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RESEARCH ARTICLE

Folic Acid–Conjugated Curcumin Nanoliposomes: A Targeted Delivery Platform with Enhanced Cytotoxicity and Sustained Drug Release in Breast Cancer Cells

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Abstract

Introduction: Breast cancer remains one of the most prevalent and fatal malignancies among women, emphasizing the need for safer and more effective therapeutic approaches. Curcumin is a multifunctional natural compound with proven anticancer activity; however, its low solubility and rapid systemic elimination restrict its clinical application. In this study, folic acid–linked PEGylated nanoliposomes (FA-Lipo-Cur) were developed to enhance curcumin delivery and cellular uptake in folate receptor–overexpressing MDA-MB-231 breast cancer cells. **Materials and Methods:** Curcumin-loaded, folate-conjugated nanoliposomes were fabricated via the thin-film hydration method followed by post-insertion of DSPE-PEG (3350)-FA. The formulations were characterized for particle size, ζ-potential, polydispersity index (PDI), morphology (SEM/TEM), encapsulation efficiency (EE%), and in-vitro drug release kinetics. Cytotoxicity was assessed using the MTT assay after 48 h exposure to FA-Lipo-Cur, non-targeted Lipo-Cur, and free curcumin. **Results:** Both formulations produced spherical vesicles with uniform morphology and high encapsulation efficiency (>75%). The mean particle size of FA-Lipo-Cur was 250 ± 7.7 nm, with a ζ-potential of −25.1 ± 2.1 mV and PDI of 0.16 ± 0.02, indicating excellent colloidal stability and homogeneity. In-vitro release studies demonstrated sustained curcumin release over 48 h, with a slower release rate for FA-Lipo-Cur compared to Lipo-Cur, attributed to steric stabilization by the PEG–folate corona. In cytotoxicity assays, FA-Lipo-Cur exhibited the lowest IC_{50} (33 ± 3.3 µg/mL) compared with Lipo-Cur (45 ± 3.9 µg/mL) and free curcumin (55 ± 4.1 µg/mL) ($p < 0.05$). When folate receptors were pre-saturated with free folic acid, the IC_{50} of FA-Lipo-Cur increased to 47.5 ± 3.2 µg/mL, confirming that enhanced cytotoxicity arose primarily from receptor-specific targeting rather than non-specific uptake. **Conclusion:** The FA-Lipo-Cur system therefore represents a promising, biocompatible nanocarrier platform for targeted breast cancer therapy, warranting further pharmacokinetic and in-vivo tumor model evaluation.

Keywords: Folic acid- conjugated nanoliposomes- curcumin- drug release- targeted drug delivery

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Introduction

Cancer is a group of diseases marked by uncontrolled cell proliferation driven by genetic and epigenetic changes, which can invade nearby tissues and spread (metastasize) to distant organs [1-5]. Breast cancer remains a major cause of cancer-related mortality among women worldwide, and therapeutic progress is often constrained by dose-limiting toxicities and the emergence of drug resistance [6]. Natural products with pleiotropic anticancer activities have attracted attention as adjuncts or alternatives to conventional chemotherapeutics. Curcumin, a polyphenolic constituent of Curcuma longa, exhibits antiproliferative, pro-apoptotic, anti-inflammatory, and anti-metastatic effects across diverse tumor models. However, its clinical translation has been hindered by extremely low aqueous solubility, rapid metabolic clearance, and poor systemic bioavailability, which collectively limit effective intratumoral exposure [7]. Curcumin has been shown in triple-negative breast cancer (TNBC) models to interfere with multiple oncogenic signaling pathways, including NF-κB, STAT3 and PI3K/AKT, as well as to promote oxidative stress-driven cytotoxicity. For example, in TNBC cell lines such as MDA-MB-231, curcumin suppresses the nuclear translocation and activity of NF-κB p65/p50 and down-regulates downstream targets such as cyclin D1, MMP-1 and VEGF, thereby attenuating proliferation, invasion and survival [8]. Concurrently, curcumin inhibits STAT3 phosphorylation and blocks PI3K/AKT activation two pathways known to support cell survival, chemoresistance and metabolic reprogramming in TNBC leading to enhanced apoptosis and reduced resistance in preclinical breast-cancer models [9]. In addition, curcumin elevates intracellular reactive oxygen species (ROS) and decreases antioxidant defense, creating oxidative stress that triggers mitochondrial damage and apoptotic cascades. This multifactorial assault on survival networks underscores curcumin's pleiotropic anticancer potential, although the exact interplay among these pathways in TNBC in vivo remains insufficiently defined [8]. In the treatment of TNBC, standard regimens such as anthracyclines (e.g., Doxorubicin), taxanes (e.g., Paclitaxel) and platinum agents (e.g., Carboplatin) have well-documented efficacy: for example, the addition of a platinum agent to anthracycline-/taxane-based neoadjuvant chemotherapy has increased pathological complete response (pCR) rates from ~35 % to over 50 % in TNBC [10]. In contrast, curcumin is largely in a preclinical or early-phase combinatorial stage: for example, a cell-line study showed that curcumin plus paclitaxel induced higher apoptosis in MDA-MB-231 cells compared to either agent alone [11]. However, curcumin's absolute cytotoxic concentrations tend to be much higher (e.g., ~60 μg/mL) compared to typical chemotherapy IC_{50} values in the low μM range, and there is a lack of direct comparative clinical trials against standard agents. Thus, while curcumin has mechanistic promise (anti-inflammatory, pro-apoptotic, NF-κB/STAT3/PI3K-AKT modulation), it remains far from replacing anthracyclines/taxanes/platinum in standard TNBC therapy, and any translational claim requires caution. Such innovations highlight the role of advanced materials in addressing critical challenges in environmental sustainability. Nanocarrier-based delivery offers a rational strategy to overcome these barriers [12-17]. Approaches utilizing nanotechnology for targeted drug delivery have been investigated, such as the adsorption of Levodopa on boron nitride nanotubes as a potential vehicle [18]. This highlights similarities in enhancing drug efficacy through nanostructured carriers. Liposomes, in particular, are biocompatible vesicles capable of encapsulating hydrophobic cargos like curcumin within their lipid bilayer while shielding them from premature degradation [19-21]. Beyond passive accumulation via the enhanced permeability and retention (EPR) effect, decorating liposomal surfaces with ligands for tumor-associated receptors can promote receptor-mediated endocytosis and improve intracellular drug delivery [22]. Folic acid (FA) is a small, stable, and inexpensive ligand that binds folate receptors (FRs), which are overexpressed in subsets of breast and other solid tumors and minimally expressed in most normal tissues features that make FA an attractive moiety for active targeting [23]. It should be noted that the expression of the folate receptor is highly heterogeneous among breast cancer subtypes, which has implications for targeting strategies. For example, the gene encoding folate receptor α (FOLR1/FRα) shows significantly higher mRNA and protein levels in triple-negative/basal breast cancers compared with ER+ or HER2+ subtypes, but still demonstrates wide intra-subtype variability i.e., a subset of TNBCs express high FRα, while others do not [24]. Additionally, while FRα is the predominant isoform exploited for tumour targeting, folate receptor β (FOLR2/FRβ) is primarily expressed on activated macrophages and in inflammatory environments rather than tumour epithelial cells, which raises concerns about specificity and possible uptake by non-malignant cells [25]. Finally, liposomal systems decorated with folate may still be taken up by cells in an antigen-independent manner (via endocytosis, enhanced permeability and retention (EPR) or non-folate-receptor mediated binding), yet the manuscript does not discuss this possibility nor the risk that folate-receptor targeting may not yield the expected selectivity in the context of such heterogeneity. In this study, folic acid–conjugated, curcumin-loaded nanoliposomes (FA-Lipo-Cur) were designed and optimized to improve the delivery and anticancer efficacy of curcumin against MDA-MB-231 cells, a representative model of aggressive triple-negative breast cancer (TNBC). We hypothesized that folate functionalization would facilitate receptor-mediated uptake and thereby enhance the cytotoxic potency of liposomal curcumin compared with non-targeted and free formulations. To validate this, FA-Lipo-Cur was prepared by the thin-film hydration method, characterized for particle size, ζ-potential, polydispersity index, and encapsulation efficiency, and its in vitro cytotoxicity was systematically evaluated using the MTT assay. The overall objective was to establish a biocompatible and targeted nanoliposomal platform capable of enhancing curcumin's therapeutic performance

in a clinically relevant TNBC model.

Materials and Methods

Materials

DSPC (1,2-distearoyl-sn-glycero-3-phosphocholine) was purchased from Lipoid GmbH (Ludwigshafen, Germany). DSPE-mPEG(3350) and DSPE-PEG (3350)-Folate were obtained from Avanti Polar Lipids (Alabaster, AL, USA). Curcumin was sourced from Cell Pharma GmbH (Germany). Cholesterol, MTT [3- (4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide], sodium pentanesulphonate, and sodium edetate were acquired from Sigma-Aldrich. Isopropanol, methanol, and chloroform were supplied by Merck. RPMI-1640 and DMEM media, fetal bovine serum, and penicillin/streptomycin were obtained from Invitrogen Corporation. The MDA-MB-231 cell line was provided by the National Cell Bank of Iran (Pasteur Institute of Iran).

Preparation of nanoliposomes

Curcumin-free (blank) and curcumin-loaded nanoliposomes were prepared using the thin-film hydration technique. Lipid components were mixed at an 80:15:5 molar ratio (DSPC:cholesterol:PEG-lipid) by dissolving 200 mg DSPC, 20 mg cholesterol, and 60 mg DSPE-mPEG(3350) in 25 mL chloroform:methanol (2:1, v/v). The organic solution was transferred into a round-bottom flask and evaporated under reduced pressure using a rotary evaporator (50 °C, 100 rpm) until a uniform thin lipid film formed on the flask wall. The residual solvent was removed by nitrogen purging for 5 min. The dried lipid film was hydrated with phosphate-buffered saline (PBS, pH 7.2) containing 9 mg curcumin (for the drug-loaded group) or plain PBS (for the blank control). The mixture was gently agitated at 50 °C in a water bath for 30 min to facilitate lipid swelling and vesicle formation. The resulting multilamellar vesicles were sonicated briefly to obtain nanosized liposomes. For the folate-targeted formulation (FA-Lipo-Cur), 6 mg DSPE-PEG(3350)-Folate was incorporated into the pre-formed PEGylated liposomes by post-insertion at 50 °C, maintaining the overall PEG-lipid content consistent with the non-targeted control. Accordingly, control liposomes (Lipo-Cur) were prepared in parallel using 46 mg DSPE-mPEG(3350) (equivalent to the total PEG-lipid in the targeted system: 40 mg mPEG-DSPE + 6 mg FA-PEG-DSPE). Finally, the curcumin-loaded liposomes were purified by Sephadex G-50 gel filtration (pre-equilibrated with PBS, pH 7.2) to remove unencapsulated curcumin. The purified vesicles were stored at 4 °C for further characterization.

Characterization of nanoliposomes

The mean hydrodynamic diameter (Z-average), PDI, and ζ-potential of the liposomal dispersions were measured using a Zetasizer Nano ZS3600 (Malvern Instruments, UK). Prior to analysis, samples were briefly sonicated in a bath sonicator (25 °C, 60 W) and diluted 1:50 in PBS (pH 7.4). Instrument performance was verified with a polystyrene nanosphere standard (DTS5050, Malvern). The morphology of both Lipo-Cur and FA-Lipo-Cur was assessed by Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM). For SEM, a drop of the diluted dispersion was placed on a silicon stub, air-dried, and sputter-coated with a thin Au/Pd layer before imaging. For TEM, a drop was deposited on a carbon-coated copper grid, wicked to remove excess, air-dried, and examined to capture high-resolution images of the vesicles. To minimize non-specific adsorption of curcumin to plastic and glassware, all tubes and pipette tips were pre-rinsed with 0.1% (v/v) Tween-80 solution, and blank liposome suspensions were used as adsorption controls. The encapsulation efficiency (EE%) was determined by the ultracentrifugation method. Briefly, 1 mL of each liposomal suspension was ultracentrifuged at 120,000 × g (≈45,000 rpm) for 60 min at 4 °C using a Beckman 90Ti rotor to ensure complete sedimentation of liposomes. The supernatant containing unencapsulated curcumin was carefully collected and quantified by UV–Vis spectrophotometry at 425 nm against appropriate blank liposome controls. The efficiency of pelleting was confirmed by verifying negligible lipid content in the supernatant of blank formulations. Drug concentration was determined from a calibrated curcumin standard curve prepared in PBS containing a small amount of Tween-80 to avoid adsorption, with appropriate dilution and sample-replacement corrections applied. EE% was calculated as:

EE%= (Initial Drug−Unentrapped Drug)/Initial Drug×100

where Initial Drug is the amount introduced into the formulation and Unencapsulated Drug is the concentration measured in the supernatant.

In vitro drug-release

The release of curcumin from non-targeted liposomes (Lipo-Cur) and folate-conjugated liposomes (FA-Lipo-Cur) was evaluated using a dialysis diffusion method under verified sink conditions. Briefly, 1 mL of each formulation was loaded into a pre-soaked dialysis bag (MWCO 12 kDa) and immersed in 50 mL of phosphate-buffered saline (PBS, pH 7.4) containing 0.5% (w/v) Tween-80 to maintain sink conditions and prevent drug adsorption to the membrane. The system was maintained at 37 ± 0.5 °C with gentle shaking (100 rpm). Sink conditions were verified by ensuring that the cumulative concentration of released curcumin never exceeded 20% of its solubility in PBS (pH 7.4, 0.5% Tween-80, 120 µg/mL at 37 °C). To confirm stability of curcumin during the 48 h study, a control solution of free curcumin (10 µg/mL) was incubated under identical conditions, and no visible precipitation or significant degradation (<5% loss at 425 nm) was observed. At predetermined time intervals (0.5, 1, 2, 4, 6, 12, 24, 36, and 48 h), 1 mL of the release medium was withdrawn and replaced immediately with an equal volume of fresh PBS (37 °C). Cumulative release values were corrected for sample replacement using the standard dilution formula. Curcumin concentration was quantified by UV–Vis spectrophotometry at 425 nm against blank liposome controls and converted to cumulative release (%) using a standard calibration curve.

Cumulative Release (%) = (Q_n / Q_total) × 100

where Q_n is the cumulative amount of drug released at time n, and Q_total is the total amount of drug initially encapsulated in the formulation.

In vitro cytotoxicity studies

Cytotoxic activity of curcumin-loaded nanoliposomes was assessed in MDA-MB-231 breast cancer cells using the MTT assay. Cells were maintained at 37 °C in a humidified 5% CO_2 incubator in growth medium supplemented with 10% fetal bovine serum (FBS), 100 mg/mL streptomycin, 100 U/mL penicillin, and 0.25 mg/mL amphotericin B. For assays, 1.0×10^4 cells/well were seeded into 96-well plates containing 200 µL of medium and allowed to adhere for 24 h. The medium was then replaced with either fresh medium (negative control) or treatment medium containing graded concentrations of curcumin equivalent to 5, 10, 20, 40, 60, 80, and 100 µg/mL. All concentrations were expressed in terms of curcumin content, calculated from the encapsulation efficiency and drug loading of each formulation. During the 48-h treatment, cells were maintained in medium containing 2% FBS to sustain viability while minimizing serum-protein binding of curcumin. For free curcumin, the compound was dissolved in dimethyl sulfoxide (DMSO) and diluted with culture medium to a final DMSO concentration below 0.1% (v/v) to avoid solvent-induced cytotoxicity. After exposure, the medium was replaced with 180 µL of fresh medium and 20 µL of MTT solution (5 mg/mL in PBS), followed by 3 h incubation to allow formazan formation. Reactions were terminated and crystals solubilized by adding 200 µL of DMSO per well. Absorbance at 570 nm was recorded using an AccuReader microplate reader (M965 Series, Metertech, Taipei, Taiwan). To confirm the role of folate receptor–mediated uptake in the cytotoxicity of FA-Lipo-Cur, a receptor-blocking assay was performed. Prior to treatment, MDA-MB-231 cells were pre-incubated with free folic acid (1 mM in complete medium) for 2 h at 37 °C to saturate surface folate receptors. After pre-incubation, cells were treated with FA-Lipo-Cur, Lipo-Cur, free curcumin, or the standard chemotherapeutic drug doxorubicin at equivalent curcumin concentrations (5–100 µg/mL for curcumin formulations, 0.1–5 µg/mL for doxorubicin) for 48 h, followed by the standard MTT procedure as described above. Cells without folic-acid pre-treatment served as the unblocked control group. Doxorubicin was used as a positive control to provide a benchmark for cytotoxic potency relative to curcumin formulations. Differences in cell viability and IC_{50} values between blocked and unblocked cells were statistically analyzed to determine whether the enhanced cytotoxicity of FA-Lipo-Cur was dependent on folate receptor binding.

Statistical Analysis

All experiments were performed in triplicate, representing three independent formulation batches (biological replicates), each measured three times (technical replicates). All quantitative data are expressed as mean ± SD (n = 3). Statistical analysis was performed using SPSS version 19, and curve fitting for IC_{50} determination was carried out in GraphPad Prism version 6.0 (GraphPad Software Inc., San Diego, CA). One-way ANOVA followed by Tukey's post hoc test was applied for multiple group comparisons (Free Cur, Lipo-Cur, FA-Lipo-Cur, FA-Lipo-Cur (blocked), and Doxorubicin). Differences were considered statistically significant at $p < 0.05$.

Results

Physicochemical characterization

Both formulations produced spherical vesicles with limited aggregation in SEM/TEM micrographs (Figure 1). Dynamic light scattering showed narrow size distributions (PDI ≤ 0.18) and moderately negative surface charge, consistent with colloidal stability in PBS. As summarized in Table 1, the mean hydrodynamic diameter of FA-Lipo-Cur was modestly smaller than that of Lipo-Cur (250 ± 7.7 nm vs. 265 ± 9.2 nm; n=3), with a slightly lower PDI (0.16 ± 0.02 vs. 0.18 ± 0.02). The ζ-potential magnitude was marginally higher for FA-Lipo-Cur (−25.1 ± 2.1 mV) compared with Lipo-Cur (−22.4 ± 1.9 mV). Both systems achieved high curcumin encapsulation, with efficiencies of 82.3 ± 3.1 % (FA-Lipo-Cur) and 79.5 ± 2.6 % (Lipo-Cur). Collectively, these data indicate that folate decoration slightly decreases particle size and polydispersity while preserving high drug

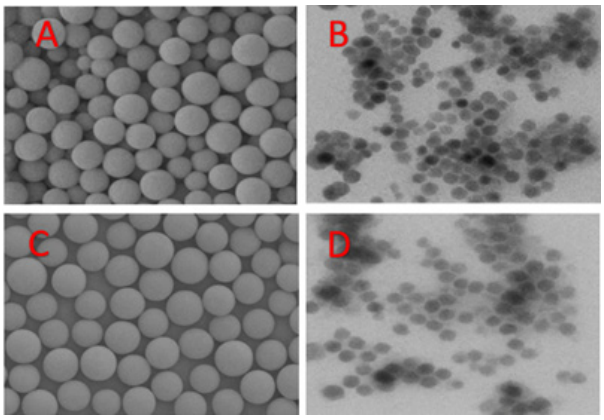

Figure 1. Representative Micrographs of (A,C) SEM and (B,D) TEM. Panels A–B correspond to FA-Lipo-Cur, and panels C–D to Lipo-Cur. Both formulations show spherical vesicles with minimal aggregation; FA-Lipo-Cur displays slightly smaller, more uniformly dispersed particles. Scale bars: A, C = 200 nm; B, D = 100 nm.

Table 1. Physicochemical Properties of Curcumin Nanoliposomes (mean ± SD, n = 3)

Formulations	Mean size (nm)	PDI	ζ-potential (mV)	Encapsulation Efficiency (%)
Lipo-Cur	265 ± 9.2	0.18 ± 0.02	−22.4 ± 1.9	79.5 ± 2.6
FA-Lipo-Cur	250 ± 7.7	0.16 ± 0.02	−25.1 ± 2.1	82.3 ± 3.1

Table 2. IC_{50} Values and Statistical Significance for Curcumin Formulations Before and After Folate Receptor Blocking (MDA-MB-231 cells, 48 h).

Formulations	IC_{50} (µg/mL)	p-value (vs Free Cur)
Free Cur	55±4.1 µg/mL	-
Lipo-Cur	45±3.9 µg/mL	-
FA-Lipo-Cur (unblocked)	33 ±3.3µg/mL	*
FA-Lipo-Cur (blocked with folic acid)	47.5 ± 3.2µg/mL	-
Doxorubicin (References)	1.2 ± 0.08µg/mL	-

FA-Lipo-Cur exhibited a significantly lower IC_{50} compared to Free Cur ($p < 0.05$), confirming folate receptor–mediated cytotoxic enhancement. However, the IC_{50} value of Doxorubicin (1.2 µg/mL) remained markedly lower than those of curcumin formulations, underscoring that FA-Lipo-Cur functions as a biocompatible, targeted adjunct therapy rather than a substitute for potent cytotoxic drugs. Each value represents mean ± SD from three independent experiments; IC_{50} values were calculated by nonlinear regression ($R^2 \geq 0.95$).

loading and a sufficiently negative ζ-potential to support dispersion stability.

In vitro drug-release

As illustrated in Figure 2, both liposomal formulations demonstrated a sustained, diffusion-controlled release pattern over a 48-hour period. The non-targeted Lipo-Cur exhibited a faster release rate than FA-Lipo-Cur, resulting in higher cumulative release values at intermediate and late time points. In contrast, the folate-conjugated liposomes showed a more gradual and controlled release, consistent with enhanced bilayer rigidity and steric stabilization conferred by the PEG–folate corona. Free curcumin, on the other hand, displayed an immediate burst effect and reached nearly complete release within the first few hours. The difference in release kinetics between Lipo-Cur and FA-Lipo-Cur was statistically significant from the mid-phase of the experiment onward ($p < 0.05$), confirming that folate conjugation effectively modulates the diffusion rate without impeding sustained drug release. The slower diffusion from FA-Lipo-Cur may be attributed to steric hindrance and reduced membrane permeability induced by the surface-bound PEG–folate layer, which acts as a diffusion barrier while maintaining structural integrity.

In vitro cytotoxicity studies

The cytotoxic effects of free curcumin and its liposomal formulations were evaluated against MDA-MB-231 breast cancer cells using the MTT assay. As illustrated in Figure 3, cell viability decreased in a concentration-dependent manner for all formulations, with the FA-Lipo-Cur group exhibiting the most pronounced reduction in cell viability across all tested concentrations (5–100 µg/mL). Both liposomal formulations demonstrated greater cytotoxicity than free curcumin, indicating improved intracellular delivery and enhanced bioavailability. Quantitative analysis of the IC_{50} values (Table 2) revealed that FA-Lipo-Cur exhibited the lowest IC_{50} (33 ± 3.3 µg/mL), followed by Lipo-Cur (45 ± 3.9 µg/mL) and free curcumin (55 ± 4.1 µg/mL). Statistical comparison confirmed that the decrease in IC_{50} for FA-Lipo-Cur was significant ($p < 0.05$) relative to free curcumin, suggesting that folate conjugation effectively enhanced the anticancer potency of the nanoliposomes through folate-receptor-mediated uptake. To further confirm the role of folate receptor targeting, a receptor-blocking assay was performed. Pre-incubation of MDA-MB-231 cells with excess free folic acid (1 mM, 2 h) markedly reduced the cytotoxicity of FA-Lipo-Cur, resulting in an increased IC_{50} value (47.5 ± 3.2 µg/mL) that became statistically comparable to non-targeted Lipo-Cur ($p > 0.05$). This loss of cytotoxic advantage upon receptor saturation strongly supports that the enhanced efficacy of FA-Lipo-Cur originates primarily from folate receptor-mediated internalization rather than non-specific uptake or PEGylation effects. For comparative context, the standard chemotherapeutic agent doxorubicin displayed an IC_{50} of approximately 1.2 ± 0.08 µg/mL under similar experimental conditions, demonstrating substantially higher intrinsic cytotoxic potency. Nevertheless, the FA-Lipo-Cur formulation offers the advantage of biocompatibility, multi-targeted activity, and receptor-specific uptake, suggesting potential application as a complementary or adjuvant therapy to reduce the required dose and systemic toxicity of conventional chemotherapeutic drugs. These findings collectively confirm that the targeted FA-Lipo-Cur system provides improved cytotoxic efficacy against triple-negative breast cancer cells and that this enhancement is mechanistically linked to folate receptor–specific targeting, distinguishing it from non-targeted and free curcumin formulations while offering translational potential for safer combination regimens in cancer therapy.

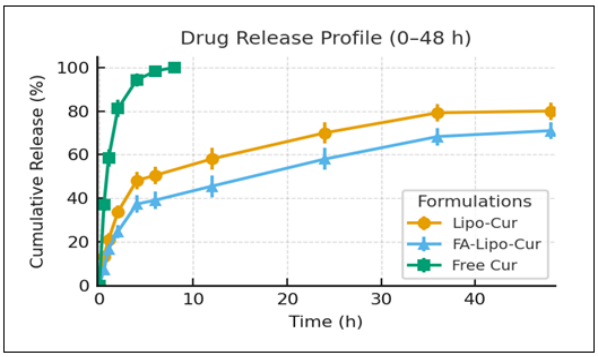

Figure 2. Cumulative Curcumin Release Profiles for Free Cur, Lipo-Cur, and FA-Lipo-Cur in PBS (pH 7.4) at 37 °C over 0–48 h (mean ± SD, n = 3). curves are displayed to highlight the rapid burst of free drug versus the sustained release from liposomes, with FA-Lipo-Cur releasing more slowly than Lipo-Cur.

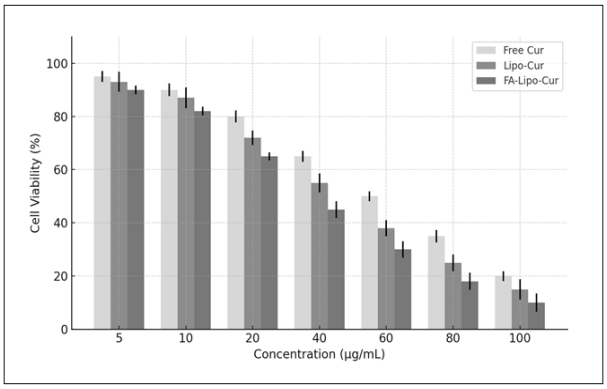

Figure 3. Comparative IC_{50} values of Free Cur, Lipo-Cur, and FA-Lipo-Cur determined in MDA-MB-231 cells (mean ± SD, n = 3). Error bars represent standard deviations. FA-Lipo-Cur exhibits the lowest IC_{50}, confirming its superior cytotoxic potency due to folate-targeted uptake.

Discussion

Given the central importance of public health to society, cancer remains a major global health challenge, underscoring the need to use effective, evidence-based treatment methods to improve patient outcomes [26-29]. Across cancers and especially in breast cancer, one of the most prevalent malignancies among women worldwide advances in chemotherapy, radiotherapy, and targeted agents notwithstanding, treatment is still limited by systemic toxicity, drug resistance, and suboptimal efficacy [6, 30-32]. These challenges highlight the critical need for advanced drug delivery systems that can enhance the therapeutic index of anticancer drugs by improving their solubility, stability, and bioavailability, while simultaneously reducing off-target side effects [33]. Nanocarrier-based delivery platforms have emerged as a promising approach to overcome these limitations, offering improved pharmacokinetics, controlled release, and the potential for active tumor targeting [3, 34-36]. Among nanocarriers, liposomes have attracted considerable attention for breast cancer therapy due to their biocompatibility, capability to encapsulate both hydrophilic and hydrophobic drugs, and the option for surface modification with targeting ligands [37]. By functionalizing liposomes with moieties such as folic acid, selective delivery to cancer cells overexpressing folate receptors can be achieved, thereby improving intracellular drug accumulation while minimizing uptake by normal cells [13]. Such targeted delivery is particularly advantageous for compounds like curcumin, a natural polyphenol with broad anticancer properties but poor aqueous solubility and rapid systemic elimination, which have limited its clinical application [38]. In our study, both Lipo-Cur and FA-Lipo-Cur produced spherical, well-dispersed vesicles with narrow size distributions (PDI $\leq$ 0.18), moderately negative ζ-potentials (supporting colloidal stability), and high encapsulation efficiencies. The FA-decorated system displayed a slightly smaller and more uniform hydrodynamic diameter than the non-targeted counterpart (~250 nm vs. ~265 nm), a feature expected to favour more reproducible behaviour and possibly enhanced tumour accumulation via the enhanced permeability and retention (EPR) effect. In release testing, free curcumin showed a rapid burst release, whereas both liposomes provided sustained, diffusion-dominated release over 48 h; the FA-liposomes released more slowly, consistent with tighter bilayer packing and steric stabilisation imparted by the PEG–folate corona. Functionally, these physicochemical and kinetic advantages translated into superior antiproliferative activity: FA-Lipo-Cur exhibited the lowest IC_{50} compared with Lipo-Cur and free curcumin, likely due to folate receptor–mediated uptake combined with maintenance of more effective intracellular drug levels. The receptor-blocking study confirmed that pre-saturation of folate receptors with excess free folic acid abolished the cytotoxic advantage of FA-Lipo-Cur, thereby supporting the conclusion that the enhanced efficacy arises primarily from receptor-specific internalisation rather than non-specific uptake or simply PEGylation/size effect [39]. Nevertheless, important limitations must be acknowledged. First, the relatively high IC_{50} values (in the range of tens of µg/mL) reflect the intrinsic low potency and solubility limitations of curcumin itself as reported in other studies [40]. Therefore, the aim of our work was not to replace conventional cytotoxic agents such as doxorubicin, but rather to improve curcumin's bioavailability and tumour cell uptake via ligand-guided nanocarriers. A second limitation is that the present study is confined to in-vitro evaluation using a single breast cancer cell line (MDA-MB-231). While the trend across independent experiments supports consistent enhancement of cellular uptake (~20% improvement) and targeting efficiency, in-vivo pharmacokinetics, tumour accumulation, and tumour-growth inhibition studies are required to confirm therapeutic relevance. Moreover, no direct head-to-head comparison with standard chemotherapeutic agents was made in a biological model; hence, clinical translation of FA-Lipo-Cur should be interpreted with caution until validated in appropriate animal models or combination regimens. On a positive note, the results emphasise that folate-conjugated nanoliposomes can enhance the delivery of challenging compounds like curcumin by improving intracellular uptake, moderating release kinetics, and preserving high drug loading and stability. In this context, FA-Lipo-Cur represents a robust and scalable platform for targeted curcumin delivery in breast cancer. Importantly, given curcumin's favourable safety profile and multi-target activity, this platform may be particularly suited for adjuvant or combination therapy strategies, for example co-delivery with standard chemotherapeutics to reduce dose and toxicity while retaining efficacy [41].

In conclusion, folic acid conjugation markedly enhanced the delivery and anticancer activity of curcumin-loaded liposomes. The FA-Lipo-Cur system provides high encapsulation, sustained release, and efficient receptor-mediated uptake, translating into improved cytotoxic potency versus free curcumin and non-targeted liposomes. By improving solubility, stabilising the payload and

increasing tumour selectivity, this targeted nanoliposomal platform offers a robust and scalable approach for breast cancer therapy and a strong foundation for advanced, combination-ready treatment strategies.

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Consent for publication

All authors have given consent for publication.

Conflict of interest

The authors declare no potential conflict of interest.

Originality Declaration for Figures

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