Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

# Biomimetic hybrid Liposome–Exosome nanoparticle: Shining a light on Theranostic agents in Breast cancer — A Systematic Review

**Zahrasadat Mehrabi**
Islamic Azad University of Medical Sciences
**Abdulwahab Teflischi Gharavi**
Kharazmi University
**Seyyed Mohammad Reza Mehrabi**
Pasteur Institute of Iran
**Saeed Irian**
Kharazmi University
**Mohammad Ali Shokrgozar**
shokrgozar1967@gmail.com

Pasteur Institute of Iran

**Research Article**

**Keywords:** Breast cancer, Nanotechnology, Drug delivery systems, Hybrid, Exosome, Liposome, Systematic review

**Posted Date:** July 8th, 2026

**DOI:** https://doi.org/10.21203/rs.3.rs-10218836/v1

**License:** This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

**Additional Declarations:** No competing interests reported.

# Biomimetic hybrid Liposome–Exosome nanoparticle: Shining a light on Theranostic agents in Breast cancer — A Systematic Review

Zahrasadat Mehrabi[1], Abdulwahab Teflischi Gharavi[2], Seyyed Mohammad Reza Mehrabi[3], Saeed Irian[2], Mohammad Ali Shokrgozar[4*]

[1] Faculty of Pharmacy, Islamic Azad University of Medical Sciences, Tehran, Iran

[2] Department of Cell and Molecular Sciences, Faculty of Biological Sciences, Kharazmi University, Tehran, Iran

[3] Nanobiotechnology Department, Pasteur Institute of Iran, Tehran, Iran

[4*] National Cell Bank of Iran, Pasteur Institute of Iran, Tehran, Iran

[*] Corresponding author

shokrgozar1967@gmail.com

## Abstract

Conventional chemotherapy for breast cancer is often constrained by systemic toxicity, limited tumor specificity, and uneven drug distribution. Exosome–liposome hybrid nanoplatforms have explored as a promising alternative approach by combining the natural biological functions of exosomes with the tunable structure of liposomes. Herein, a systematic review was carried out in accordance with PRISMA guidelines, searching through Web of Science, PubMed, and Scopus databases. Our assessment focused on the design, characterization, therapeutic efficacy, and overall quality of these hybrids in breast cancer therapy. The reviewed studies showed that exosome–liposome hybrid nanovesicle enhance cellular uptake, tumor targeting ability, enable lysosomal escape, stability, drug loading capacity, and support multimodal therapeutic effects including chemotherapy, phototherapy, and immunomodulation. Of note, in vivo studies in mouse models reported meaningful tumor suppression, prolonged survival rate, reduced metastasis, and a favorable safety profile. However, several limitations were observed including poor reporting of experimental controls, inconsistent hybridization and cargo-loading protocols, and a lack of long-term stability data which hinder comparability and slow down the clinical translation. Overall, exosome–liposome hybrid nanovesicle represent a biologically synergistic and versatile platform for breast cancer treatment. Notably, advancing these systems into clinical trials will require standardized methods, rigorous bias control, thorough pharmacokinetic and immunological studies, and scalable manufacturing processes that comply with Good Manufacturing Practice (GMP) standards.

**Keywords:** Breast cancer, Nanotechnology, Drug delivery systems, Hybrid, Exosome, Liposome, Systematic review

## 1. Introduction

Breast cancer, as the most important women health issue, develops due to abnormal breast cells dividing[1]. Today, the majority of chemotherapeutic medicines clinically recruited for breast cancer therapy are delivered through a target-off mechanism resulting in an ineffective drug delivery toward the breast cancer cells eventually leading to serious chemotherapy side effects and several non-specific toxicities [2, 3].

The clinical application of anti-tumor chemotherapy drugs is challenged by factors such as tumor heterogeneity and physiological barriers[4, 5].Although researchers have made significant efforts to design and fabricate diverse carriers to overcome specific physiological barriers, the development of carriers capable of effectively delivering anticancer agents continues to present considerable challenges [6]. In this context, using nano-scaled delivery systems may enhance drug accumulation in tumor site[7]. However, Traditional nanocarriers exhibit limited specificity, suboptimal biodistribution, and inadequate biocompatibility, making them susceptible to immune system recognition and rapid clearance. Notably, lack of precise targeting mechanisms can limit their therapeutic efficacy[9 ,8].

Exosomes as a subclass of extracellular vesicles are membranous vesicles composed of various lipids such as cholesterol, proteins including tetraspanins, diverse range of nucleic acids such as microRNAs, siRNAs, also exosomes surface decorated with myriad agent like growth factors[10-12] The terminology used to describe them reflects their biogenesis, specifically their origin from endosomal compartments, and typically measure less than 200 nanometers in diameter [13]. Significantly, these nanoscale vesicles may serve as more effective delivery platforms due to their inherent characteristics, lower toxicity, targeted delivery capabilities, protection of the cargo, enhanced stability within the bloodstream, and improved endosomal escape [14-16]

Although the utilization of exosomes as delivery vehicles offers numerous advantages, most existing exosome-loading techniques are hindered by low encapsulation efficiency, limited loading capacity, fast clearance rate, heterogeneity in biomarker composition, storage instability ,and limited production scalability[17]. thereby complicating the attainment of clinically relevant drug dosages [18]. Even achieving a loading capacity of 10%,requires an impractically large quantity of exosomes to deliver standard therapeutic doses [19]. Furthermore, current loading methodologies often fail to accommodate large biomolecules effectively, as the creation of transient pores or membrane disruption can compromise or destroy the vesicular structure[20]. For instance, approaches such as electroporation frequently lead to cargo aggregation around the exosomes rather than genuine intravesicular loading, underscoring the challenges associated with achieving consistent and controlled encapsulation[21].

On the other hand, liposome as a synthetic nanocarrier have been studied as an effective tool for encapsulating and delivering molecules in the treatment of various diseases including breast cancer. Liposomes are mainly composed of phospholipids which are

molecules consisting of a hydrophilic head and hydrophobic tail groups. This amphiphilic feature makes liposomes as an ideal drug carrier for both aqueous and lipophilic agent. Liposome size ranges vary from 20 nanometers to 2.5 micrometers. They may consist of one or several concentric or non-concentric bilayer membranes. In addition, liposomes are widely used mainly because of their biocompatibility, stability, ease of synthesis, high drug-loading efficiency, enhanced bioavailability, and the safe excipients utilized in their structure [22, 23] [24-26].

The emergence of nanotechnology has created significant opportunities in drug delivery; however, drug delivery still encounters challenges. As synthetic nanoparticle including liposome go through the bloodstream to its target, they face myriad physiological and cellular barriers to eliminate foreign molecules from the body. In this regard, some obstacles that nanoparticle experienced through body include rapid clearance of synthetic nanoparticles by the immune system, accumulation in organs like kidney reduce their uptake by cancerous cells, and some of its materials may cause potential toxicity. Of note, this toxicity can limit the clinical application of nanoparticles. Therefore, researchers still face barriers in developing liposomes effectively as drug-delivery systems in clinical settings [27-29].

Interestingly, hybridization of exosomes with synthetic nanoparticles through hybridization techniques has led to the development of innovative drug delivery systems, specifically exosome-based hybrid systems[30]. Hybrid nanoparticles composed of exosomes and liposomes combine the inherent properties of both vesicle that signify an advancement in nanomedicine[31]. In fact, by combining the properties of both nanoparticles, a new type of nanoparticle for drug delivery is created; not only offers high drug-loading capacity, stability, and the ability to modify surface features such as surface charge and targeting ability, but also shows excellent biocompatibility, higher cellular uptake, and low immunogenicity. Altogether, it provides a new insight into nanoparticle-based drug delivery systems[29, 32].

The exosomal component has biological functionalities, including biocompatibility, targeted cellular recognition and internalization, on the other hand, the liposome provides a stable vesicle with the capacity for controlled release of the cargo [31, 33]. Furthermore, exosomes possess intrinsic biological features such as cell-specific targeting ability, immunomodulatory effects, and the ability to traverse biological barriers, which collectively enhance the efficacy of these hybrid delivery systems[34, 35]

collectively, exosome-liposome hybrid nanoparticles have demonstrated a significant potential features in several biomedical sectors, including drug delivery, gene therapy, regenerative medicine, and molecular imaging[36].

Here in this study, we specifically aim to evaluate several critical aspects related to the use of exosome in breast cancer treatment. These include the quality assessment of both in vivo and in vitro experiments, regarding the study design methodology, statistical analysis, and reporting quality. in addition, the stability of exosomes in relation to the storage

condition, the selection of animal models and their relevance to breast cancer, as well as the source, isolation, and characterization techniques of exosomes is examined. The investigation further extends to the properties of liposomes, including their synthesis methods, lipid molar ratios, storage conditions, and characterization approaches. Moreover, the study explores the formation of exosome-liposome hybrids, focusing on the exosomes to liposomes ratio, the nature of their cargoes, cargo loading methods, and the techniques employed for hybrid characterization and validation. Finally, the therapeutic potential of the exosome-liposome complexes in breast cancer is critically assessed. By addressing these specific components, this systematic review offers a comprehensive and updated overview of the therapeutic applications of exosome-liposome hybrids in the context of breast cancer treatment.

Of note, the table below compares the features that each nanoparticle has individually [27, 29, 32, 37, 38]

| Feature | Liposome | Exosome | Exosome–Liposome Hybrid |
|---|---|---|---|
| Targeting ability | Limited; depends on ligand engineering | Natural targeting ability | Combination of engineering and intrinsic targeting |
| Encapsulation efficiency | High Encapsulation efficiency | Limited Encapsulation capacity | Improved Encapsulation capacity |
| Biocompatibility and Immunogenicity | may induce immune responses | High biocompatibility and low immunogenicity | High biocompatibility with reduced immunogenicity |
| Stability | Moderate stability | High stability in biological fluids | Enhanced stability |
| Production and Scalability | Relatively simple and scalable | Complex isolation and purification processes | Moderate complexity with improved scalability |
| Surface Modification | Easy surface modification | Limited engineering flexibility | Flexible surface engineering |
| Cellular Uptake Efficiency | Moderate uptake | High cellular uptake | Enhanced uptake efficiency |

## 2. Methods

### 2.1. Systematic review and criteria for exclusion and inclusion data

This systematic review was organized according to the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines [39]. On 31 July 2025 a survey was

conducted using the Web of Science, PubMed, and Scopus databases as bibliographic datasets. The search queries used were “exosomes” AND “liposome” AND “hybrid” AND “breast cancer”, which were derived from several relevant published studies and the Medical Subject Headings [40] Database. The articles retrieved from the three databases were pooled and imported to EndNote, where duplicates were removed. Initially, abstracts were retrieved, and then a refined list was generated based on predefined inclusion or exclusion criteria. Next, the related full texts were downloaded and assessed for eligibility. Criteria were utilized in a manner that included only original research publications written in English, with the search keywords in both the title and the abstract. In the first stage, the entire existing reports on the application of exosome-liposome hybrid for breast cancer therapy were included. At this stage, reports that were not original such as reviews, letters, commentaries, conference proceedings and Book chapters, were excluded. Additionally, non-peer-reviewed articles and non-English articles were also excluded. At last, for final included articles, both in vitro and in vivo experiments were included. Summary of this approach is reported as a flowchart in (**Figure 1**).

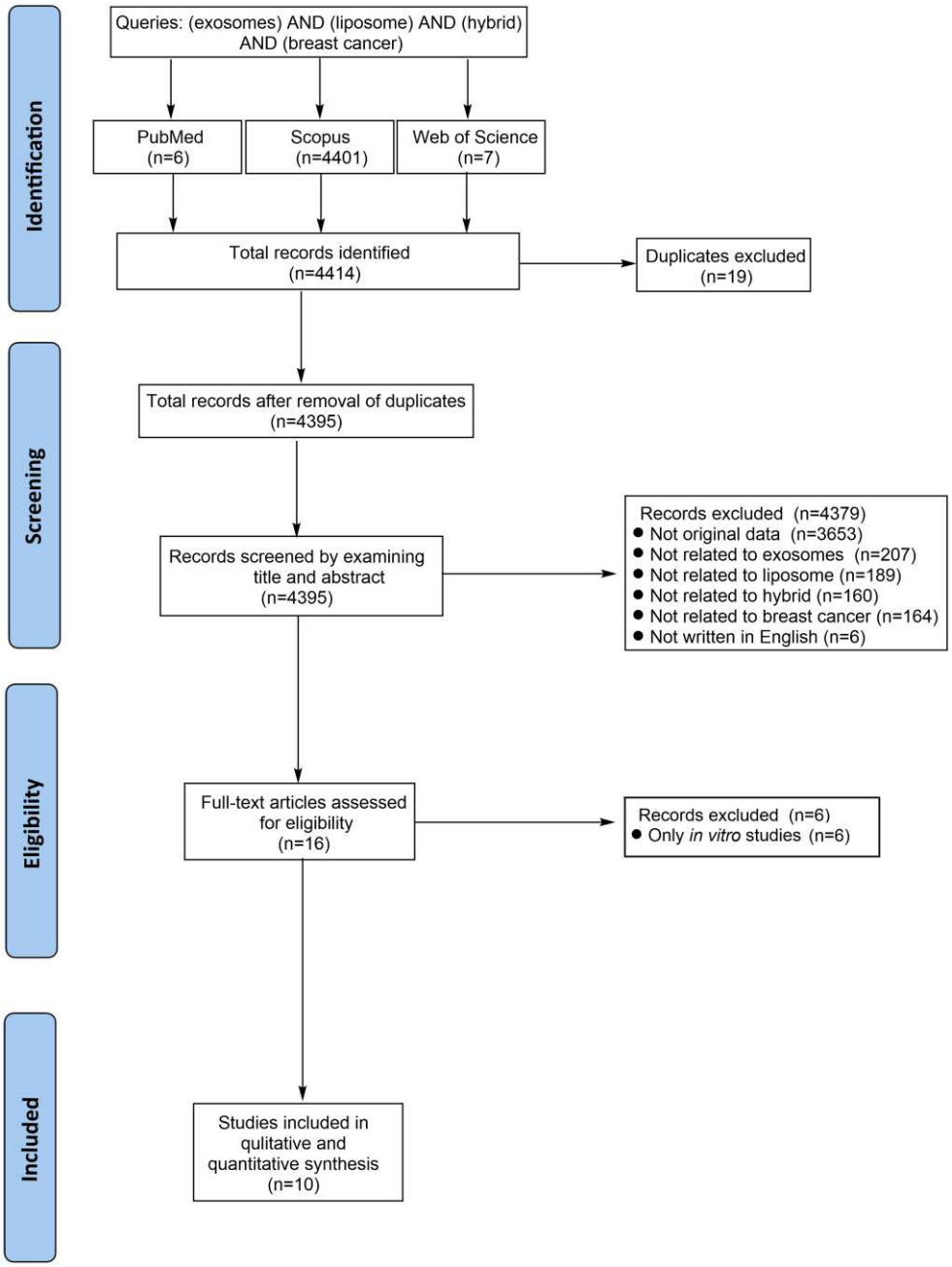



**Figure 1.** PRISMA diagram of systematic review

## 2.2. Quality assessment of studies

The gathered studies were evaluated based on their study design, with particular attention on assessing the quality of in vivo experiments. Nevertheless, the precise nature of the in vitro studies necessitates the development of comprehensive guidelines to ensure their quality[41]. Furthermore, the risk of bias in the in vivo experiments reported in these articles was assessed using the Systematic Review Centre for Laboratory Animal Experimentation (SYRCLE) risk of bias assessment tool [42].

### 2.3. DATA collection sources

The study design and findings were independently extracted as data for this systematic review. Information was obtained from the main text, tables, figures, supplementary materials, and references to ensure comprehensive data collection.

The study design included comprehensive information regarding the animal models utilized, such as sex and species, age, disease model, weight, rout of administration and hybrid name. In addition, details regarding the exosomes, including the extraction and characterization methods, donor cell type, modifications, storage conditions, and cargo loading, were extracted. Moreover, liposome configurations such as their compositions, molar ratio, synthetic methods, liposome modifications, their cargoes and liposome characterization techniques were evaluated. Furthermore, details of exosome-liposome hybrid platforms including methods of hybridization, the ratio between exosomes and liposomes, hybrid cargoes, methods of characterization and validation of hybrid platforms were also studied in this systematic review.

Additionally, information regarding the stability and release conditions of hybrid platforms was gathered. Besides, all in vitro, in vivo, and ex vivo results were fully collected from the included studies. Following the analysis of these results, they were presented qualitatively in the tables.

## 3. Results

### 3.1. Study selection and analysis

All 4414 articles gathered according to our queries were consolidated into EndNote 2025.2 software. After excluding 19 duplicate papers, the titles and abstracts of the remaining 4,395 articles were examined. 4,379 publications were removed for their absence of original data, comprising books, reviews, and editorial pieces (n = 3,653), papers irrelevant to exosomes (n = 207), liposomes (n = 189), hybrids (n = 160), breast cancer (n = 5), and articles not authored in English (n = 6). The complete text of the remaining 16 papers was assessed for eligibility criteria, leading to the elimination of 6 publications that lacked either in vivo or in vitro data. A total of 10 reports were incorporated into this systematic review based on

the year of publication (**Figure 2A**), and the main country in which the studies were conducted (**Figure 2B**) [43-52].

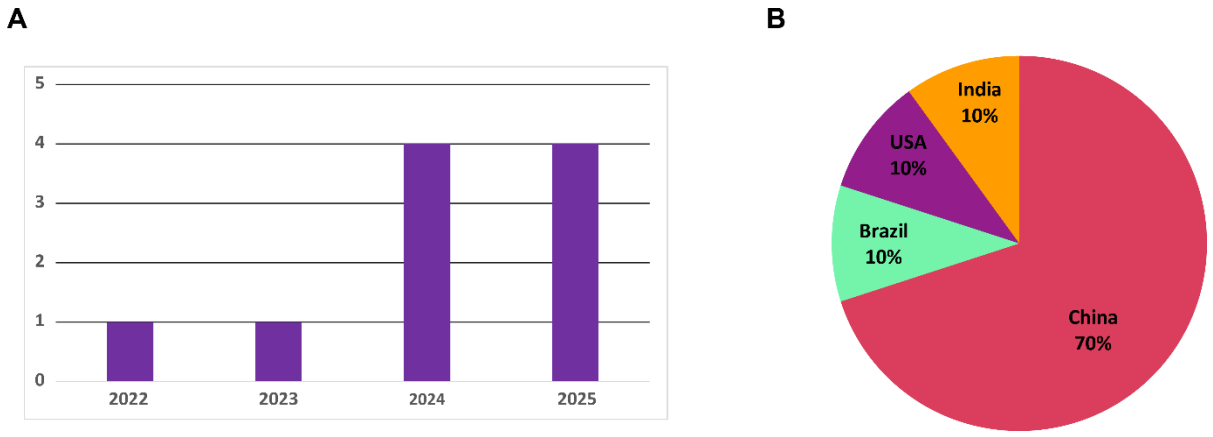



**Figure 2. Studies analysis.** Distribution of the publications based on the **A.** The year of the publication and **B.** The country of study

In addition, we summarized the details of the studies in Tables 1 through 5. The key features of the included research are outlined in **Table 1**, which highlights the variety of platforms and scientific techniques used to develop the exosome-liposome hybrid drug delivery systems for breast cancer treatments. Exosome sources for hybrid nanoplatforms were covered in **Table 2**, which also included the characterization method and the storage condition. **Table 3** describes the liposomal component of hybrid platforms. In **Table 4** we presented how the ten different research reports combined liposomes and exosomes to create hybrid nanovesicles. Finally, we included all of the information regarding the physical features, release, stability, and encapsulation efficiency of exosome-liposome hybrid systems in **Table 5**.

### 3.2. Reporting the quality of the *In vivo* experiments

In biomedical research, the reliability and validity of study outcomes are considered highly crucial for advancing scientific knowledge. Also, methodological biases and poor reporting can lead to skewed outcomes, misleading estimates, and hinder the reproducibility of studies. Thus, we assessed both outcomes and methodologies for in vivo sections as shown in **Table 6**.

All 10 studies provided essential details regarding animal models, such as age, gender, and species, indicating a low risk for baseline generation bias [43-52].

**a) Selection bias**

Selection bias was assessed through three items: random sequence generation, baseline characteristics, and allocation concealment [42]. All ten included studies reported similar baseline characteristics between groups and were therefore judged as low risk for this item[43-52]. Random sequence generation was described adequately in eight studies and rated low risk [43, 44, 46-51], whereas in the remaining two studies including Li 2025 and Verma 2025 it remained unclear[45, 52]. However, allocation concealment was not reported in any of the studies, resulting in an unclear risk rating across the entire set of studies [43-52].

**b) Performance bias**

Performance bias was evaluated by random housing and blinding of investigators[42]. None of the studies provided information on whether animals were housed randomly during the experiment that led to unclear risk for random housing in all ten studies[43-52]. Moreover, the blinding of personnel was not mentioned in any of the ten studies and thus remained as unclear risk [43-52] .

**c) Detection bias**

Detection bias was assessed via random outcome assessment and blinding of outcome assessors[42]. Random selection of animals for outcome assessment was clearly described and rated low risk in six studies[43, 46, 49-52]. However, this bias remained unclear in the remaining four studies[44, 45, 47, 48]. In addition, only Zhen et al. declared the blinding of outcome assessors in their work [44], while the remaining nine studies provided no such information and hence rated as unclear risk status for this bias[43, 45-52].

**d) Attrition bias**

All ten studies adequately addressed incomplete outcome data. Exclusions, animal losses, or missing data were either minimal or appropriately explained and accounted for in the analyses. Accordingly, attrition bias was judged as low risk across all included studies[43-52].

**e) Reporting bias**

There was no evidence of selective outcome reporting in any of the studies. All pre-specified primary and secondary outcomes listed in the methods sections were fully reported in the results, with no unexplained omission of outcomes. Reporting bias was therefore rated low risk for the ten studies, reflecting a high level of transparency in outcome reporting[43-52].

**f) Other biases**

According to the SYRCLE risk of bias tool, potential additional sources of bias such as conflicts of interest, inappropriate influence from funders, or major design flaws should be identified and reported in each study [42]. Only the studies by Guo et al. and Xu et al. were

assessed as having a low risk of other sources of bias [43, 50]. On the other hand, the three studies conducted by Zhen et al., Hu et al., and Verma et al. were judged to be at high risk due to reported conflicts of interest [44, 46, 52]. The remaining five studies either explicitly declared the absence of such issues or provided insufficient details in their disclosures; Consequently, the risk of other sources of bias in these studies was rated as unclear [43-52].

### 3.3. The Designing of the studies

The characteristics of the included studies were demonstrated in **Table 1**. As shown in the included studies, the exosomes which was used to form the hybrids came from several sources: macrophages [43-45], tumor cells [46-48, 50, 51], platelets [49] or human plasma [52]. As demonstrated in the titles of these studies, the hybrid formulations encompass a range of formation strategies, incorporating chemotherapeutics, photothermal /photodynamic agents, immunomodulatory compounds, and responsive nanomaterials designed for enhanced therapeutic performance[43-52].

Animal model characteristics, including their strain, sex, age, weight, and routes of administration are systematically outlined. Seven studies employed female Balb/C mice aged 5–8 weeks with tail vein injections as the primary administration route[43, 44, 46, 47, 49-51]. Although, Li et al utilized Balb/C mice with tail vein injections as the primary administration route, no mention of the age and sex of the mice was made[45]. In addition, Sulthana et al. employed 5-6 weeks female C57BL6 mice with tail vein injections as the primary administration route[48]. SD rats were utilized for systemic evaluation in the work of Verma et al [52].

For each study, the presence of in vitro and in vivo components is clearly specified[43-52]. In vitro investigations across the studies comprise detailed characterizations of hybrid nanovesicles, drug release kinetics, colloidal stability, intracellular uptake, ROS measurements, apoptotic markers, lysosomal escape, ICD evaluations, and assessments of cytotoxicity, migration, wound healing and biocompatibility. In addition, macrophage repolarization, photothermal and photodynamic performance, and immune activation assays such as dendritic cell maturation and cytokine expression were included as in vitro tests in the included studies [43-52].

The in vivo study parameters of breast tumor-bearing mice/rats models were also specified in **Table 1**. Administration strategies, dosing routes, and physiological conditions of the animals are consistently documented, and as mentioned above all of ten included studies administrated their hybrid system through the animal models vein tails [43-52]. The names of the exosome-liposome hybrid systems included REV@SR780Fe@LEV-RS17 [43], P-I@M1E/AAL [44], DOX@EDHPA [45], IG Exo-Lipo [46], HF-pHSL/EV-DOX [47], LEV [48], DCHL [49], E-cLip-DTX/si [50], TEX-Liposome-PTX [51], and MB-EXO-ICG-LIPO-VCs/ PLB-EXO-LIPO-VCs [52], with each representing tailored designs aimed at improving tumor targeting, drug delivery efficiency, and multimodal therapeutic outcomes.

**Table 1.** The main designing of the included studies

| First author | Title of study | In vitro study | In vivo study | | | | | Cell line | Hybrid name |
|---|---|---|---|---|---|---|---|---|---|
| | | | model | sex | age | weight | Rout of administration | | |
| Yawen Guo[43] | Acidity-activatable dynamic hybrid nanoplatforms derived from extracellular vesicles of M1 macrophages enhance cancer immunotherapy through synergistic triple immunotherapy | Characterization of hybrid- drug release profile-cellular uptake-Measurement of ROS production-Examination of lipid peroxidation-Detection of mtDNA escape-cell viability assays-examination of ICD[1]-activation of DCs[2]-analysis of macrophage repolarization-Western blot | Balb/C | F | 6 weeks | 20-22 g | Tail vein | BMDCs[3]-RAW264.7-4T1-HUVECs-L929-L-02-MCF-7-MDA-MB-231 | REV@ SR780Fe@LEV-RS17 |
| Xueyan Zhen [44] | Biointerface-Engineered Hybrid Nanovesicles for Targeted Reprogramming of Tumor Microenvironment | Characterization of hybrid-evaluartion of the stability and hemolysis of hybrid-evalutaion of the Photothermal and Photodynamic Performance effect-Cellular Internalization- | Balb/C | F | 6 weeks | 18 g | Tail vein | 4T1 | P-I@M1E/AAL |

[1] immunogenic cell death
[2] dendritic cells
[3] Bone marrow-derived dendritic cells

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Macrophage Repolarization- evaluation of the Anticancer and Hypoxia - | | | | | | | |
| Yunyan Li [45] | Construction of biomimetic hybrid nanovesicles based on M1 macrophage-derived exosomes for therapy of cancer | Characterization of hybrid- measurement- colloidal stability assay- drug release kinetic- Phagocytosis of tumor cells cytotoxicity assay- ICD examination | Balb/C | N/R | N/R | ~ 19 g | Tail vein | Raw264.7- 4T1 | DOX@EDHPA |
| Jiawei Hu [46] | An engineered-exosome disguised multifunctional nanoplatform for enhanced chemo-photothermal therapy of triple-negative breast cancer | Characterization of hybrid- drug release profile-evaluation of the photothermal efficiency- Cytotoxicity assay- Cellular uptake assay- Apoptosis assay- Intracellular ROS generation- Expression of HSP90- | Balb/C | F | 6-8 weeks | N/R | Tail vein | 4T1 | IG Exo-Lipo |
| Luiza Marques Paschoal Barbosa [47] | Hybrid Nanosystem Formed by DOX-Loaded Liposomes and Extracellular Vesicles from MDA-MB-231 Is Effective against Breast Cancer Cells with Different Molecular Profiles | Characterization of hybrid- Storage Stability- Release Study- Sulforhodamine B Assay- Cellular Uptake- Migration Test- Wound-Healing Assay- | Balb/C | F | 6-7 weeks | 18 g | Tail vein | MDA-MB-231- MCF7- SKBR3 | HF-pHSL/EV-DOX |
| Shoukat h Sulthana [48] | Maximizing liposome tumor delivery by hybridizing with tumor- | Characterization of hybrid- stability test-Cellular uptake study- | C57BL6 | F | 5-6 weeks | ~ 25 g | Tail vein | 4T1- J774A.1 | LEV |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | derived extracellular vesicles | Biocompatibility assay- cytokine release assay- | | | | | | | |
| Shipeng Ning [49] | Platelet-derived exosomes hybrid liposomes facilitate uninterrupted singlet oxygen generation to enhance breast cancer immunotherapy | Characterization of hybrid- Photodegradation of DPBF[4] for ROS detection- drug release profile- invitro targeting ability- Mitochondrial integrity assay- Intracellular H2O2 Detection- Intracellular Uninterrupted ROS Generation- Induction of immunologic cell death (ICD)c- anticancer effect | Balb/C | F | 5-6 weeks | N/R | Tail vein | 4T1 | DCHL |
| Minhao Xu [50] | ROS-Responsive Biomimetic Nanocomplexes of Liposomes and Macrophage-Derived Exosomes for Combination Breast Cancer Therapy | Characterization of hybrid- Intracellular ROS Detection- evaluation of the Escape Macrophage Uptake- Cellular Uptake-evaluation of the Lysosomal Escape- Cell Viability assay- Cell Cycle Arrest and Apoptosis assay- release profile | Balb/C | F | 5-6 weeks | N/R | Tail vein | RAW264.7-4T1 | E-cLip-DTX/si |
| Haiqin Huang [51] | Synergistic strategy with hyperthermia therapy-based immunotherapy | Characterization of hybrid-evaluation of the | Balb/C | F | 5-6 weeks | N/R | Tail vein | 4T1- NIH 3T3 - HUVEC | TEX-Liposome-PTX |

[4] 1, 3-Diphenylisobenzofuran

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | and engineered exosomes liposomes targeted chemotherapy prevents tumor recurrence and metastasis in advanced breast cancer | biocompatibility- antitumor and Photothermal assays- cellular uptake and targeting ability- release profile | | | | | | | |
| Nidhi Verma [52] | Exosomes fused liposomes: formulation, stability studies and theranostic evaluation for breast cancer applications | Characterization of hybrid- Storage stability studies- release profile- cytotoxicity assay- Cellular uptake study- Intracellular ROS generation analysis- Apoptosis assessment- Caspase-3/7 activity assay- Safety evaluation | SD [5]rat | F | 60-70-days | 200-220 g | Tail vein | MCF7 | 1. MB-EXO-ICG-LIPO-VCs<br>2. PLB-EXO-LIPO-VCs |

[5] Sprague-Dawley

### 3.4. The exosome of exosome-liposome hybrid nanoplatform

A comprehensive overview of the source and preparation methods of exosomes employed in the construction of exosome–liposome hybrid nanoplatforms for breast cancer therapy across all 10 included studies are presented in **Table 2**[43-52].

The donor cell sources are diverse, in detail, M1-polarized macrophages were employed as exosome source of three studies[43-45]. Hu et al., Sulthana et al. and Huang et al. isolated their exosomes from murine 4T1 breast cancer cells [46, 48, 51]. In addition, human triple-negative breast cancer MDA-MB-231 cell derived exosomes were used by Barbosa et al. [47]. Xu et al. employed RAW264-derived exosomes to perform their delivery system[50]. Finally, Ning et al and Verma et al. utilized platelet- and human plasma- derived exosomes, respectively[49, 52].

Ultracentrifugation remained the most widely adopted isolation technique among all exosome isolating methods, here used by six studies[43-45, 49-51]. While PEG-based precipitation methods were utilized by Barbosa et al. and Verma et al. [47, 52], ultrafiltration was applied by Sulthana and colleagues to isolate their exosomes [48]. Finally, Hu et al mentioned that their exosomes were isolated by a commercial quick exosome isolation kit[46].

The majority of the reported exosomes were used in their native, unmodified form without genetic or cellular engineering [43-45, 47-52]. However, a notable exception was a study that genetically engineered 4T1-derived exosomes with SIRPα knockdown to improve immune evasion and tumor targeting [46].

Additionally, in most studies, exosomes were not pre-loaded with therapeutic cargo prior to hybridization with liposomes; instead, functional molecules were typically incorporated into the final hybrid nanoparticles or liposomes[43-51]. The only clear instance of direct cargo loading into exosomes was reported by Verma et al. in which, human plasma-derived exosomes were loaded with methylene blue and/or the CDK4/6 inhibitor palbociclib[52].

Characterization of isolated exosomes generally involved a combination or employing one of transmission electron microscopy (TEM), dynamic light scattering (DLS), nanoparticle tracking analysis (NTA), Western blotting or SDS-PAGE for exosomal markers, and BCA or Bradford assays for protein quantification of exosomes[43-45, 47-52]. Additionally, Verma et al utilized scanning electron microscopy (SEM) and scanning probe microscopy (SPM) for further characterization of exosomes[52], while Barbosa et al. applied nano flow cytometry technique to characterize their exosomes[47]. Finally, Hu et al. did not clearly mention of the method used for exosome characterization [46].Long-term storage at −80 °C was explicitly reported in three studies[44, 48, 51], whereas the remaining studies did not specify storage conditions[43, 45-47, 49, 50, 52].

**Table 2.** The Exosome of hybrid nanoparticles

| First author | Donor cell type | Isolation method | Modification | cargo | Cargo loading | Characterization | storage |
|---|---|---|---|---|---|---|---|
| Yawen Guo[43] | $M_1$ macrophages | Ultracentrifugation | No | no | no | Western blotting | N/R |
| Xueyan Zhen [44] | $M_1$ macrophages | Ultracentrifugation | No | no | no | TEM-DLS- BCA protein quantification kit- western blot-ELISA | −80 °C |
| Yunyan Li [45] | $M_1$ macrophages | Ultracentrifugation | No | no | no | TEM-DLS- Western blot- | N/R |
| Jiawei Hu [46] | 4T1 | quick exosome isolation kit | Yes (SIRP α gene engineered) | no | no | N/R | N/R |
| Luiza Marques Paschoal Barbosa [47] | MDA-MB-231 | PEG precipitation method | No | no | no | DLS-NTA-BCA Protein Assay Kit- Nanoflow Cytometry- | N/R |
| Shoukath Sulthana [48] | 4T1 | Ultrafiltration | No | no | no | DLS-Bradford assay- SDS PAGE- and immunoblotting experiments | −80 °C |
| Shipeng Ning [49] | Platelet | Ultracentrifugation | No | no | no | TEM- western blot- DLS | N/R |
| Minhao Xu [50] | RAW264.7 | Ultracentrifugation | No | no | no | TEM- PALS particle size potentiometer- BCA assay kit- Western blot- SDS-PAGE | N/R |
| Haiqin Huang [51] | 4T1 | Ultracentrifugation | No | no | no | SDS-PAGE-TEM-DLS | −80 °C |
| Nidhi Verma [52] | Human Plasma | PEG precipitation method | No | Methylene blue/ Palbociclib | incubation | DLS- SPM[6]- HR-TEM- HR-SEM- | N/R |

[6] Scanning probe microscopy

### 3.5. The liposome of exosome-liposome hybrid nanoplatform

Although Guo et al. did not clearly point out their liposomal composition [43], the majority of formulations include neutral or zwitterionic phosphatidylcholines such as soy lecithin, S100 PC, DPPC, DOPE, which combined with cholesterol for membrane rigidity and stability, dominate the compositions[44-52]. PEGylated lipids (DSPE-PEG2000) appear to confer stealth properties, while specialized lipids such as cationic DC-cholesterol, anionic DSPG, or pH-sensitive CHEMS are employed in specific cases to enable electrostatic interactions or endosomal escape(**Table3**)[53].

Thin-film hydration technique remains the most preferred synthesis method of liposome which used in seven studies, reflecting its simplicity, reproducibility, and scalability[44, 46-49, 51, 52]. Only one study employed a microfluidic chip approach, indicating that precision nanoparticle engineering is still emerging in this hybrid field. Notably, Guo et al. did not report of their synthesis method in their work[43].

Molar or mass ratios were reported in seven studies, which typically showed phospholipid-to-cholesterol ratios of 4:1 to 10:1, with PEG-lipids kept as ≤10 mol% of total liposomal compositions [44, 47-52].

Functional modifications of the liposomes are relatively uncommon among these studies [43, 46, 48, 49, 51, 52]. Most formulations are simple; however, targeted liposomes include AS1411 aptamer conjugation and anisamide sigma-receptor ligand incorporation which were recruited by Zhen et al and Li et al., respectively[44, 45]. In addition, Barbosa et al. and Xu et al used controllable responsive features such as pH-sensitive hydrazone-PEG or CHEMS and ROS-responsive chitosan-nanobubble complexes like CS-NBC [47, 50].

Theranostic cargoes, present in 6 studies [46, 48-52], consists primarily of passively encapsulated agents such as chemotherapeutic drugs including docetaxel [50] or paclitaxel [51]. Additionally, near-infrared photosensitizers including IR780 [46], DPDPy-CPO [49], or fluorescent/near-infrared dyes such as Alexa or DiR[48] and ICG [52] are utilized for imaging or phototherapy. Although, Guo et al, used REV+SR780Fe as a liposomal cargo, they did not report the loading method [43]. Notably, the remaining three studies used completely unloaded liposomes, implying that drug incorporation occurred predominantly after or during the hybridization step with exosomes [44, 45, 47].

Characterization of liposome relying almost exclusively on TEM for morphology and DLS for hydrodynamic diameter and polydispersity [43-45, 48-52]. Additional methods such as nanoflow cytometry, fluorescence microscopy, or phase-analysis light scattering appear only sporadically [44, 47, 50, 52]. It is worth noting that Hu et al. did not report on their characterization method [46]. Long-term storage conditions are universally unreported across all entries, representing a notable gap in translational documentation[43-52].

**Table 3.** Liposomal contents of hybrid nanoparticles

| First author | Lipid composition | Molar ratio | Synthesis method | Modification | cargo | Cargo loading | Characterization | Storage |
|---|---|---|---|---|---|---|---|---|
| Yawen Guo[43] | N/R | N/R | N/R | No | REV+SR780Fe | N/R | TEM-DLS | N/R |
| Xueyan Zhen [44] | Lecithin- cholesterol-DSPE-$PEG_{2000}$-Aapt | 4:1 | Thin-film hydration | AS1411 aptamer | No | No | Fluorescence microscope-TEM-DLS | N/R |
| Yunyan Li [45] | DSPE- Hyd-$PEG_{2000}$ -AA | N/R | N/R | pH-sensitive-target anisamide (AA) | No | No | TEM-DLS | N/R |
| Jiawei Hu [46] | cholesterol-$S_{100}$ PC | N/R | Thin-film hydration | No | MPEG-TK-IR780+GA | Passive loading | N/R | N/R |
| Luiza Marques Paschoal Barbosa [47] | DOPE- CHEMS-DSPE-$PEG_{2000}$ | 5.7:3.8:0.5 | Thin-film hydration | pH-sensitive | No | No | Nanoflow Cytometry-protein content | N/R |
| Shoukath Sulthana [48] | 1. DSPG-cholesterol<br>2. DSPG-DSPE-PEG cholesterol | 1. 70:30<br>2. 55:10:30 | Thin-film hydration | No | Alexa or DiR | Passive loading | DLS | N/R |
| Shipeng Ning [49] | DPPC-cholesterol | 27 mg:2.8 mg | Thin-film hydration | No | CPO-DPDPy | Passive loading | TEM-DLS | N/R |
| Minhao Xu [50] | soybean lecithin- DC-cholesterol | 8:1(mass ratio) | microfluidic chip | CS-NBC | DTX | Passive loading | PALS particle size potentiometer-TEM- SDS-PAGE | N/R |
| Haiqin Huang [51] | soy lecithin-cholesterol | 10:1 w/w | Thin-film hydration | No | PTX | Passive loading | DLS-TEM | N/R |
| Nidhi Verma [52] | DSPE-PEG 2000 -DPPC cholesterol | 1mg:8mg:7.7mg | Thin-film hydration | No | ICG | Passive loading | TEM,DLS,SAD,SPM,FTIR | N/R |

### 3.6. Exosome-liposome hybrid nanoplatform

**Table 4** provides a details of the hybridization technique employed to merge exosomes with liposomes for breast cancer across ten studies[43-52]. The predominant approach is membrane fusion method, which utilized in eight studies. These studies typically incorporate one or a combination of incubation [43, 51] or mechanical processes. Precisely, sonication was utilized in four studies[43-45, 52], freeze-thaw cycles in two studies[44, 46], and membrane extrusion employed in six studies[43-45, 49-51], to facilitate lipid bilayer intermixing and vesicle reassembly. Notably two studies utilized thin-film hydration-based hybridization method in which exosomes were incorporated during liposome formation through lipid film rehydration[47, 48].

Furthermore, liposome-to-exosome ratios are specified in only six studies, with a strong preference for 1:1 equivalence by volume, weight, particle number, or mass ratio, suggesting an optimal balance for integrating exosomal-liposomal hybrid delivery systems[43, 44, 47, 48, 52]. However, only one study explored variable protein-to-phospholipid mass ratios (1:5 to 1:100), indicating systematic optimization to achieve desired surface biomarker density for tumor targeting[51].

Post-hybridization modification of hybrid systems was infrequent, with only Guo et al., employing DSPE-PEG2000-maleimide-linked RS17 targeting peptide for functional enhancement of their exosome-liposome hybrid platform[43]. Additionally, theranostic cargoes were dominantly inherited only from the liposomal fraction in four studies[43, 46, 49, 51]. Verma et al., also mentioned that the theranostic cargoes were inherited from both liposomal and exosomal fraction into their nanoplatform[52]. On the contrary, Zhen et al. used post-insertion through sonication-extrusion method to insert IR780 photosensitizer and PFTBA as oxygen carrier into their engineered hybrid nanocarrier[44]. Moreover, Li et al., and Barbosa et al., utilized remote loading via pH gradients to load Doxorubicin into hybrid delivery systems[45, 47]. Finally, a single study combined both strategy form liposomal content and simple incubation for loading Bcl-2 siRNA into nanocomplexes of liposomes and macrophage-derived exosomes for breast cancer therapy[50].

Post-hybridization characterization of hybrid nanocarriers consistently emphasizes physical attributes. In this context TEM and/or DLS were employed in all ten studies for morphology and size distribution assessment of hybrid nanoplatforms[43-52]. Additionally, biochemical assessments, such as SDS-PAGE and immunoblotting assays for exosomal protein markers were commonly utilized in four studies[44, 48, 50, 51]. Moreover, advanced tools such as nanoparticle tracking analysis (NTA), nanoflow cytometry, high-resolution TEM/SEM, and scanning probe microscopy were used frequently across the selected studies (**Table 4**).

The successful merging of the hybrid nanoparticles were verified through a multi-method approach [54]. In this context, fluorescence resonance energy transfer (FRET) were utilized in four studies to confirm lipid mixing of the hybrid nanoparticles [44, 48-50]. Fourier-transform

infrared (FT-IR) spectroscopy were also employed in three studies for chemical bond analysis[44, 45, 52]. Li et al and Barbosa et al., also used Nanoflow cytometry to validate the fusion of liposome and exosome membranes[45, 47]. This is while Huang et al., validated the hybridization by using confocal laser scanning microscopy (CLSM)[51]. In addition, SDS-PAGE/immunoblotting, and BCA assay were also utilized by three studies to verify protein transfer and validation of hybridization[44, 47, 48]. Finally, Guo et al., and Hu et al., did not report on the method used to validate the hybridization in their nanoplatforms[43, 46].

Overall, the storage conditions for the final hybrids were notably underreported, with only one study specifying a storage temperature of 4 °C [48].

**Table 4.** Hybrid nanoplatforms used in the selected studies**.**

| First author | Method of hybridization | Liposome/ exosome ratio | Modification | Cargo | Cargo loading | characterization | Method of validation | Storage |
|---|---|---|---|---|---|---|---|---|
| Yawen Guo[43] | membrane fusion technology (incubation, sonication, extrusion) | 1:1 v/v | Yes (DSPE-$PEG_{2000}$-Mal-RS17 peptide) | Inherited from liposome | No | TEM-DLS-(UV–vis) spectrometer - atomic absorption spectrometer | N/R | N/R |
| Xueyan Zhen [44] | membrane fusion technology (sonication-freeze-thaw circles-extrusions) | 1:1 W/W | No | IR780 PFTBA | Post-insertion loading (sonication–extrusion) | TEM-DLS- SDS-PAGE | FRET[7]-fluorescence microscope-FT-IR spectrometer-SDS-PAGE-Western blot | N/R |
| Yunyan Li [45] | membrane fusion technology (sonication, extrusion) | N/R | No | DOX | Remote loading | TEM-DLS | Nanoflow cytometry-FT-IR spectrometer- | N/R |
| Jiawei Hu [46] | membrane fusion technology (freeze-thaw circles) | N/R | No | Inherited from liposome | No | Flow Nano Analyzer-DLS-TEM | N/R | N/R |
| Luiza Marques Paschoal Barbosa [47] | Thin film hydration–based hybridization method | $7.99 \times 10^{10}$ particles 1:1 v/v | No | DOX | Remote loading | BCA -DLS-NTA Nanoflow -Assay Cytometry | BCA Assay, Nanoflow Cytometry | N/R |
| Shoukath Sulthana [48] | Thin film hydration–based hybridization method-extrusion | 1:1 number/number | No | DiR | N/R | Bradford assay-SDS PAGE-immunoblotting experiments-DLS | FRET, SDS-PAGE, immunoblot experiment | 4 °C |
| Shipeng Ning [49] | membrane fusion technology (extrusion) | N/R | No | Inherited from liposome | No | TEM-DLS | FRET | N/R |
| Minhao Xu [50] | membrane fusion technology (extrusion) | N/R | No | Inherited from liposome | Incubation (Bcl-2 siRNA) | PALS particle size potentiometer-TEM-SDS-PAGE | FRET | N/R |

[7] fluorescence resonance energy transfer

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | +Bcl-2 siRNA | | | | |
| Haiqin Huang [51] | membrane fusion technology (incubation and extrusion) | mass ratio of protein and phos pholipid of the prepared exosomes biomimetic liposome was 1:100, 1:20, and 1:5 | No | Inherited from liposome | No | DLS-TEM- SDS-PAGE- | CLSM | N/R |
| Nidhi Verma [52] | membrane fusion technology (Vortex[8]/Vortex and sonication[9]) | 1:1 | No | Inherited from liposome and exosome | No | DLS- SPM- HR-TEM- HR-SEM- | FT-IR spectrometer | N/R |

[8] MB-EXO-ICG-LIPO-VCs
[9] PLB-EXO-LIPO-VCs

### 3.7. Stability and functionality assessment of Exosome-Liposome Hybrid nanoplatforms

Across all ten included studies, the physicochemical stability of liposome, exosome, and hybridized nanovesicles was evaluated based on particle size, zeta potential, poly dispersity index (PDI), colloidal stability, drug release at different pH and encapsulation efficiency (**Table5**). Of note, all the ten studies had performed verification experiments to underscore the emerging field of exosome–liposome hybrid nanoplatforms, highlighting their application in breast cancer through diverse modalities such as immunotherapy, chemotherapy, photothermal/photodynamic treatments, and theranostics [43-52].

Within this body of work, Guo et al. described a hybrid system that exhibited strong ICG fluorescence and progressive cellular uptake, as confirmed by CLSM and flow cytometry. Moreover, the release and conversion of $Fe^{3+}$ to $Fe^{2+}$ resulted in marked GPX4 inhibition and a pronounced increase in intracellular lipid ROS, particularly under acidic conditions combined with irradiation. This oxidative stress activated the cGAS/STING pathway, promoted immunogenic cell death, suppressed 4T1 breast cancer cell proliferation, and produced the highest levels of 8-OH-dG. When evaluated in vivo, the same hybrid demonstrated efficient tumor targeting, substantial tumor growth inhibition, prolonged survival, enhanced apoptosis, robust antitumor immune activation, reduced risks of metastasis and recurrence, and a favorable biosafety[43].

A complementary immunomodulatory approach, reported by Zhen et al., showed a hybrid which was efficiently internalized by cells and actively repolarized M2-like macrophages toward an M1-like antitumor phenotype. Upon NIR808 irradiation, singlet oxygen and ROS production increased markedly, lysosomal escape was achieved, and apoptosis reached 54.50%, while hemolysis risk remained low. These effects translated into 4T1 tumor bearing mice that exhibited an effective tumor accumulation, irradiation-dependent tumor suppression, prolonged survival, sustained macrophage repolarization, and good biocompatibility[44]. In another study, Li et al. focused on immune engagement at the cellular level, wherein their hybrid enhanced phagocytosis, induced apoptosis, inhibited tumor cell proliferation, and redirected M2-like macrophages toward an antitumor phenotype, thereby strengthening immune responses, although the corresponding in vivo outcomes were not clearly described[45].

The therapeutic potential of photothermal and oxidative mechanisms was further illustrated by Hu et al., whose hybrid demonstrated effective tumor targeting in vitro, strong photothermal performance under NIR irradiation, efficient ROS generation, and pronounced tumor cell apoptosis and cytotoxicity. The in vivo outcomes showed a successful low-temperature photothermal therapy, with the highest levels of apoptosis and necrosis among treatment groups, while maintaining targeting capability[46].

Safety-oriented observations were emphasized by Barbosa et al.; Wherein they reported that the hybrid did not produce a significant cytotoxicity difference compared to other formulations. Although free DOX showed a higher cellular uptake, the hybrid exhibited the weakest wound-healing effect. Furthermore, in vivo results supported these findings, as biochemical parameters and histopathological assessments revealed no significant differences between groups, suggesting an acceptable level of safety[47].

Targeting performance and compatibility were highlighted by Sulthatna et al.; They confirmed through fluorescence imaging and flow cytometry, that their hybrid possessed clear targeting ability and maintained biocompatibility across a wide concentration range, while ex vivo results were not clearly reported[48]. Ning et al., experiments also confirmed cellular uptake and demonstrated mitochondrial damage, hydrogen peroxide generation, impairment of the electron transport chain, strong antitumor activity, and induction of immunogenic cell death. In vivo, these effects were accompanied by prolonged circulation, superior tumor targeting, reduced tumor burden, extended survival, suppression of secondary tumor growth, and the development of strong immune memory responses[49]. Xu et al. extended these findings by showing that their hybrid achieved excellent cellular uptake, reduced clearance by the mononuclear phagocyte system, enabled lysosomal escape, and supported ROS-responsive drug release, resulting in a stronger tumor inhibition than DTX. In vivo evaluation revealed enhanced tumor accumulation, favorable circulation profiles, reduced MPS uptake, effective tumor suppression without body-weight loss, increased apoptosis, decreased Bcl-2 expression, and absence of major organ damage[50].

The importance of formulation ratios was demonstrated by Huang et al., who reported that the targeting ability was significantly enhanced when the exosome-to-liposome ratio reached or exceeded 1/20, without inducing cytotoxicity and while maintaining high biocompatibility. In vivo outcomes also approved that an optimized formulation exhibited a pronounced lung-targeting behavior, with improved targeting and adhesion at higher ratios, although additional details were not provided[51]. Verma et al. presented a hybrid system that displayed higher intracellular fluorescence, elevated ROS production, stronger induction of apoptosis, mitochondrial-mediated apoptosis, and caspase activation in MCF-7 cells compared to the controls, resulting in significant cytotoxicity. In vivo results also revealed that the application of hybrid nanoplatforms reduced histopathological alterations, demonstrated prolonged circulation and tumor-targeting behavior, decreased tumor size and angiogenesis, and maintained biochemical and hematological safety[52].

**Table 5.** Stability assessment of performed hybrid nanoplatform

| First author | Characterization | | | | | | | | | Stability | | | Release(pH=7.4) | | | Encapsulation efficiency | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Size(nm) | | | Zeta | | | PDI | | | | | | | | | | | |
| | Lip | Exo | H | Lip | Exo | H | Lip | Exo | H | Lip | Exo | H | Lip | Exo | H | Lip | Exo | H |
| Yawen Guo[43] | N/R | N/R | 123.8±2.8 | N/R | N/R | -16.7±0.7 | N/R | N/R | 0.23±0.02 | N/R | N/R | stable | N/R | N/R | ≤22.5%(48h) | N/R | N/R | N/R |
| Xueyan Zhen [44] | 92.20 | 95.84 | 138.60 | -7.36 | -13.72 | -29.83 | N/R | N/R | N/R | Stable | N/R | Stable | N/R | N/R | N/R | N/R | N/R | 87.51±1.49% |
| Yunyan Li [45] | 98.15±0.239 | 167±1.206 | 122.4±0.608 | -15.57±0.851 | -33.43±2.108 | -18.53±0.611 | 0.201±0.011 | 0.2±0.008 | 0.167±0.007 | stable | N/R | stable | ≤50% | N/R | ≤50%(24h) | 75.51±0.90 | 46.14±0.96 | 83.45±1.15 |
| Jiawei Hu [46] | N/R | N/R | 156 ± 2 | N/R | N/R | 3 ± 1 | N/R | N/R | N/R | N/R | N/R | N/R | N/R | N/R | 60.74 ± 6.48 % (336h) | N/R | N/R | IR780: 96.89 ± 1.19 % GA: 93.27 ± 1.87 % |
| Luiza Marques Paschoal Barbosa [47] | N/R | 181.6 ± 11.5 | 140.2 ± 2.7 | N/R | N/R | −8.2 ± 1.5 | N/R | N/R | 0.102 ± 0.033 | N/R | N/R | stable | N/R | N/R | 29.2±5.7%(24h) | N/R | N/R | 88.9 ± 2.4 |
| Shoukath Sulthana [48] | 120±6 | 10-50 | 140±5 | -31±0.8 | -15±0.3 | -36±0.6 | N/R | N/R | 0.145 ± 0.02 | stable | N/R | stable | N/R | N/R | N/R | N/R | N/R | N/R |
| Shipeng Ning [49] | ~90 | ~140 | ~70 | -8.2 | N/R | -15.3 | N/R | N/R | N/R | N/R | N/R | stable | N/R | N/R | ≤30%(48h) | N/R | N/R | DPDPY=82.3% CPO=34.7% |
| Minhao Xu [50] | 55-80 | 70-95 | 110 | 36 | -9 | 24 | N/R | N/R | N/R | N/R | N/R | stable | N/R | N/R | N/R | 96.5±2.6% | N/R | 91.1±1.34% |
| Haiqin Huang [51] | 95.77 ± 0.54 | 106.63±1.24 | 120.64 ± 1.66<br>123.82 ± 1.08<br>133.41 ± 2.50 | -26.69 ± 1.17 | -37.45±1.53 | -30.30 ± 0.19<br>-35.01 ± 2.41<br>-40.80 ± 1.83 | 0.067 ± 0.009 | 0.145 ± 0.028 | 0.115 ± 0.048<br>0.186 ± 0.043<br>0.228 ± 0.021 | N/R | N/R | N/R | ~70% | N/R | ~70% | 97.58±1.36% | N/R | ~97% |
| Nidhi Verma [52] | 141.5 ± 1.5 | 30.2 ± 3.4 | MB:175±2.1<br>PLB:194.5±2.8 | -32.34±0.06 | -22±0.05 | MB: -28.33±0.04<br>PLB: -30.31±0.05 | 0.120±0.01 | 0.103 ± 0.01 | MB:0.225±0.02<br>PLB: 0.234±0.01 | Stable | Low stable | stable | N/R | ≤50%(24h) | ≤50%(24h) | 97.11±0.56% | MB-EXO:80.91 ± 0.60<br>PLB-EXO:75.45 ± 0.23 | MB:78.91 ± 0.17 ICG: 94.06 ± 0.02 PLB: 86.70 ± 0.21 |

**Table 6.** SYRCLE risk of bias assessment tool was employed to assess risk of bias

| First author | Year | Selection bias | | | Performance bias | | Detection bias | | Attrition bias | Reporting bias | Other |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Sequence generation | Baseline generation | Allocation concealment | Random housing | Blinding | Random outcome assessment | Blinding | Incomplete outcome data | Selective outcome reporting | Other sources of bias |
| Yawen Guo[43] | 2024 | Low Risk | Low Risk | Unclear Risk | Unclear Risk | Unclear Risk | Low Risk | Unclear Risk | Low Risk | Low Risk | Low Risk |
| Xueyan Zhen [44] | 2024 | Low Risk | Low Risk | Unclear Risk | Unclear Risk | Unclear Risk | Unclear Risk | Low Risk | Low Risk | Low Risk | High Risk |
| Yunyan Li [45] | 2025 | Unclear Risk | Low Risk | Unclear Risk | Unclear Risk | Unclear Risk | Unclear Risk | Unclear Risk | Low Risk | Low Risk | Unclear Risk |
| Jiawei Hu [46] | 2025 | Low Risk | Low Risk | Unclear Risk | Unclear Risk | Unclear Risk | Low Risk | Unclear Risk | Low Risk | Low Risk | High Risk |
| Luiza Marques Paschoal Barbosa [47] | 2024 | Low Risk | Low Risk | Unclear Risk | Unclear Risk | Unclear Risk | Unclear Risk | Unclear Risk | Low Risk | Low Risk | Unclear Risk |
| Shoukath Sulthana [48] | 2024 | Low Risk | Low Risk | Unclear Risk | Unclear Risk | Unclear Risk | Unclear Risk | Unclear Risk | Low Risk | Low Risk | Unclear Risk |
| Shipeng Ning [49] | 2023 | Low Risk | Low Risk | Unclear Risk | Unclear Risk | Unclear Risk | Low Risk | Unclear Risk | Low Risk | Low Risk | Unclear Risk |
| Minhao Xu [50] | 2025 | Low Risk | Low Risk | Unclear Risk | Unclear Risk | Unclear Risk | Low Risk | Unclear Risk | Low Risk | Low Risk | Low Risk |
| Haiqin Huang [51] | 2021 | Low Risk | Low Risk | Unclear Risk | Unclear Risk | Unclear Risk | Low Risk | Unclear Risk | Low Risk | Low Risk | Unclear Risk |
| Nidhi Verma [52] | 2025 | Unclear Risk | Low Risk | Unclear Risk | Unclear Risk | Unclear Risk | Low Risk | Unclear Risk | Low Risk | Low Risk | High Risk |

## 4. Discussion

This systematic review provides a comprehensive evaluation of exosome–liposome hybrid nanoplatforms for breast cancer therapy based exclusively on ten different selected original articles published during 2022 -2025. Collectively, these studies show that combining naturally derived exosomes with synthetic liposomes can be strategically impressive method to overcome the challenges of the traditional nanocarriers, especially regarding tumor targeting, immune system modulation, and the integration of multiple therapeutic functions[43-52].

**Hybridization effect**

Across the included studies, hybrid nanovesicles consistently showed better cellular uptake, increased tumor targeting, and therapeutic effectiveness compared to the free drugs or the non-hybrid vesicle. The role of exosomes was clear to target specific cell types, enhance immune system interaction, and improve intracellular processes such as escaping the lysosomes. At the same time, the liposomal part contributed structural stability, allowed for versatile drug loading, and in many instances, enabled release triggered by specific stimuli.

**Therapeutic efficacy**

Therapeutically, the hybrids covered a wide range of treatment methods, such as chemotherapy, photothermal therapy, photodynamic therapy, immunotherapy, and the combined theranostic approaches. Many hybrid showed effects such as ROS-induced apoptosis, mitochondrial dysfunction, GPX4 inhibition, and activation of antitumor immune responses. A common observation was the repolarization of macrophages toward an M1-like phenotype and the induction of immunogenic cell death, especially in macrophage-derived or engineered exosome systems. In vivo studies, mainly using 4T1 tumor-bearing Balb/C mice, demonstrated significant tumor growth suppression, extended survival, decreased metastatic potential, and low systemic toxicity. Notably, several studies reported maintained body weight and no major organ damage, indicating preliminary biosafety.

**Reporting quality**

Despite these impressive outcomes, critical methodological limitations were identified through SYRCLE-based risk of bias assessment. The evidence base in this field is limited by significant methodological weaknesses, including widespread unclear or high risk of bias in selection, performance, and detection domains. Although attrition and reporting biases were well controlled, the lack of information on allocation concealment poses a major risk of selection bias. Additionally, the absence of random housing and blinding measures raises serious concerns regarding performance bias, potentially leading to systematic differences in animal care favoring experimental groups. Furthermore, the frequent lack of assessor blinding and inconsistent random outcome assessment contribute to a moderate-to-high risk of detection bias, particularly affecting subjective measures like tumor volume in cancer models.

These limitations should be carefully considered when evaluating the reliability and strength of the preclinical findings.

In summary, the studies which were reviewed indicate that exosome–liposome hybrid nanoplatforms offer a biologically synergistic and technologically adaptable approach for breast cancer treatment, showing improved targeting ability, multimodal therapeutic potential, and an acceptable and proper short-term safety in preclinical models. However, challenges such as inconsistent methodologies, insufficient bias control, limited data on stability, and lack of clinical trials hinder their clinical translation. Moreover, progress in this area depends on establishing standardized experimental protocols, thorough pharmacokinetic and immunological assessments, and well-structured translational research to effectively connect experimental nanomedicine with clinical breast cancer therapy.

## 5. Conclusion

Exosome–liposome hybrid nanoplatforms have recently gained attention as a promising approach for breast cancer treatment. Research has shown that exosomes can be engineered for targeted drug delivery and imaging, Meanwhile, liposomes offer advantages such as structural flexibility, improved stability, and the ability to carry both hydrophilic and hydrophobic therapeutic agents at the same time. Exosomes are particularly valued for their natural biocompatibility and targeting features which makes them particularly attractive for biomedical use. However, several challenges remain unchanged, including limited long-term stability, difficulties in loading therapeutic cargo efficiently, poor retention in the body after administration, and obstacles to large-scale production for clinical use. Hybrid systems that merge exosomes with liposomal structures have shown encouraging early results. Nevertheless, translating these systems into clinical practice is hindered by the lack of standardized drug-loading protocols, limited preclinical evidence, and the need for production processes that comply with Good Manufacturing Practice (GMP) standards.

Looking ahead, further advances may come from integrating exosomes with functionalized liposomal structures. In this regard, these platforms could enable precise, non-invasive diagnostics and personalized treatment by combining therapies, imaging agents within a single system. Moreover, further investigative efforts, comprehensive comparative analyses, and rigorously designed clinical trials are imperative to fully harness the clinical potential of exosome–liposome hybrids in breast cancer.

## Author contributions

Zahrasadat Mehrabi: Conceptualization, Methodology, Writing, Abdulwahab Teflischi Gharavi: Writing, Visualization, Methodology, Seyyed Mohammad Reza Mehrabi: Reviewing Saeed Irian: Writing- Reviewing and Editing, Mohammad Ali Shokrgozar: Supervision

## Funding

The authors declared that financial support was not received for this work and/or its publication.

## Data availability

No datasets were generated during the current study.

# Declarations

## Ethics approval and consent to participate

Not applicable.

## Consent for publication

Not applicable.

## Competing interests

The authors declare no competing interests.

## Author details

1. Faculty of Pharmacy, Islamic Azad University of Medical Sciences, Tehran, Iran
2. Department of Cell and Molecular Sciences, Faculty of Biological Sciences, Kharazmi University, Tehran, Iran
3. Nanobiotechnology Department, Pasteur Institute of Iran, Tehran, Iran
4. National Cell Bank of Iran, Pasteur Institute of Iran, Tehran, Iran

# References

1. Samad, M.A., et al., *Breast Cancer: Molecular Pathogenesis and Targeted Therapy.* MedComm, 2025. **6**(10): p. e70404.
2. Li, Y., et al., *Cancer cell membrane coated PLGA nanoparticles as biomimetic drug delivery system for improved cancer therapy.* Colloids and Surfaces B: Biointerfaces, 2023. **222**: p. 113131.
3. Wang, J. and S.G. Wu, *Breast Cancer: An Overview of Current Therapeutic Strategies, Challenge, and Perspectives.* Breast Cancer (Dove Med Press), 2023. **15**: p. 721-730.
4. Zafar, A., et al., *Advancements and limitations in traditional anti-cancer therapies: a comprehensive review of surgery, chemotherapy, radiation therapy, and hormonal therapy.* Discov Oncol, 2025. **16**(1): p. 607.
5. Sunilkumar, M.M., et al., *Health-Related Suffering and Palliative Care in Breast Cancer.* Curr Breast Cancer Rep, 2021. **13**(4): p. 241-246.
6. Xiao, P., et al., *Milk Exosome-Liposome Hybrid Vesicles with Self-Adapting Surface Properties Overcome the Sequential Absorption Barriers for Oral Delivery of Peptides.* ACS Nano, 2024. **18**(32): p. 21091-21111.

7. Huang, D., et al. *Nanodrug Delivery Systems Modulate Tumor Vessels to Increase the Enhanced Permeability and Retention Effect*. Journal of Personalized Medicine, 2021. **11**, DOI: 10.3390/jpm11020124.
8. Bao, J., et al., *The Fate of Nanoparticles In Vivo and the Strategy of Designing Stealth Nanoparticle for Drug Delivery.* Curr Drug Targets, 2021. **22**(8): p. 922-946.
9. Min, H.Y. and H.Y. Lee, *Molecular targeted therapy for anticancer treatment.* Exp Mol Med, 2022. **54**(10): p. 1670-1694.
10. Gharavi, A.T., et al., *The role of macrophage subtypes and exosomes in immunomodulation.* Cell Mol Biol Lett, 2022. **27**(1): p. 83.
11. Kalluri, R. and K.M. McAndrews, *The role of extracellular vesicles in cancer.* Cell, 2023. **186**(8): p. 1610-1626.
12. Liu, Y.J. and C. Wang, *A review of the regulatory mechanisms of extracellular vesicles-mediated intercellular communication.* Cell Commun Signal, 2023. **21**(1): p. 77.
13. Welsh, J.A., et al., *Minimal information for studies of extracellular vesicles (MISEV2023): From basic to advanced approaches.* J Extracell Vesicles, 2024. **13**(2): p. e12404.
14. Gharavi, A.T., et al., *Harnessing exosomes as a platform for drug delivery in breast cancer: A systematic review for in vivo and in vitro studies.* Molecular Therapy Oncology, 2024. **32**(2).
15. Wessler, S. and N. Meisner-Kober, *On the road: extracellular vesicles in intercellular communication.* Cell Commun Signal, 2025. **23**(1): p. 95.
16. Wang, J., M.M. Barr, and A.M. Wehman, *Extracellular vesicles.* Genetics, 2024. **227**(4).
17. Moradi, Z., et al., *Unlocking the potential of exosomes: overcoming challenges in retention time, stability and targeting.* Nanomedicine, 2026: p. 1-19.
18. Ducrot, C., et al., *Hybrid extracellular vesicles for drug delivery.* Cancer Lett, 2023. **558**: p. 216107.
19. Chen, Z., et al., *Encapsulation and assessment of therapeutic cargo in engineered exosomes: a systematic review.* Journal of Nanobiotechnology, 2024. **22**(1): p. 18.
20. Hao, X., et al., *Exosomes as drug delivery systems in glioma immunotherapy.* Journal of Nanobiotechnology, 2024. **22**(1): p. 340.
21. Chen, T., et al., *Extracellular vesicles as vital players in drug delivery: a focus on clinical disease treatment.* Frontiers in Bioengineering and Biotechnology, 2025. **Volume 13 - 2025**.
22. Ye, Y.-T., et al., *Nanoarchitecting intelligently encapsulated designs for improved cancer therapy.* Frontiers in Bioengineering and Biotechnology, 2025. **Volume 13 - 2025**.
23. Paolino, D., et al., *Improved anti-breast cancer activity by doxorubicin-loaded super stealth liposomes.* Biomaterials Science, 2024. **12**(15): p. 3933-3946.
24. Fidan, Y., et al., *Recent advances in liposome-based targeted cancer therapy.* J Liposome Res, 2024. **34**(2): p. 316-334.
25. Nsairat, H., et al., *Liposomes: structure, composition, types, and clinical applications.* Heliyon, 2022. **8**(5): p. e09394.
26. Wang, S., et al., *Liposomes for Tumor Targeted Therapy: A Review.* Int J Mol Sci, 2023. **24**(3).
27. Chen, L., et al., *Recent progress in targeted delivery vectors based on biomimetic nanoparticles.* Signal Transduction and Targeted Therapy, 2021. **6**(1): p. 225.
28. Li, L., et al., *Exosome-liposome hybrid nanoparticle codelivery of TP and miR497 conspicuously overcomes chemoresistant ovarian cancer.* J Nanobiotechnology, 2022. **20**(1): p. 50.
29. Liu, A., et al., *Research progress in membrane fusion-based hybrid exosomes for drug delivery systems.* Frontiers in Bioengineering and Biotechnology, 2022. **Volume 10 - 2022**.
30. Yang, Q., et al., *Exosome-based delivery strategies for tumor therapy: an update on modification, loading, and clinical application.* Journal of Nanobiotechnology, 2024. **22**(1): p. 41.

31. Azimizonuzi, H., et al., *A state-of-the-art review of the recent advances of theranostic liposome hybrid nanoparticles in cancer treatment and diagnosis.* Cancer Cell International, 2025. **25**(1): p. 26.
32. Lu, J., et al., *Advanced strategies to evade the mononuclear phagocyte system clearance of nanomaterials.* Exploration (Beijing), 2023. **3**(1): p. 20220045.
33. Xie, X., et al., *Hybrid Exosome-Liposome Nanoparticles for Dual Modulation of Neuroinflammation and Lipid Metabolism in Ischemic Stroke.* ACS Nano, 2025. **19**(37): p. 33567-33586.
34. Wu, D., et al., *The blood–brain barrier: Structure, regulation and drug delivery.* Signal Transduction and Targeted Therapy, 2023. **8**(1): p. 217.
35. Kumar, M.A., et al., *Extracellular vesicles as tools and targets in therapy for diseases.* Signal Transduct Target Ther, 2024. **9**(1): p. 27.
36. Li, J., J. Wang, and Z. Chen, *Emerging role of exosomes in cancer therapy: progress and challenges.* Molecular Cancer, 2025. **24**(1): p. 13.
37. Alturki, M.S., et al., *A multi-stage computational pipeline for repurposing FDA-approved drugs: application to EGFR C797S-mutant NSCLC.* Front Chem, 2026. **14**: p. 1753911.
38. Large, D.E., et al., *Liposome composition in drug delivery design, synthesis, characterization, and clinical application.* Adv Drug Deliv Rev, 2021. **176**: p. 113851.
39. Page, M.J., et al., *The PRISMA 2020 statement: an updated guideline for reporting systematic reviews.* Bmj, 2021. **372**: p. n71.
40. Ramesh, P., R. Karuppasamy, and S. Veerappapillai, *Machine learning driven drug repurposing strategy for identification of potential RET inhibitors against non-small cell lung cancer.* Med Oncol, 2022. **40**(1): p. 56.
41. Tran, L., et al., *Quality assessment tools used in systematic reviews of in vitro studies: A systematic review.* BMC Med Res Methodol, 2021. **21**(1): p. 101.
42. Hooijmans, C.R., et al., *SYRCLE's risk of bias tool for animal studies.* BMC Med Res Methodol, 2014. **14**: p. 43.
43. Guo, Y., et al., *Acidity-activatable dynamic hybrid nanoplatforms derived from extracellular vesicles of M1 macrophages enhance cancer immunotherapy through synergistic triple immunotherapy.* Journal of Nanobiotechnology, 2024. **22**(1): p. 430.
44. Zhen, X., et al., *Biointerface-Engineered Hybrid Nanovesicles for Targeted Reprogramming of Tumor Microenvironment.* Advanced Materials, 2024. **36**(41): p. 2401495.
45. Li, Y., et al., *Construction of biomimetic hybrid nanovesicles based on M1 macrophage-derived exosomes for therapy of cancer.* Chinese Chemical Letters, 2024. **36**: p. 109942.
46. Hu, J., et al., *An engineered-exosome disguised multifunctional nanoplatform for enhanced chemo-photothermal therapy of triple-negative breast cancer.* Materials & Design, 2025. **251**: p. 113633.
47. Paschoal Barbosa, L.M., et al., *Hybrid Nanosystem Formed by DOX-Loaded Liposomes and Extracellular Vesicles from MDA-MB-231 Is Effective against Breast Cancer Cells with Different Molecular Profiles.* Pharmaceutics, 2024. **16**(6).
48. Sulthana, S., D. Shrestha, and S. Aryal, *Maximizing liposome tumor delivery by hybridizing with tumor-derived extracellular vesicles.* Nanoscale, 2024. **16**.
49. Ning, S., et al., *Platelet-derived exosomes hybrid liposomes facilitate uninterrupted singlet oxygen generation to enhance breast cancer immunotherapy.* Cell Reports Physical Science, 2023. **4**(7): p. 101505.
50. Xu, M., et al., *ROS-Responsive Biomimetic Nanocomplexes of Liposomes and Macrophage-Derived Exosomes for Combination Breast Cancer Therapy.* International Journal of Nanomedicine, 2025. **20**(null): p. 5161-5180.
51. Huang, H., et al., *Synergistic strategy with hyperthermia therapy based immunotherapy and engineered exosomes-liposomes targeted chemotherapy prevents tumor recurrence and metastasis in advanced breast cancer.* Bioeng Transl Med, 2022. **7**(2): p. e10284.

52. Verma, N., et al., *Exosomes fused liposomes: formulation, stability studies and theranostic evaluation for breast cancer applications.* Int J Pharm, 2025. **682**: p. 125907.
53. Tenchov, R., J.M. Sasso, and Q.A. Zhou, *PEGylated Lipid Nanoparticle Formulations: Immunological Safety and Efficiency Perspective.* Bioconjugate Chemistry, 2023. **34**(6): p. 941-960.
54. Gilroy, K.D., et al., *Bimetallic Nanocrystals: Syntheses, Properties, and Applications.* Chemical Reviews, 2016. **116**(18): p. 10414-10472.

## Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- GraphicalAbstract.jpg