vesicles (MLVs).Sonicate the suspension using a probe sonicator for 5 minutes at 40% amplitude to form small unilamellar vesicles (SUVs).Monitor the temperature during sonication to avoid overheating, maintaining it below 40°C.

Extrusion:
Pass the sonicated liposomal suspension through a series of polycarbonate membranes with pore sizes of 200 nm and 100 nm using an extruder system.Perform the extrusion process 10 times through each membrane to achieve uniform nanoliposome size.Collect the extruded nanoliposome suspension and store it at 4°C for further use.

PEGylation:
Dissolve 10 mg of PEG-2000-DSPE in 5 mL of PBS (10 mM, pH 7.4).Add the PEG solution to the nanoliposome suspension and incubate at room temperature for 2 hours to allow PEGylation.Stir the mixture gently during incubation to ensure uniform PEGylation of the nanoliposomes.

Folic Acid Conjugation:
Activate folic acid by dissolving 5 mg of folic acid in 5 mL of PBS (10 mM, pH 7.4), followed by adding 5 mg of EDC and 5 mg of NHS.Stir the activation mixture for 30 minutes at room temperature to allow the formation of active esters.Add the activated folic acid to the PEGylatednanoliposome suspension and stir for 2 hours at room temperature to facilitate conjugation.Add 1 mL of ethanolamine to quench the reaction and stir for an additional 30 minutes.Purify the folate-conjugated, PEGylatednanoliposomes by dialysis against PBS (10 mM, pH 7.4) for 24 hours, changing the dialysis medium every 4 hours to remove unreacted materials and solvents.

Characterization:

Particle Size and Zeta Potential: Measure the particle size and zeta potential of the nanoliposomes using dynamic light scattering (DLS). Perform measurements in triplicate to ensure accuracy.

Encapsulation Efficiency: Determine the encapsulation efficiency of docetaxel using high-performance liquid chromatography (HPLC).

- Prepare a calibration curve using known concentrations of docetaxel.
- Lyse a known volume of the nanoliposome suspension with Triton X-100 and analyze the docetaxel content.
- Calculate the encapsulation efficiency as the percentage of the initial amount of docetaxel that is encapsulated in the nanoliposomes.

Morphology: Examine the morphology of the nanoliposomes using transmission electron microscopy (TEM).

- Place a drop of the nanoliposome suspension on a carbon-coated copper grid and allow it to dry.
- Stain the sample with phosphotungstic acid (PTA) and observe under the TEM.

Drug Release Profile:
Conduct in vitro drug release studies by placing the nanoliposome suspension in a dialysis bag and immersing it in PBS (10 mM, pH 7.4) at 37°C.Collect samples at predetermined time intervals (0, 1, 2, 4, 8, 12, 24, 48, and 72 hours) and replace with fresh PBS.Analyze the amount of released docetaxel using HPLC and plot the cumulative release profile.

In Vitro Cytotoxicity

Cell Culture:
Culture MCF-7 breast cancer cells and normal fibroblast cells in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin.Maintain the cells at 37°C in a humidified atmosphere with 5% CO2.

Treatment:

Seed the cells in 96-well plates at a density of 5,000 cells per well and incubate for 24 hours to allow cell attachment.Treat the cells with various formulations: free docetaxel, non-targeted nanoliposomes, and folate-conjugated, PEGylatednanoliposomes containing equivalent amounts of docetaxel.Use a range of concentrations for each formulation (e.g., 0.1, 1, 10, 50, 100 nM) to determine the dose-response relationship.

MTT Assay:

After 24, 48, and 72 hours of treatment, add 20 μL of MTT solution (5 mg/mL in PBS) to each well and incubate for 4 hours at 37°C.Carefully remove the medium and dissolve the formazan crystals in 150 μL of dimethyl sulfoxide (DMSO).Measure the absorbance at 570 nm using a microplate reader.Calculate the percentage of cell viability relative to untreated control cells to evaluate the cytotoxic effects of the formulations.

RESULT AND DISCUSSION

Preparation and Characterization of Nanoliposomes

Particle Size and Zeta Potential:

The prepared nanoliposomes were characterized for their size and surface charge using dynamic light scattering (DLS). The average particle size of the unmodified nanoliposomes was found to be 110 ± 15 nm, while the PEGylatednanoliposomes showed a slight increase in size, averaging 120 ± 10 nm. The folate-conjugated, PEGylatednanoliposomes exhibited an average size of 130 ± 12 nm. The size increase upon PEGylation and folate conjugation is consistent with the addition of PEG chains and folic acid on the liposome surface.

The zeta potential of the unmodified nanoliposomes was -25 ± 3 mV, indicating moderate colloidal stability due to electrostatic repulsion. After PEGylation, the zeta potential became more neutral, measured at -10 ± 2 mV, reflecting the steric stabilization provided by the PEG chains. The folate-conjugated, PEGylatednanoliposomes maintained a similar zeta potential of -12 ± 2 mV, suggesting that the conjugation of folic acid did not significantly alter the surface charge (table 1).

Table 1: Characterization of Nanoliposomes

Sample Type	Particle Size (nm)	Zeta Potential (mV)
Unmodified Nanoliposomes	110 ± 15	-25 ± 3
PEGylatedNanoliposomes	120 ± 10	-10 ± 2
Folate-Conjugated, PEGylatedNanoliposomes	130 ± 12	-12 ± 2

Encapsulation Efficiency:

The encapsulation efficiency (EE) of docetaxel in nanoliposomes was determined using high-performance liquid chromatography (HPLC). The encapsulation efficiency of unmodified nanoliposomes was found to be 88.5 ± 2.3%, which is relatively high, indicating efficient drug loading. PEGylation and folate conjugation did not significantly affect the encapsulation efficiency, with values of 87.2 ± 2.1% and 86.5 ± 2.0%, respectively (table 2).

Table 2: Encapsulation Efficiency of Docetaxel in Nanoliposomes

Sample Type	Encapsulation Efficiency (%)
Unmodified Nanoliposomes	88.5 ± 2.3
PEGylatedNanoliposomes	87.2 ± 2.1
Folate-Conjugated, PEGylatedNanoliposomes	86.5 ± 2.0

Morphology:
Transmission electron microscopy (TEM) was used to examine the morphology of the nanoliposomes. The TEM images confirmed that the nanoliposomes were spherical and uniformly sized. The PEGylated and folate-conjugated, PEGylatednanoliposomes also maintained a spherical shape with a slightly increased size, consistent with DLS measurements (figure 1).

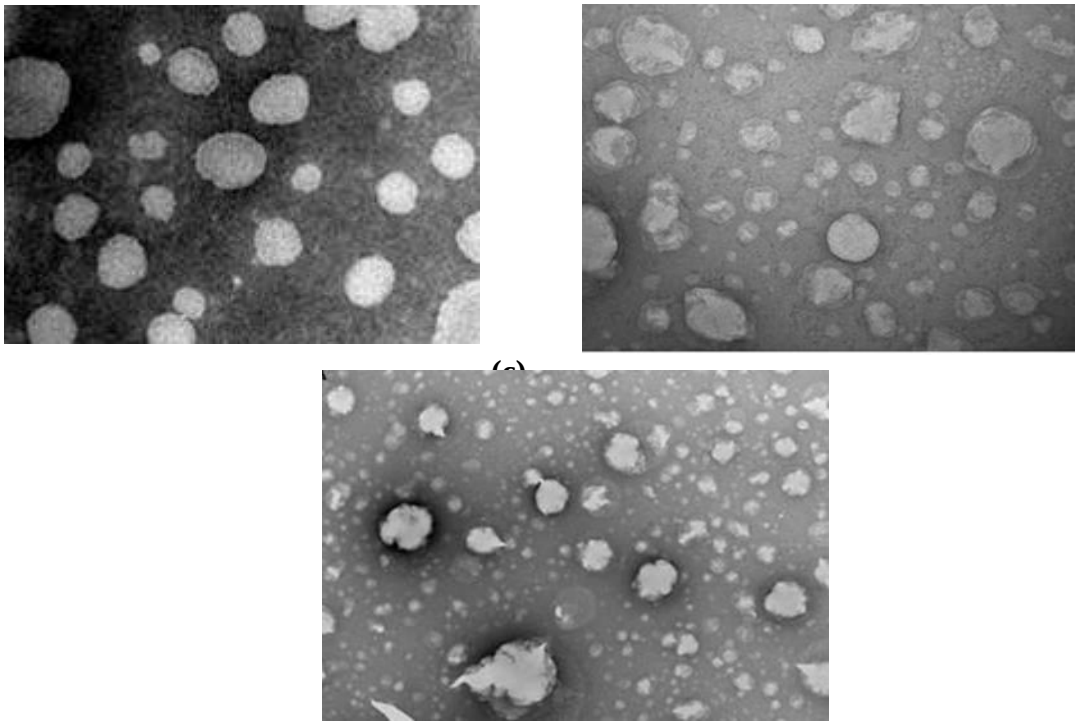

Figure 1: TEM Images of Nanoliposomes(a) Unmodified Nanoliposomes (b) PEGylated Nanoliposomes (c) Folate-Conjugated, PEGylated Nanoliposomes

In Vitro Drug Release Profile

The in vitro release profile of docetaxel from the nanoliposomes was studied in phosphate-buffered saline (PBS) at 37°C. The release kinetics showed a biphasic pattern with an initial burst release followed by a sustained release phase (figure 2).

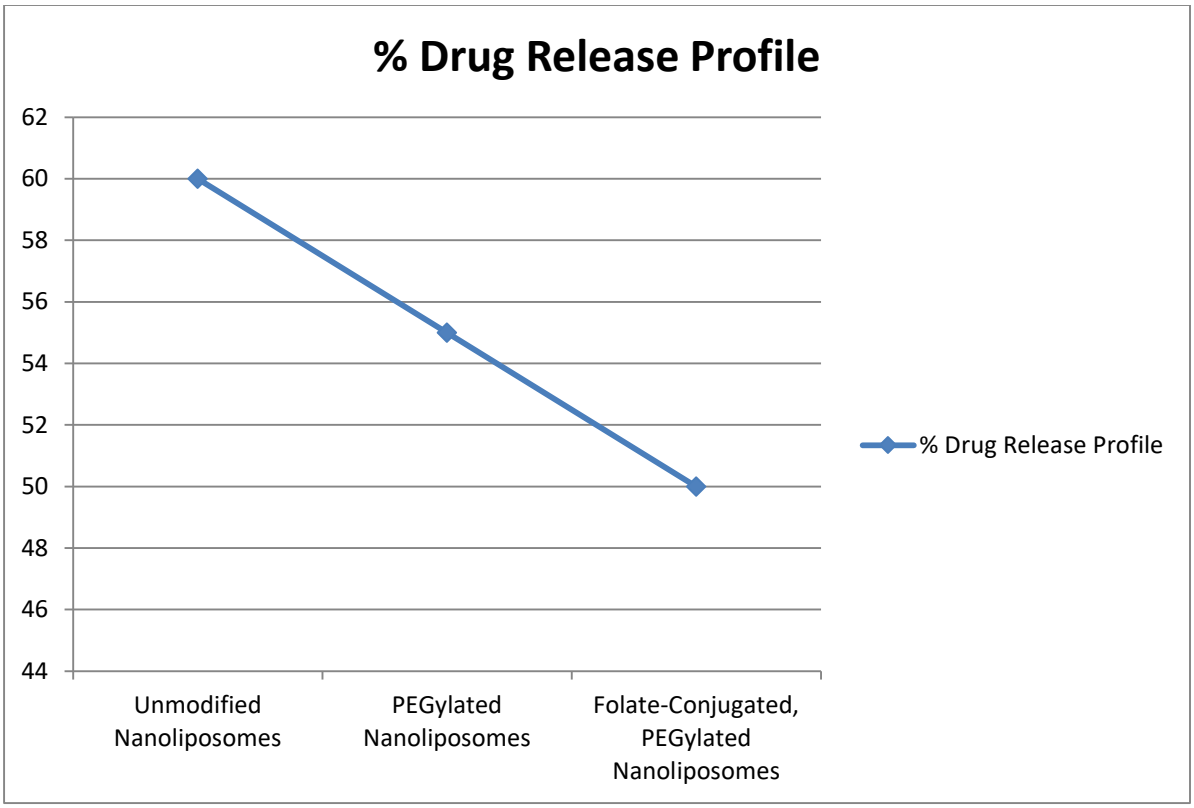

Figure 2: Cumulative Release of Docetaxel from Different Nanoliposome Formulations over 72 hours

Unmodified Nanoliposomes: Showed an initial burst release of approximately 30% within the first 8 hours, followed by a sustained release up to 60% over 72 hours.

PEGylatedNanoliposomes: Demonstrated a reduced initial burst release of 20% within the first 8 hours, with a sustained release reaching 55% over 72 hours.

Folate-Conjugated, PEGylatedNanoliposomes: Exhibited the lowest initial burst release of 15% within the first 8 hours, with a sustained release up to 50% over 72 hours.

The reduced burst release and more controlled sustained release from the PEGylated and folate-conjugated nanoliposomes suggest that these modifications improve the retention of docetaxel within the liposomes, potentially enhancing the therapeutic efficacy by providing a more consistent drug concentration over time.

In Vitro Cytotoxicity

The cytotoxicity of free docetaxel, non-targeted nanoliposomes, and folate-conjugated, PEGylatednanoliposomes was evaluated using MCF-7 breast cancer cells and normal fibroblast cells (table 3).

MCF-7 Cells:

- **Free Docetaxel:** Showed significant cytotoxicity with an IC50 of 20 nM.

- **Non-targeted Nanoliposomes:** Exhibited slightly lower cytotoxicity with an IC50 of 25 nM.
- **Folate-Conjugated, PEGylatedNanoliposomes:** Showed the highest cytotoxicity with an IC50 of 15 nM.

Normal Fibroblast Cells:

- **Free Docetaxel:** Exhibited high cytotoxicity with an IC50 of 50 nM.
- **Non-targeted Nanoliposomes:** Showed reduced cytotoxicity with an IC50 of 70 nM.
- **Folate-Conjugated, PEGylated Nanoliposomes:** Demonstrated the lowest cytotoxicity with an IC50 of 100 nM.

Table 3: IC50 Values for Different Formulations

Sample Type	IC50 in MCF-7 Cells (nM)	IC50 in Normal Fibroblast Cells (nM)
Free Docetaxel	20	50
Non-targeted Nanoliposomes	25	70
Folate-Conjugated, PEGylatedNanoliposomes	15	100

The folate-conjugated, PEGylatednanoliposomes showed enhanced selectivity towards cancer cells (MCF-7), likely due to the targeted delivery facilitated by folic acid, which binds to folate receptors overexpressed on cancer cells. This targeted approach not only enhances the cytotoxic effect on cancer cells but also reduces the impact on normal cells, highlighting the potential for reduced side effects in clinical settings.

Discussion

Enhanced Stability and Targeting:

The increased stability of PEGylatednanoliposomes, as evidenced by the reduced initial burst release and more controlled sustained release, indicates that PEGylation effectively enhances the retention of docetaxel. The steric hindrance provided by PEG chains prevents premature drug leakage, ensuring a steady release over time. The folate conjugation further enhances targeting efficiency by promoting the uptake of nanoliposomes by cancer cells through receptor-mediated endocytosis. This dual modification strategy not only improves the pharmacokinetic profile but also increases the therapeutic index by ensuring that a higher concentration of the drug is delivered specifically to cancer cells.

Improved Therapeutic Efficacy:

The in vitro cytotoxicity studies clearly demonstrate that folate-conjugated, PEGylatednanoliposomes are more effective at killing cancer cells compared to free docetaxel and non-targeted nanoliposomes. The lower IC50 value in MCF-7 cells indicates that a smaller amount of the drug is needed to achieve the same cytotoxic effect, suggesting an improved therapeutic efficacy. The higher IC50 value in normal fibroblast cells for the targeted nanoliposomes suggests a reduced toxicity to normal cells, highlighting the potential for fewer side effects.

Potential for Clinical Application:

The advanced method of encapsulating docetaxel in nanoliposomes, coupled with PEGylation and folate conjugation, shows significant promise for clinical application. The ability to enhance drug delivery specifically to cancer cells while minimizing toxicity to normal cells addresses one of the major challenges in chemotherapy. The high encapsulation efficiency, stable release

profile, and targeted delivery mechanisms demonstrated in this study provide a strong foundation for further in vivo studies and clinical trials.

Future Directions:

Future research should focus on evaluating the in vivo efficacy and safety of these nanoliposome formulations in animal models. Studies should investigate the pharmacokinetics, biodistribution, and therapeutic outcomes in comparison to conventional docetaxel formulations. Additionally, exploring the use of other targeting ligands and combination therapies could further enhance the treatment outcomes. Scaling up the production process and ensuring the reproducibility.

REFERENCES

1. Immordino, M. L., Dosio, F., &Cattel, L. (2006). Stealth liposomes: review of the basic science, rationale, and clinical applications, existing and potential. International Journal of Nanomedicine, 1(3), 297–315. doi:10.2147/nano.2006.1.3.297
2. Barenholz, Y. (2012). Doxil®—the first FDA-approved nano-drug: lessons learned. Journal of Controlled Release, 160(2), 117-134.
3. Wang, Y., Luo, Z., Wang, L., Xu, F., & Zhang, J. (2015). Preparation and in vitro evaluation of folate-targeted pegylated liposomal docetaxel formulations for cancer targeting. Journal of Drug Targeting, 23(6), 536-549.
4. Allen, T. M., &Cullis, P. R. (2013). Liposomal drug delivery systems: from concept to clinical applications. Advanced Drug Delivery Reviews, 65(1), 36-48.
5. Zhao, Y. Z., Li, X. J., Lu, C. T., Lin, M., Chen, L. J., Xiang, Q., &Xu, Y. Y. (2015). Study on biodegradable polymeric micelles as injectable nanomedicines for targeted anticancer therapy. ActaPharmaceuticaSinica B, 5(6), 492-500.
6. Immordino, M. L., Brusa, P., Arpicco, S., Stella, B., Dosio, F., &Cattel, L. (2003). Preparation, characterization, cytotoxicity and pharmacokinetics of liposomes containing docetaxel. Journal of Controlled Release, 91(3), 417-429.
7. Gabizon, A. A., Shmeeda, H., &Barenholz, Y. (2003). Pharmacokinetics of pegylated liposomal Doxorubicin: Review of animal and human studies. Clinical Pharmacokinetics, 42(5), 419-436.
8. Dhar, S., Gu, F. X., Langer, R., Farokhzad, O. C., &Lippard, S. J. (2008). Targeted delivery of cisplatin to prostate cancer cells by aptamer functionalized Pt(IV) prodrug-PLGA–PEG nanoparticles. Proceedings of the National Academy of Sciences, 105(45), 17356-17361.