

Targeted Nanoliposomal Delivery of Dichloroacetate Promotes Uptake and Phenotypic Reprogramming of THP-1-Derived Tumor-Associated Macrophages

Win Darmanto

windarmanto@fst.unair.ac.id

Airlangga University

Sri Puji Astuti Wahyuningsih

Airlangga University

Firli Rahmah Primula Dewi

Airlangga University

Ally Kafesa

Airlangga University

Mochammad Aqilah Herdiansyah

Airlangga University

Rr. Aulia Rahmawati Kusuma Putri

Airlangga University

Liew Kai Bin

Cyberjaya University

Intan Zarina Zainol Abidin

Cyberjaya University

Article

Keywords: Deoxycholic acid, nanoliposomes, tumor-associated macrophages, macrophage repolarization, immunometabolism

Posted Date: August 12th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-10463937/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Additional Declarations: No competing interests reported.

Version of Record: A version of this preprint was published at Scientific Reports on October 21st, 2026.
See the published version at <https://doi.org/10.1038/s41598-026-73503-w>.

Targeted Nanoliposomal Delivery of Dichloroacetate Promotes Uptake and Phenotypic Reprogramming of THP-1-Derived Tumor-Associated Macrophages

Win Darmanto^{*1,2}, Sri Puji Astuti Wahyuningsih¹, Firli Rahmah Primula Dewi¹, Ally Kafesa^{1,4}, Mochammad Aqilah Herdiansyah¹, Rr. Aulia Rahmawati Kusuma Putri¹, Liew Kai Bin³, Intan Zarina Zainol Abidin³

¹ Faculty of Science & Technology, Airlangga University, Jl. Soekarno Kecamatan Mulyorejo, Surabaya, Jawa Timur, 60115, Indonesia

² Institute of Technology Science and Health Insan Cendikia Medika Jombang, Jl. Kemuning Candimulyo, Jombang, Jawa Timur, 61419, Indonesia

³ Faculty of Pharmacy, Cyberjaya University, Pesiaran Bestari, 63000, Selangor Darul Ehsan, Malaysia

⁴ Medical Laboratory Technology Study Program, Institut Kesehatan Rajawali, Jl. Cihanjuang No. 303, Kabupaten Bandung Barat, Jawa Barat, 40162, Indonesia

*Corresponding author: windarmanto@fst.unair.ac.id

Contributing authors: kafeally@gmail.com

Abstract

Tumor-associated macrophages (TAMs) predominantly exhibit an immunosuppressive M2 phenotype that promotes tumor progression. Reprogramming TAMs toward the pro-inflammatory M1 phenotype represents a promising strategy for cancer immunotherapy. This study developed targeted deoxycholic acid (DCA)-loaded nanoliposomes to enhance macrophage uptake and induce M2-to-M1 repolarization. Methods: DCA-loaded nanoliposomes were prepared by thin-film hydration and characterized using dynamic light scattering (DLS), zeta potential analysis, transmission electron microscopy (TEM), and high-performance liquid chromatography (HPLC). Cellular uptake was evaluated by fluorescence microscopy, cytotoxicity by the MTT assay, and macrophage polarization by flow cytometry using CD206 and CD80 as M2 and M1 markers, respectively. Results: The nanoliposomes exhibited a mean particle size of 158 nm, a polydispersity index of 0.28, a zeta potential of -12.8 mV, spherical morphology, and a high encapsulation efficiency of 82.94%. The formulation demonstrated concentration-dependent cytotoxicity with an IC_{50} of 13.47 μ M. Fluorescence microscopy confirmed enhanced intracellular uptake of targeted nanoliposomes. Flow cytometry revealed a significant decrease in CD206⁺ macrophages (76.8% to 21.7%) and an increase in CD80⁺ macrophages (18.6% to 68.3%), indicating efficient macrophage repolarization. Conclusions: Targeted DCA-loaded nanoliposomes exhibit favorable physicochemical properties, efficient intracellular delivery, and potent immunometabolic activity, effectively reprogramming macrophages from the M2 to the M1 phenotype. These findings support their potential as a macrophage-targeted nanotherapeutic platform for cancer immunotherapy.

Keywords: Deoxycholic acid; nanoliposomes; tumor-associated macrophages; macrophage repolarization; immunometabolism

1. Introduction

The majority of immune cells in the tumor microenvironment (TME), which can make up 30–50% of the total tumor mass in a variety of solid malignancies, are tumor-associated macrophages (TAMs). The majority of TAMs have a phenotype similar to M2 macrophages, which are involved in angiogenesis, immunosuppression, invasion, metastasis, and immunotherapy and chemotherapy resistance[1]. In cancer patients, a poor prognosis and lower survival rates are also correlated with high levels of TAM infiltration [2]. Thus, a crucial tactic in cancer immunotherapy at the moment is reprogramming TAMs from the protumor M2 phenotype to the proinflammatory and anticancer M1 phenotype[3]. Over the last five years, numerous research have shown that medication delivery and metabolic manipulation based on nanotechnology can successfully change the phenotype of TAMs. According to Shi et al. (2024) [4], TAMs can be repolarized toward the M1 phenotype by regulating particular metabolic and immunological pathways using a nanoparticle approach[5]. This strategy is seen to be promising since it can get around the drawbacks of traditional treatments, which solely target tumor cells without changing the immunosuppressive environment. By inducing TAM polarization toward the M1 phenotype and greatly increasing drug accumulation at tumor locations[3], this delivery method improved anticancer immune activity. These findings support the use of liposomes as a successful delivery system for TME macrophage function modulation.

Nanomedicines that target TAMs are a very promising strategy for next-generation cancer therapy. TAM phenotypic modification by nanoparticle delivery methods can boost immune responses and stop tumor growth, according to Zheng et al. In the meantime[6], Bulbake et al. emphasized the significance of TAM metabolic reprogramming since metabolic alterations play a major role in the transition from M2 to M1 phenotype[7]. Mannose-modified nanoparticles that targeted macrophage receptors were shown by Paurevic et al. to be more successful than non-targeted systems at increasing cellular uptake and triggering anticancer immune responses[8]. During the same time frame, Palencia-Campos et al. demonstrated that TAMs in a pancreatic cancer model could be reprogrammed by lipid nanosystems [9], which greatly reduced tumor development and liver metastasis[10]. Moreover, recent analyses have determined that liposome and nanolipid platforms are among the most promising approaches for getting past drug delivery obstacles in the intricate tumor microenvironment.

Dichloroacetate (DCA) is one metabolic substance that may be useful for TAM reprogramming [11]. By activating the pyruvate dehydrogenase complex, DCA, an inhibitor of pyruvate dehydrogenase kinase (PDK), can change cellular metabolism from aerobic glycolysis to oxidative phosphorylation[11][12]. The activation of pro-inflammatory macrophages and the decrease of immunosuppressive characteristics in the TME are known to be significantly influenced by this metabolic regulation[13]. Nonetheless, there are still a number of issues with DCA's over-the-counter use, including its non-specific distribution, short circulation duration, poor tissue penetration, and the requirement for comparatively high doses to have the best biological effects. Because of their superior encapsulating properties, high biocompatibility, and capacity to be tailored to improve medication delivery to target cells, nanoliposomes provide an answer to these problems. By employing certain ligands to target the surface of nanoliposomes, receptors expressed on TAMs can recognize them more readily, increasing particle internalization and intracellular drug concentration. Recent research has demonstrated that the successful reprogramming of macrophages and the activation of anticancer immune responses depend on TAMs' enhanced absorption of nanoparticles[14].

The creation of DCA-loaded nanoliposomes designed especially to target TAMs is a very intriguing strategy in light of these advancements. It is anticipated that this approach will improve cellular metabolic modulation, boost macrophage uptake of DCA, and cause a change in the TAM phenotype from M2 to M1. Thus, as a novel immunometabolic approach for cancer treatment based on tumor microenvironment manipulation, this study attempts to assess the capacity of targeted DCA-loaded nanoliposomes to enhance TAM uptake and induce macrophage reprogramming in vitro.

2. Materials and methods

2.1 Material

Dichloroacetate (DCA) sodium salt ($\geq 99\%$ purity) was purchased from Sigma-Aldrich. Hydrogenated soy phosphatidylcholine (HSPC), cholesterol, and DSPE-PEG2000 were obtained from eggs. Mannose-conjugated DSPE-PEG2000 (DSPE-PEG2000-Mannose) was used as a macrophage-targeting ligand. Chloroform and methanol (HPLC grade) were purchased from Merck. Human monocytic THP-1 cells were obtained from Swiss Type Culture Collection. RPMI-1640 medium, fetal bovine serum (FBS), penicillin-streptomycin, phosphate-buffered saline (PBS), phorbol 12-myristate 13-acetate (PMA), interleukin-4 (IL-4), and interleukin-13 (IL-13) were purchased from Thermo Fisher Scientific. Antibodies against CD80⁺, CD206⁺, TNF- α , and TGF- β were obtained from BioLegend. All reagents used were of analytical grade.

2.2 Methods

2.2.1 Preparation of DCA-Loaded Nanoliposomes

Dichloroacetate (DCA)-loaded nanoliposomes were prepared using the thin-film hydration technique. Briefly, hydrogenated soy phosphatidylcholine (HSPC), cholesterol, DSPE-PEG2000, and DSPE-PEG2000-mannose were dissolved in chloroform:methanol (2:1, v/v) at a molar ratio of 60:35:3:2. The organic solvent was evaporated under reduced pressure using a rotary evaporator at 45°C to form a thin lipid film. The lipid film was hydrated with DCA solution (100 mM in PBS, pH 7.4) at 60°C for 30 min with continuous stirring. The resulting multilamellar vesicles were sonicated and extruded through polycarbonate membranes of decreasing pore sizes (400, 200, and 100 nm) to obtain uniformly sized nanoliposomes. Unencapsulated DCA was removed by dialysis against PBS for 24 h at 4°C. Blank nanoliposomes and non-targeted DCA-loaded nanoliposomes were prepared similarly but without mannose ligand incorporation.

2.2.2 Characterization of Nanoliposomes

Particle size, polydispersity index (PDI), and zeta potential were measured using dynamic light scattering (DLS). Measurements were performed in triplicate at 25°C after dilution of samples in ultrapure water. Morphological characteristics were examined by scanning electron microscopy (SEM) and transmission electron microscopy (TEM). A drop of nanoliposome suspension was placed on a carbon-coated copper grid, negatively stained with 2% phosphotungstic acid, air-dried, and observed under TEM. Encapsulation efficiency (EE%) and drug loading (DL%) were determined using high-performance liquid chromatography (HPLC). The amount of encapsulated DCA was quantified after disrupting liposomes with methanol.

2.2.3 Cellular Uptake of Nanoliposome-DCA

Evaluation of cellular uptake, nanoliposomes were labeled with the fluorescent probe Dil. TAM-like macrophages were seeded in 24-well plates at a density of 2×10^5 cells/well and incubated with Dil-labeled nanoliposomes equivalent to 1 mM DCA. After 1, 4, 8, and 24 h of incubation, cells were washed three times with cold PBS to remove extracellular nanoparticles. Cellular uptake was analyzed using fluorescence microscopy and flow cytometry. The mean fluorescence intensity (MFI) was quantified to compare uptake efficiency among free dye, non-targeted nanoliposomes, and mannose-targeted nanoliposomes.

2.2.4 Cytotoxicity Assay

The cytotoxicity of DCA-loaded nanoliposomes was evaluated using the MTT assay. TAM-like macrophages were seeded in 96-well plates at 1×10^4 cells/well and incubated overnight. Cells were treated with free DCA, blank nanoliposomes, non-targeted DCA-loaded nanoliposomes, and targeted DCA-loaded nanoliposomes at DCA concentrations of 0.25, 0.5, 1, 2, and 4 mM for 24 and 48 h. Following treatment, 20 μ L of MTT solution (5 mg/mL) was added to each well and incubated for 4 h. The resulting formazan crystals were dissolved in dimethyl sulfoxide (DMSO), and absorbance was measured at 570 nm using a microplate reader. Cell viability was expressed as a percentage relative to untreated control cells.

2.2.5 Flow Cytometric Analysis of Macrophage Polarization

Following treatment, cells were harvested and stained with fluorochrome-conjugated antibodies against CD80 (M1 markers), as well as CD206 (M2 markers). Samples were incubated in the dark for 30 min at 4°C, washed with PBS containing 2% FBS, and analyzed using a flow cytometer. The percentages of CD80⁺ and CD206⁺ cells were determined. The ratio of M1 to M2 markers was used as an indicator of macrophage reprogramming.

3. Result and Discussion

3.1 Synthesis and Physicochemical Characterization of Targeted DCA-Loaded Nanoliposomes

The physicochemical characteristics of nanocarriers, such as particle size, size distribution, surface charge, shape, and drug encapsulation efficiency, are crucial in determining their therapeutic efficacy[15]. Together, these factors control intracellular drug delivery, cellular internalization, circulatory behavior, and nanoparticle stability. In order to verify that the designed DCA-loaded nanoliposomes had the structural characteristics necessary for effective macrophage targeting and subsequent biological activity, a thorough physicochemical evaluation was carried out.

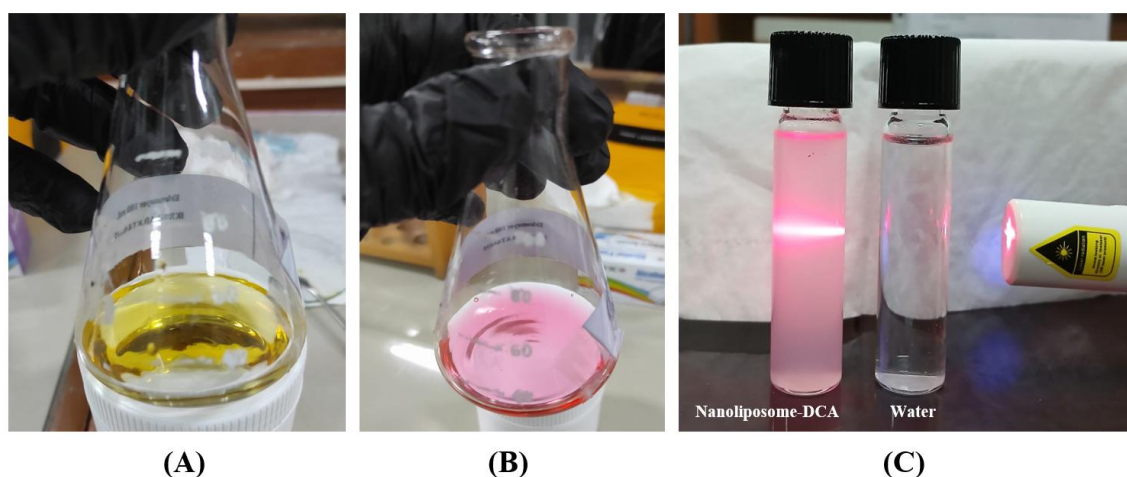


Fig. 1 Macroscopic appearance and Tyndall effect of DCA nanoliposomes. (A) Unlabeled DCA nanoliposome, (B) Dil-labeled DCA nanoliposome, (C) Tyndall effect observed upon laser irradiation of the nanoliposome suspension.

Prior to fluorescent labeling, the produced DCA nanoliposome suspension had a translucent yellow appearance (**Fig. 1A**). The suspension turned a uniform pink after the lipophilic fluorescent probe Dil was added (**Fig. 1B**), suggesting that the dye had successfully partitioned into the phospholipid bilayer. Dil is a commonly utilized fluorescent tracer for lipid-based nanocarriers and membrane research because it is very hydrophobic and preferentially localizes within the lipid membrane rather than the aqueous phase. A clear Tyndall effect was seen when the nanoliposome suspension was exposed to a laser beam (**Fig. 1C**). Colloidal particles scattered throughout the suspension scattered incident light, creating the visible light path. A typical optical feature in colloidal systems, the Tyndall effect is frequently employed as a quick qualitative indicator of stable colloidal dispersion and nanoparticle production. Preliminary proof that the generated DCA nanoliposomes are nanoscale vesicles rather than a real molecular solution is thus provided by the detection of a strong Tyndall effect. The interaction between the nanoscale liposomal particles scattered across the aqueous media and the fluctuating electromagnetic field of the incident laser beam is the source of the observed Tyndall effect. Each nanoliposome acts as a secondary radiating dipole due to oscillations of the electron cloud and dipole moments inside the lipid bilayer caused by the alternating electric field. The visible scattering path typical of the Tyndall effect is produced by these generated oscillations, which reemit light in various directions. The particle size, concentration, refractive index contrast between the nanoliposomes and the surrounding medium, and incident light wavelength all affect how much of this scattering occurs. The presence of a persistent colloidal dispersion of nanosized vesicles with dimensions adequate to interact with visible light is thus qualitatively confirmed by the clear Tyndall effect seen in the DCA nanoliposome suspension. For nanoliposomes that are between 100 and 200 nm in size, the scattering mechanism typically occurs at the boundary between Rayleigh and Mie scattering [16] in the nanoliposome-DCA formulation. The Mie scattering contribution starts to take over because the particle size is not significantly smaller than the visible light wavelength ($\approx 400\text{--}700\text{ nm}$). Compared to considerably tiny particles, Mie scattering results in a brighter, easier-to-see laser trail [17]. Because water molecules are too tiny to cause noticeably strong visible light scattering, this explains why nanoliposome suspensions show a strong Tyndall effect whereas pure water does not.

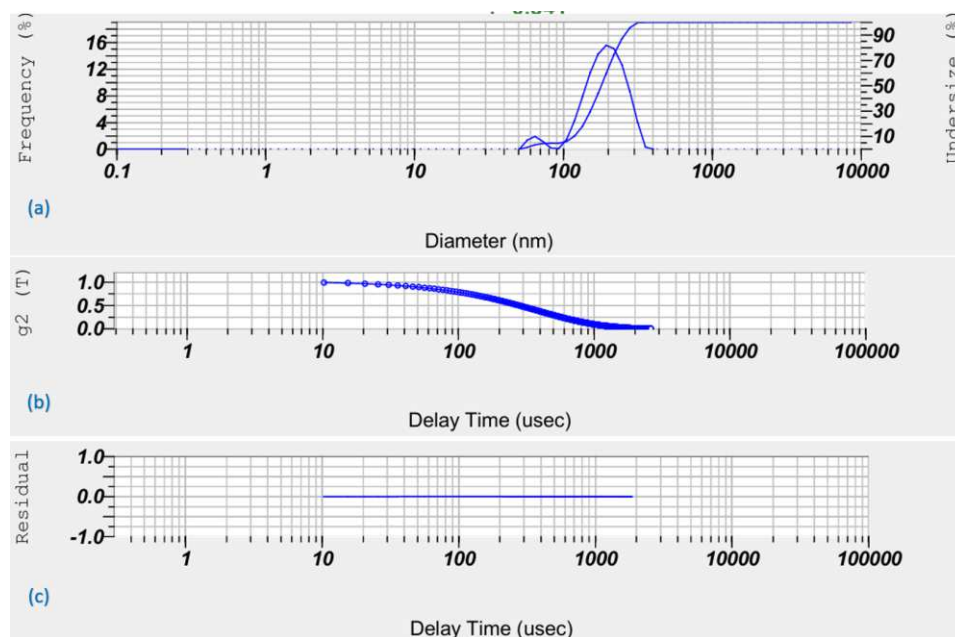


Fig. 2 Dynamic light scattering characterization of targeted DCA-loaded nanoliposomes: (a) particle size distribution, (b) autocorrelation function, and (c) residual fitting.

The hydrodynamic characteristics of the engineered DCA-loaded nanoliposomes were evaluated by dynamic light scattering (DLS) to determine particle size distribution and assess formulation homogeneity. As shown in **Fig. 2a**, the nanoliposomes exhibited a predominantly monomodal particle size distribution, with the major population centered at approximately 155.1 ± 0.34 nm. Only a minor shoulder was observed below 100 nm, indicating the presence of a negligible fraction of smaller vesicles that did not substantially influence the overall size distribution[7]. The absence of multiple dominant peaks suggests that the thin-film hydration followed by size reduction successfully produced a relatively homogeneous nanoliposomal formulation. The average particle diameter obtained in this study falls within the optimal size range (100–200 nm) for nanocarrier-mediated drug delivery[15]. Nanoparticles of this dimension are widely recognized to exhibit prolonged circulation, enhanced colloidal stability, and efficient cellular internalization by macrophages through endocytic pathways[18]. Furthermore, nanoparticles within this size range are sufficiently small to penetrate the abnormal vasculature of solid tumors via the enhanced permeability and retention (EPR) [19] effect while remaining large enough to minimize rapid renal clearance. Consequently, the observed particle size provides a favorable physicochemical basis for efficient delivery of DCA to tumor-associated macrophages (TAMs). The intensity autocorrelation curve obtained from DLS analysis (**Fig. 2b**) exhibited a smooth and continuous decay without secondary correlation phases. Such behavior is characteristic of nanoparticle suspensions containing a relatively uniform population undergoing Brownian motion[15]. The absence of irregular fluctuations or multiple decay components indicates that particle aggregation was minimal and that the measured hydrodynamic diameter accurately represents the dominant nanoliposomal population. From a physicochemical perspective, the gradual decay of the autocorrelation function reflects stable particle diffusion throughout the suspension medium[20]. Since DLS determines particle size based on translational diffusion, a single smooth decay curve is generally associated with monodisperse or narrowly dispersed nanoparticle systems. These findings therefore support the conclusion

that the prepared DCA-loaded nanoliposomes possess excellent colloidal behavior following formulation. To further evaluate the reliability of the DLS measurements, residual analysis was performed following cumulant fitting of the autocorrelation data. As illustrated in **Fig. 2c**, the residual values remained uniformly distributed around zero across the entire delay-time range, indicating excellent agreement between the experimental correlation data and the mathematical fitting model. Residual values centered near zero suggest minimal systematic deviation during curve fitting and demonstrate that the measured particle size distribution accurately reflects the physicochemical characteristics of the nanoliposomes. Conversely, large oscillations or systematic deviations would indicate poor fitting caused by aggregation, multiple particle populations, or unstable suspensions[5]. The excellent residual profile observed in this study therefore confirms the robustness and reliability of the particle size analysis.

The designed nanoliposomes successfully formed a homogenous nanosuspension with a limited size distribution (PDI = 0,28) and a mean hydrodynamic diameter of $155,1 \pm 0,34$ nm. Because they effectively utilize endocytic pathways while preserving advantageous colloidal stability, nanoparticles in the 100–200 nm range are thought to be ideal for macrophage internalization. Through the enhanced permeability and retention (EPR) effect, this particle size is also beneficial for passive accumulation within tumor tissues, raising the possibility of interaction with tumor-associated macrophages.

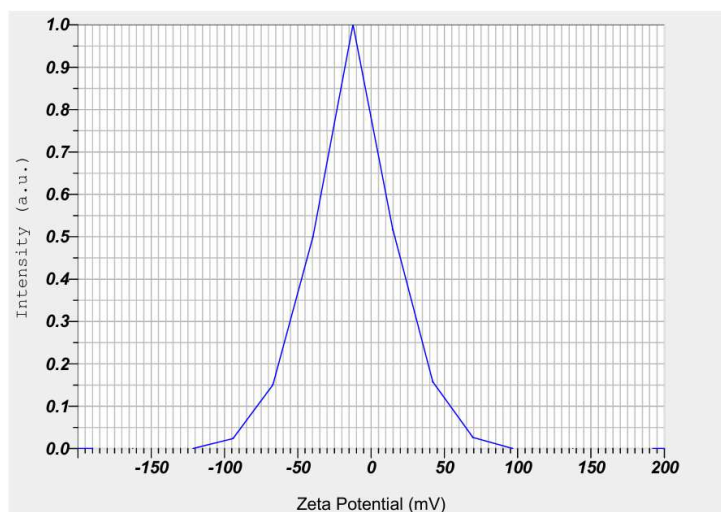


Fig. 3 Zeta potential profile of targeted DCA-loaded nanoliposomes

While limiting nonspecific protein adsorption, the fairly negative zeta potential (-12.8 ± 0.6 mV) indicates adequate electrostatic stabilization (**Fig. 3**). Compared to highly charged nanoparticles, a slightly negative surface charge has been shown to slow down quick clearance and promote longer interactions with macrophages. Furthermore, steric stability was probably aided by the addition of PEGylated lipids without sacrificing cellular absorption. In addition, the moderately negative surface may contribute to prolonged colloidal stability and facilitate receptor-mediated uptake by THP-1-derived macrophages without inducing significant nonspecific interactions with serum proteins.

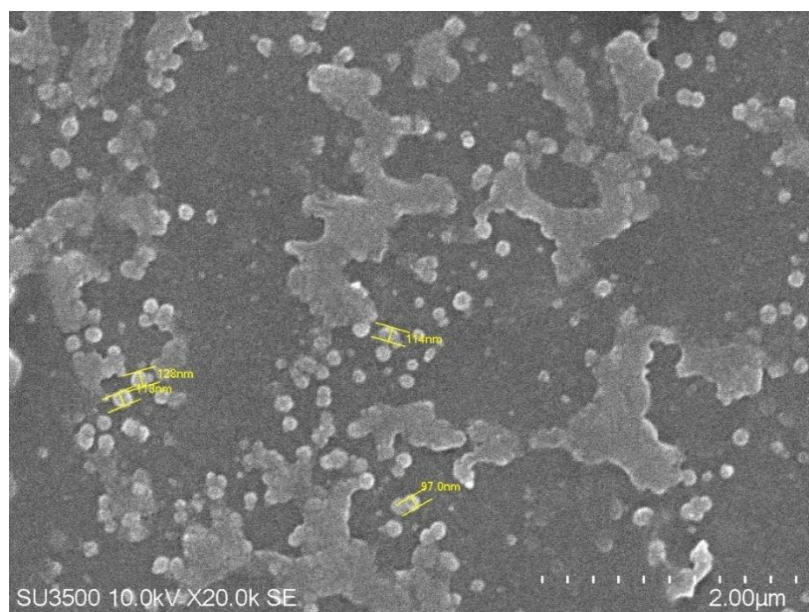


Fig. 4 Scanning electron microscopy (SEM) image showing the surface morphology of targeted DCA-loaded nanoliposomes with magnification of 20.000x

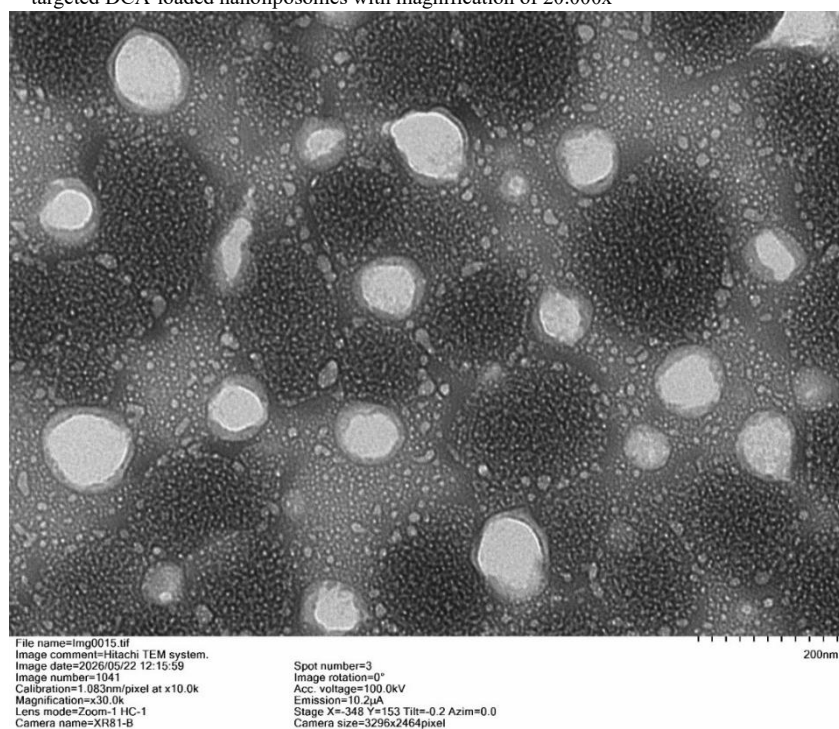


Fig. 5 Transmission electron microscopy (TEM) image of targeted DCA-loaded nanoliposomes with magnification of 10.000x

SEM analysis (**Fig. 4**) revealed the successful formation of nanosized liposomal particles distributed across the sample surface. Most particles exhibited a quasi-spherical morphology with relatively well-defined boundaries. Direct measurements of representative particles indicated diameters of approximately 97.0 nm, 114 nm, and 128 nm, confirming that the particle size was within the nanoscale range of approximately 100–130 nm. The particles appeared to be relatively uniformly distributed throughout the sample, although localized regions of mild aggregation were observed. Such aggregation is commonly attributed to sample preparation procedures for SEM, particularly dehydration under vacuum and sputter coating, which may induce partial collapse or fusion of the lipid vesicles. Consequently, the morphology observed by SEM may not fully represent the native hydrated structure of liposomes in suspension. The effective production of spherical unilamellar vesicles with uniform shape and smooth membrane architectures was further validated by TEM imaging (**Fig. 5**). The formulation procedure produced structurally stable nanoliposomes that could retain their nanoscale dimensions following hydration, as evidenced by the agreement between TEM observations and DLS measurements. Most vesicles appeared as discrete nanoparticles with diameters ranging from approximately 100 to 170 nm, which is consistent with the particle size distribution obtained from Dynamic Light Scattering (DLS) analysis. The slight variation in particle diameter observed by TEM reflects the inherent size distribution of liposomal formulations and remains within the expected nanoscale range for drug delivery applications.

The encapsulation efficiency (EE%) of DCA within the nanoliposomal formulation was determined using ultrafiltration followed by high-performance liquid chromatography (HPLC) analysis. As shown in **Fig. 6**, the chromatographic profile exhibited a single sharp and symmetrical peak with a retention time of 6.215 min, indicating the successful separation of DCA from other formulation components. The absence of interfering peaks demonstrates the high selectivity of the analytical method for DCA quantification.

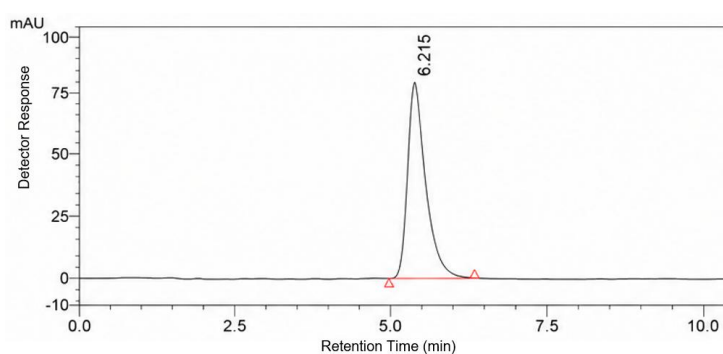


Fig. 6 Representative HPLC chromatogram of EE% nanoliposome deoxycholic acid (DCA) detected at 210 nm.

The HPLC system showed excellent chromatographic performance, with a tailing factor of 1.07 and 9136 theoretical plates, satisfying the system suitability criteria for quantitative analysis. The calibration curve demonstrated excellent linearity over the tested concentration range (0–200 ppm), yielding the regression equation $y = 14956x + 10321$ with a coefficient of determination ($R^2 = 0.9997$), confirming the reliability and accuracy of the analytical method for DCA determination. Quantitative analysis revealed that the total DCA concentration released after complete nanoliposome disruption was $500.00 \mu\text{g mL}^{-1}$, whereas the concentration of unencapsulated (free) DCA recovered in the ultrafiltrate was only $85.30 \mu\text{g mL}^{-1}$. Consequently, the encapsulated DCA concentration reached $414.70 \mu\text{g mL}^{-1}$, corresponding to an

encapsulation efficiency of 82.94%. An EE value exceeding 80% indicates that the thin-film hydration method efficiently incorporated DCA into the phospholipid bilayer. The high encapsulation efficiency can be attributed to the amphiphilic nature of deoxycholic acid, which enables favorable interactions with the phospholipid membrane through both hydrophobic interactions between its steroid backbone and lipid acyl chains, and hydrogen bonding involving its hydroxyl groups [21]. These molecular interactions stabilize DCA within the lipid bilayer and reduce premature leakage during formulation. Moreover, cholesterol incorporated into the lipid membrane likely contributed to the enhanced drug retention by increasing membrane packing density and reducing bilayer permeability[22]. Cholesterol is well known to decrease phospholipid membrane fluidity while improving membrane integrity, thereby minimizing passive diffusion of encapsulated molecules into the surrounding aqueous medium. This stabilizing effect is particularly important for maintaining DCA loading during storage and subsequent biological applications.

All of these results show that the created DCA-loaded nanoliposomes satisfy the physicochemical requirements needed for drug delivery to macrophages and offer the structural basis for assessing cellular absorption and biological reprogramming.

3.2 Cellular Uptake of DCA-Loaded Nanoliposomes by THP-1-Derived Macrophages

Efficient intracellular delivery represents one of the major challenges in nanomedicine because therapeutic efficacy depends on the amount of drug reaching intracellular targets. To evaluate whether targeted engineering improves cellular delivery, uptake of fluorescently labeled DCA-loaded nanoliposomes by THP-1-derived tumor-associated macrophages was investigated using fluorescence microscopy and flow cytometry.

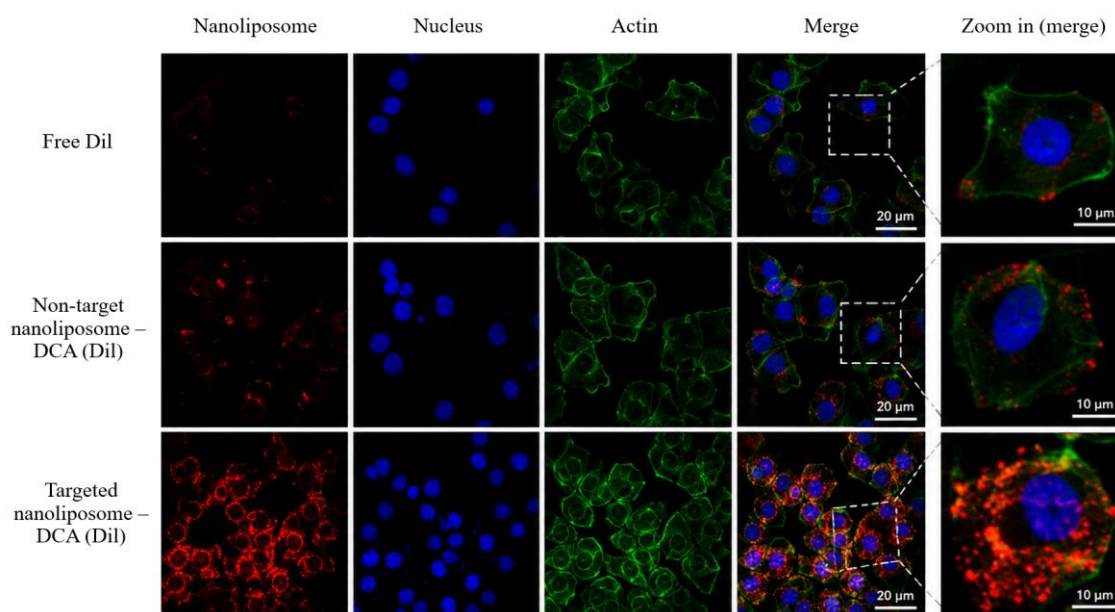


Fig. 7 Representative fluorescence microscopy images of THP-1-derived macrophages following incubation with free Dil, non-targeted DCA-loaded nanoliposomes, or targeted DCA-loaded nanoliposomes. Dil (red) was incorporated into the nanoliposome

membrane to monitor nanoparticle uptake, whereas nuclei and filamentous actin (F-actin) were stained with DAPI (blue) and phalloidin (green), respectively. The merged images and corresponding magnified regions demonstrate substantially greater intracellular accumulation of targeted nanoliposomes than free Dil or non-targeted nanoliposomes, confirming that surface functionalization significantly enhances macrophage uptake. Scale bars represent 20 μm in the merged images and 10 μm in the enlarged views.

Fluorescence imaging revealed a markedly stronger intracellular fluorescence signal (**Fig. 7**) in macrophages treated with targeted nanoliposomes compared with free DCA and non-targeted liposomes. The fluorescence was predominantly localized within the cytoplasm, suggesting successful internalization rather than surface adsorption. These observations indicate that ligand-mediated interactions substantially enhance nanoparticle uptake by macrophages. Quantitative flow cytometric analysis demonstrated a significant increase in mean fluorescence intensity (MFI) following treatment with targeted nanoliposomes. The higher intracellular fluorescence confirms that ligand functionalization effectively promotes receptor-mediated endocytosis[23]. Mannose modification is likely responsible for enhanced recognition by CD206 receptors, which are highly expressed on M2-like macrophages, thereby facilitating selective nanoparticle internalization[5]. Enhanced uptake is expected to increase intracellular DCA concentration, resulting in more effective inhibition of pyruvate dehydrogenase kinase (PDK) and subsequent metabolic reprogramming. Therefore, improved nanoparticle internalization provides a mechanistic explanation for the biological responses observed in the subsequent macrophage repolarization experiments.

3.3 Phenotypic Reprogramming of THP-1-Derived Tumor-Associated Macrophages

Since macrophage phenotype is tightly regulated by intracellular metabolism, enhanced DCA delivery is hypothesized to facilitate metabolic remodeling and promote the transition of tumor-associated macrophages from an immunosuppressive M2 phenotype toward a pro-inflammatory M1 phenotype. To verify this hypothesis, macrophage polarization was comprehensively evaluated through phenotypic marker expression, cytokine secretion, and metabolic gene analysis.

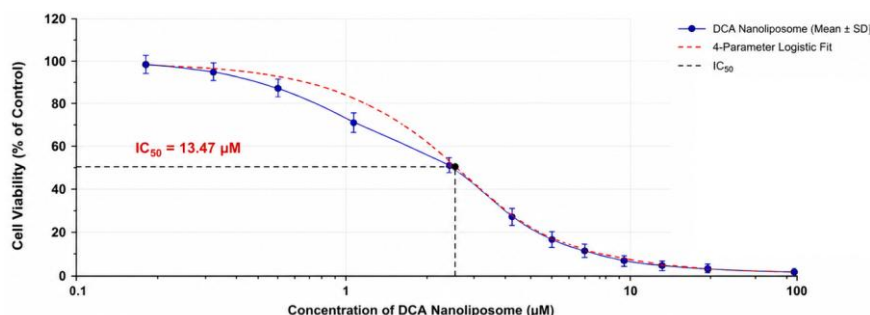


Fig. 8 Cytotoxicity profile of DCA-loaded nanoliposomes in THP-1-derived macrophages.

The cytotoxicity of DCA-loaded nanoliposomes toward THP-1-derived macrophages was evaluated using an MTT assay over a concentration range of 0.25–100 μM (**Fig. 8**). Cell viability remained above 90% at concentrations below 0.5 μM , indicating negligible cytotoxicity under low-dose exposure. A progressive reduction in cell viability was observed as the nanoliposome concentration increased, demonstrating a clear concentration-dependent response. Cell viability was determined after exposure to

increasing concentrations of DCA-loaded nanoliposomes using the MTT assay. Data are presented as the mean $\pm$ SD ($n = 3$). The dose–response relationship was fitted using a four-parameter logistic (4PL) model, yielding an IC_{50} value of 13.47 μ M. Cell viability decreased in a concentration-dependent manner, indicating that the cytotoxic effect of DCA-loaded nanoliposomes became more pronounced at higher concentrations. Nonlinear regression analysis using a four-parameter logistic model produced an IC_{50} value of 13.47 μ M, indicating that approximately half of the macrophage population remained viable at this concentration. The fitted dose–response curve closely matched the experimental data, suggesting good agreement between the mathematical model and the observed biological response. Importantly, macrophage viability remained relatively high at concentrations below the IC_{50} , whereas a sharp decline was observed at concentrations exceeding approximately 5–10 μ M, ultimately reaching less than 10% viability at the highest concentrations tested. These findings demonstrate that DCA-loaded nanoliposomes exhibit dose-dependent biological activity while maintaining acceptable cellular compatibility at lower concentrations.

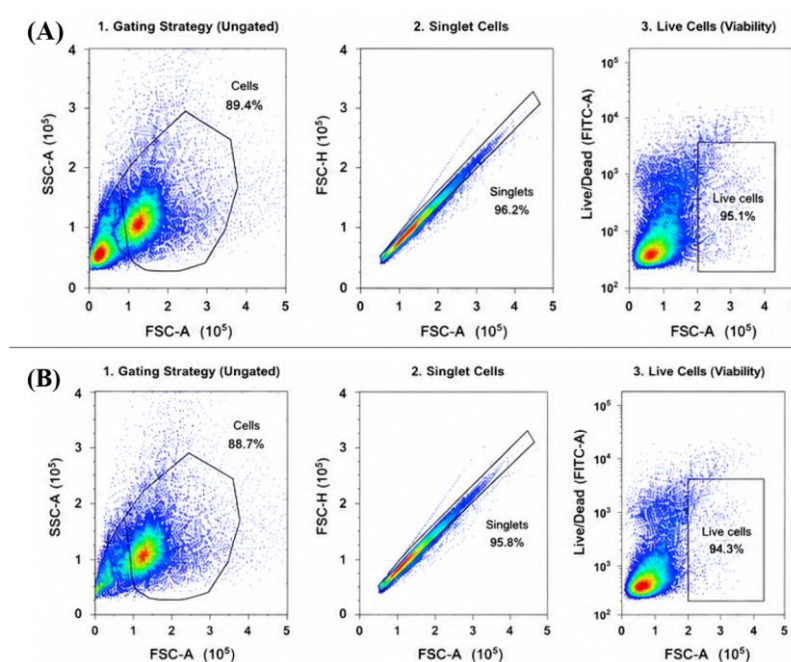


Fig. 9 Representative gating strategy used for flow cytometric analysis of THP-1-derived macrophages: (A) Control macrophages and (B) macrophages treated with DCA-loaded nanoliposome.

Following treatment with targeted DCA-loaded nanoliposomes, flow cytometric analysis showed a considerable decrease in CD206 and a significant rise in CD80 expression. Singlet discrimination using FSC-A against FSC-H was used to remove doublets after the initial cell populations were detected using FSC-A and SSC-A features to exclude debris. Following live labeling, viable cells were chosen for further examination of macrophage polarization markers (CD206 and CD80) (**Fig. 9**). These results show that THP-1-derived macrophages successfully repolarize from an M2-like phenotype to an M1 phenotype.

Interestingly, compared to free DCA, the tailored formulation generated a larger phenotypic shift, indicating that effective macrophage reprogramming requires improved intracellular drug delivery.

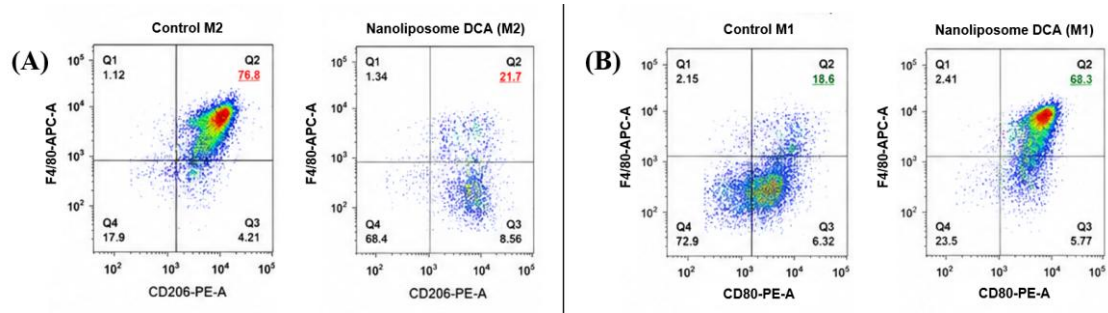


Fig. 10 Flow cytometric analysis of macrophage polarization following treatment with DCA-loaded nanoliposomes. (A) Representative dot plots of CD206 expression in M2 macrophages. (B) Representative dot plots of CD80 expression in M1 macrophages.

Untreated M2 macrophages demonstrated a high percentage of CD206⁺ cells (76.8%), as illustrated in **Fig. 10**, indicating effective polarization toward the immunosuppressive M2 phenotype. The percentage of CD206-positive macrophages significantly dropped to 21.7% after treatment with DCA-loaded nanoliposomes, indicating a 71.7% relative reduction (or 55.1 percentage points) in the M2 population. This significant decrease suggests that nanoliposomal DCA successfully reduced the immunosuppressive macrophage phenotype and inhibited the expression of M2-associated surface markers. Analysis of the M1 phenotype, however, showed the reverse pattern. Untreated macrophages showed a comparatively low number of CD80-positive cells (18.6%), but treatment with DCA-loaded nanoliposomes dramatically boosted the CD80-positive population to 68.3%. This indicates a strong stimulation of the pro-inflammatory macrophage phenotype and corresponds to a roughly 3.7-fold rise in CD80 expression.

The concurrent decrease in CD206 expression and increase in CD80 expression show that DCA-loaded nanoliposomes actively encourage phenotypic reprogramming from the M2 state toward the M1 phenotype rather than only suppressing M2 macrophages[8][5]. Because effective macrophage repolarization necessitates coordinated downregulation of anti-inflammatory markers together with elevation of pro-inflammatory markers rather than selective suppression of a particular phenotype[24], this distinction is biologically significant.

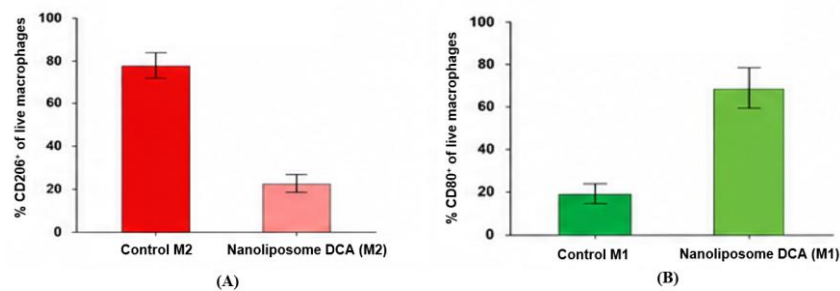


Fig. 11 Quantitative analysis of macrophage polarization following treatment with DCA-loaded nanoliposomes. Flow cytometric quantification of (A) CD206-positive (M2) macrophages and (B) CD80-positive (M1) macrophages.

The capacity of DCA-loaded nanoliposomes to alter macrophage polarization was further validated by quantitative flow cytometric analysis (**Fig. 11**). The successful formation of the alternatively activated macrophage phenotype was demonstrated by the high percentage of CD206-positive cells ($76.8 \pm 2.31\%$) in untreated M2 macrophages. The percentage of CD206-positive macrophages dropped dramatically to $21.7 \pm 1.98\%$ ($P < 0.001$) after treatment with DCA-loaded nanoliposomes, which is almost 71.7% less than the untreated control. On the other hand, after therapy, the percentage of CD80-positive macrophages—a characteristic of the classically activated M1 phenotype—rose noticeably. DCA-loaded nanoliposomes boosted CD80-positive cells from $18.6 \pm 1.72\%$ in untreated macrophages to $68.3 \pm 2.54\%$ ($P < 0.001$), a roughly 3.7-fold increase over the control group.

3.4 Proposed Mechanism of Targeted DCA-Loaded Nanoliposome-Mediated Macrophage Reprogramming

The collective findings of this study support a mechanistic model in which targeted engineering of DCA-loaded nanoliposomes enhances macrophage reprogramming through the sequential integration of receptor-mediated cellular uptake, intracellular metabolic remodeling, and immunophenotypic transition. Unlike free DCA, whose therapeutic efficacy is limited by nonspecific distribution, poor intracellular accumulation, and rapid diffusion, ligand-functionalized nanoliposomes provide a targeted delivery platform that substantially increases intracellular drug availability within tumor-associated macrophages (TAMs).

The first critical event in this mechanism is the preferential recognition of the targeted nanoliposomes by macrophage surface receptors. M2-like TAMs highly express scavenger and lectin receptors, particularly the mannose receptor (CD206)[25], which mediates receptor-dependent endocytosis. Functionalization of the nanoliposome surface with a macrophage-targeting ligand therefore facilitates selective nanoparticle binding and internalization[26]. The enhanced cellular uptake observed in this study suggests that ligand-mediated endocytosis represents the dominant pathway for nanoparticle entry, thereby overcoming one of the major limitations associated with passive diffusion [19] of free DCA. Consequently, intracellular DCA concentrations become substantially higher than those achieved by soluble drug administration. Following intracellular internalization, nanoliposomes undergo endosomal trafficking, where gradual destabilization of the lipid bilayer promotes controlled DCA release into the cytoplasm. Sustained intracellular drug release is expected to maintain effective inhibition of pyruvate dehydrogenase kinase (PDK) [27], resulting in continuous activation of the pyruvate dehydrogenase (PDH) complex. This metabolic switch redirects pyruvate flux away from aerobic glycolysis and toward mitochondrial oxidative phosphorylation (OXPHOS)[28], thereby reversing one of the hallmark metabolic adaptations characteristic of immunosuppressive TAMs[29].

The current formulation stands out from earlier macrophage-targeting liposomal systems due to both its intended biological application[30] and its advantageous physicochemical properties[31]. The targeted DCA-loaded nanoliposomes created in this work were especially made to trigger immunometabolic reprogramming of THP-1-derived tumor-associated macrophages (TAMs), whereas previous research mostly concentrated on macrophage targeting [6], immune evasion [3], or general drug administration [15].

Therefore, a physicochemical basis for the increased cellular uptake and macrophage repolarization seen in later biological tests is provided by the combination of suitable particle size, relatively negative surface charge, and spherical morphology.

Table 1 Comparison of physicochemical characteristics with previously reported liposomal formulations

No.	Liposomal formulation	DLS size (nm)	Zeta potential (mV)	TEM morphology / size	Application	Reference
1	Unmodified liposomes	96.3 ± 9.4	-16.8 ± 0.8	Spherical vesicles	Macrophage targeting	[32]
2	Arginine-modified liposomes	~100–110	-8.9 to -33.9	Spherical vesicles	Macrophage targeting	[32]
3	Mannose-functionalized liposome nanoplatform	NR	NR	Uniform spherical liposomes	Macrophage depletion	[33]
4	Macrophage membrane-coated cubosomes	Increased after coating	-26.7 ± 0.4	Spherical nanoparticles	Immune evasion	[34]
5	Conventional PEGylated liposome (reviewed formulations)	99–115	-5.9 to -16.6	Spherical liposomes	Drug delivery	[35]
6	Gold–liposome nanohybrids	138.4 ± 1.4	-12.9 ± 6.6	Liposome–gold hybrid	Cellular uptake	[36]
7	Targeted DCA-loaded nanoliposomes	158 ± xx	-12.8 ± 0.6	Spherical vesicles (TEM: 155 ± 0.3 nm)	THP-1-derived TAM reprogramming	This study

The comparison shows that the new tailored DCA-loaded nanoliposomes offer a novel application for immunometabolic reprogramming of tumor-associated macrophages while having physicochemical characteristics consistent with well-established liposomal formulations. The originality and translational potential of the current formulation are highlighted by this combination of advantageous physicochemical properties and medicinal activity.

Metabolic reprogramming initiated by PDK inhibition subsequently induces profound changes in intracellular signaling pathways[37] that regulate macrophage polarization. Reduction of glycolytic dependence decreases stabilization of hypoxia-inducible factor-1 α (HIF-1 α)[38], leading to suppression of downstream genes associated with M2 polarization. Simultaneously, restoration of mitochondrial oxidative metabolism enhances ATP production [39] and improves mitochondrial membrane potential while reducing excessive reactive oxygen species (ROS) [6][40] generation associated with dysfunctional mitochondrial respiration [41]. These mitochondrial adaptations restore metabolic homeostasis [42] and create an intracellular environment favorable for pro-inflammatory macrophage activation. The metabolic remodeling observed following DCA delivery is closely linked to transcriptional reprogramming of macrophage phenotype. Activation of oxidative metabolism promotes NF- κ B [24] and STAT1-associated inflammatory signaling while attenuating STAT6- and PPAR γ -mediated pathways that normally sustain the M2 phenotype[20][37]. As a consequence, expression of classical M1 markers, including CD80 [25] is markedly increased, whereas expression of M2-associated markers such as CD206 [25] is significantly

suppressed. These molecular changes collectively demonstrate that metabolic intervention functions as an upstream regulator of macrophage phenotype rather than merely altering cytokine secretion [43].

The current results imply that the primary factor influencing biological efficacy is improved intracellular delivery. Free DCA has intrinsic metabolic action, but it cannot sustainably suppress intracellular PDK signaling due to its limited cellular accumulation. Targeted nanoliposomes, on the other hand, offer concentrated intracellular drug deposition that intensifies metabolic disruption and results in a stronger phenotypic conversion of TAMs. This finding highlights how nanoparticle engineering optimizes medication bioavailability at the cellular level, thereby actively modulating therapeutic performance in addition to acting as a drug carrier[15]. The suggested mechanism anticipates significant alteration of the tumor immune microenvironment in addition to macrophage polarization. Through increased expression of co-stimulatory molecules, pro-inflammatory cytokines that attract cytotoxic immune cells, and decreased production of immunosuppressive mediators that typically prevent T-cell activation[10], reprogrammed macrophages improve their ability to deliver antigens[5]. Therefore, the positive feedback loop between tumor cells and immunosuppressive macrophages may be broken by converting M2-like TAMs to an M1 phenotype[34][44]. This would restore immune surveillance and enhance response to anticancer treatments[45], such as immune checkpoint blockade[3]. When considered collectively, the suggested mechanism shows that, as opposed to a single chemical event, targeted DCA-loaded nanoliposomes operate via a hierarchical biological cascade. The modified nanocarrier first improves receptor-mediated uptake, then raises intracellular DCA bioavailability, activates pro-inflammatory transcriptional networks, causes mitochondrial metabolic rewiring by blocking the PDK–PDH axis, and finally promotes stable repolarization of tumor-associated macrophages toward an antitumor phenotype. Targeted DCA-loaded nanoliposomes are positioned by this integrated mechanism as a promising immunometabolic nanotherapeutic platform that can both modify the tumor microenvironment and overcome intracellular drug delivery hurdles.

4. Conclusion

Targeted DCA-loaded nanoliposomes were successfully formulated with favorable physicochemical characteristics, including a mean particle size of 158 nm, a polydispersity index (PDI) of 0.28, a moderately negative zeta potential (−12.8 mV), spherical morphology confirmed by TEM, and a high encapsulation efficiency of 82.94%, indicating efficient drug incorporation and good colloidal stability. The nanoliposomal formulation demonstrated concentration-dependent cytotoxicity with an IC_{50} of 13.47 μ M, providing an appropriate concentration range for biological evaluation. Fluorescence microscopy confirmed enhanced intracellular uptake of targeted nanoliposomes by THP-1-derived macrophages, while flow cytometric analysis demonstrated a marked reduction in CD206⁺ (M2) macrophages accompanied by a significant increase in CD80⁺ (M1) macrophages. Collectively, these findings demonstrate that targeted DCA-loaded nanoliposomes effectively deliver DCA into macrophages and promote immunometabolic reprogramming from the M2 toward the M1 phenotype. This nanoplatform represents a promising strategy for macrophage-targeted immunotherapy and provides a strong foundation for further in vivo validation and translational development.

Acknowledgements This research is funded by the Indonesian Endowment Fund for Education (LPDP) on behalf of the Indonesian Ministry of Higher Education, Science and Technology and managed under the

EQUITY Program (Contract No. 4300/B3/DT.03.08/2025; No. 297/UN3/HK.07.00/2025) and International Research Network (IRN) scheme No. 5534/B/UN3.LPPM/PT.01.03/2025). The authors express their gratitude to the LPDP, BRIN, and Research Center for Nanotechnology Technologies (PNN) ITB, Cytogenic Laboratory of Padjadjaran University, Chemical Chemistry Laboratory of Institut Kesehatan Rajawali, for their support for research facilities and Cyberjaya University for they support for research design.

Author contributions All authors contributed to the study conception and design. Material preparation, data collection, and analysis were performed by Win Darmanto, Sri Puji Astuti Wahyuningsih, Firli Rahmah Primula Dewi, Ally Kafesa, Moch. Aqilah Herdiansyah, RR. Aulia Rahmawati Kusuma Putri, Liew Kai Bin, Intan Zarina Zainol Abidin. The first draft of the manuscript was written by Ally Kafesa and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Funding This research is funded by the Indonesian Endowment Fund for Education (LPDP) on behalf of the Indonesian Ministry of Higher Education, Science and Technology and managed under the EQUITY Program (Contract No. 4300/B3/DT.03.08/2025; No. 297/UN3/HK.07.00/2025) and International Research Network (IRN) scheme No. 5534/B/UN3.LPPM/PT.01.03/2025).

References

- [1] D. Cruceriu, O. Baldasici, O. Balacescu, and I. Berindan-Neagoe, "The dual role of tumor necrosis factor- α (TNF- α) in breast cancer: molecular insights and therapeutic approaches," Feb. 01, 2020, *Springer*. doi: 10.1007/s13402-019-00489-1.
- [2] Y. Chen *et al.*, "Enhancing cancer immunotherapy: Nanotechnology-mediated immunotherapy overcoming immunosuppression," Sep. 01, 2024, *Chinese Academy of Medical Sciences*. doi: 10.1016/j.apsb.2024.05.032.
- [3] Y. He, Q. Hong, S. Chen, J. Zhou, and S. Qiu, "Reprogramming tumor-associated macrophages in gastric cancer: a pathway to enhanced immunotherapy," *Front. Immunol.*, vol. 16, no. March, pp. 1–12, 2025, doi: 10.3389/fimmu.2025.1558091.
- [4] X. Wang *et al.*, "Sijunzi decoction enhances sensitivity of colon cancer cells to NK cell destruction by modulating P53 expression," *J. Ethnopharmacol.*, vol. 329, Jul. 2024, doi: 10.1016/j.jep.2024.118115.
- [5] B. Gao, Z. Wang, and W. Tan, "Mannosylated nanocarriers: a precision targeting strategy for tumors and infectious diseases," *Front. Pharmacol.*, vol. 17, no. March, pp. 1–27, 2026, doi: 10.3389/fphar.2026.1744292.
- [6] Y. Zhang, S. Choksi, K. Chen, Y. Pobezinskaya, I. Linnoila, and Z. G. Liu, "ROS play a critical role in the differentiation of alternatively activated macrophages and the occurrence of tumor-associated macrophages," *Cell Res.*, vol. 23, no. 7, pp. 898–914, 2013, doi: 10.1038/cr.2013.75.
- [7] U. Bulbake, S. Doppalapudi, N. Kommineni, and W. Khan, "Liposomal formulations in clinical use: An updated review," *Pharmaceutics*, vol. 9, no. 2, pp. 1–33, 2017, doi: 10.3390/pharmaceutics9020012.
- [8] M. Paurević, M. Šrajer Gajdošik, and R. Ribić, "Mannose Ligands for Mannose Receptor Targeting," *Int. J. Mol. Sci.*, vol. 25, no. 3, 2024, doi: 10.3390/ijms25031370.

- [9] M. Esteves, P. Benilde, and S. Supervisor, "Interaction of two bile acids , a cytotoxic and a cytoprotective , with biomembrane models".
- [10] L. Cassetta and J. W. Pollard, "A timeline of tumour-associated macrophage biology," *Nat. Rev. Cancer*, vol. 23, no. 4, pp. 238–257, 2023, doi: 10.1038/s41568-022-00547-1.
- [11] B. K. Min *et al.*, "Pyruvate dehydrogenase kinase is a metabolic checkpoint for polarization of macrophages to the M1 phenotype," *Front. Immunol.*, vol. 10, no. MAY, pp. 1–14, 2019, doi: 10.3389/fimmu.2019.00944.
- [12] A. K. Meyers *et al.*, "Pyruvate dehydrogenase kinase supports macrophage NLRP3 inflammasome activation during acute inflammation," *Cell Rep.*, vol. 42, no. 1, p. 111941, 2023, doi: 10.1016/j.celrep.2022.111941.
- [13] N. Anand, K. H. Peh, and J. M. Kolesar, "Macrophage Repolarization as a Therapeutic Strategy for Osteosarcoma," *Int. J. Mol. Sci.*, vol. 24, no. 3, 2023, doi: 10.3390/ijms24032858.
- [14] X. Geeraerts, E. Bolli, S. M. Fendt, and J. A. Van Ginderachter, "Macrophage metabolism as therapeutic target for cancer, atherosclerosis, and obesity," *Front. Immunol.*, vol. 8, no. March, 2017, doi: 10.3389/fimmu.2017.00289.
- [15] L. Shi and H. Gu, "Emerging nanoparticle strategies for modulating tumor-associated macrophage polarization," *Biomolecules*, vol. 11, no. 12, 2021, doi: 10.3390/biom11121912.
- [16] M. R. Jalali, D. Kaushik, S. Verma, and H. Singh, "A coupled model of finite element method and Mie theory for heat transfer inside expanded perlite vacuum insulation panels (VIPs) at high temperatures," *Int. J. Heat Mass Transf.*, vol. 219, Feb. 2024, doi: 10.1016/j.ijheatmasstransfer.2023.124885.
- [17] M. A. AbuDalo, I. R. Al-Mheidat, A. W. Al-Shurafat, C. Grinham, and V. Oyanedel-Craver, "Synthesis of silver nanoparticles using a modified Tollens' method in conjunction with phytochemicals and assessment of their antimicrobial activity," *PeerJ*, vol. 2019, no. 2, 2019, doi: 10.7717/peerj.6413.
- [18] H. Nsairat, D. Khater, U. Sayed, F. Odeh, A. Al Bawab, and W. Alshaer, "Liposomes: structure, composition, types, and clinical applications," *Heliyon*, vol. 8, no. 5, p. e09394, 2022, doi: 10.1016/j.heliyon.2022.e09394.
- [19] M. S. Gatto, M. P. Johnson, and W. Najahi-Missaoui, "Targeted Liposomal Drug Delivery: Overview of the Current Applications and Challenges," *Life*, vol. 14, no. 6, 2024, doi: 10.3390/life14060672.
- [20] Y. Sun *et al.*, "Development of Liposome containing sodium deoxycholate to enhance oral bioavailability of itraconazole," *Asian J. Pharm. Sci.*, vol. 12, no. 2, pp. 157–164, 2017, doi: 10.1016/j.ajps.2016.05.006.
- [21] T. Sousa *et al.*, "Deoxycholic acid modulates cell death signaling through changes in mitochondrial membrane properties," *J. Lipid Res.*, vol. 56, no. 11, pp. 2158–2171, 2015, doi: 10.1194/jlr.M062653.
- [22] P. K. W. C. S. P. and K. V. P. Srikoorn, "Deoxycholic acid-formulated curcumin enhances caspase 3/7-dependent apoptotic induction in cholangiocarcinoma cell lines," *J. Basic Appl. Pharmacol.*, vol. 1, no. 1, pp. 1–9, 2021.

- [23] C. Kelly, C. Jefferies, and S.-A. Cryan, "Targeted Liposomal Drug Delivery to Monocytes and Macrophages," *J. Drug Deliv.*, vol. 2011, pp. 1–11, 2011, doi: 10.1155/2011/727241.
- [24] Y. Liu *et al.*, "The role of deoxycholic acid in macrophage polarization and remodeling of the tumor microenvironment during colorectal adenoma-carcinoma transition," *Cancer Metab.*, vol. 13, no. 1, 2025, doi: 10.1186/s40170-025-00416-z.
- [25] J. Ye *et al.*, "Drug-free mannosylated liposomes inhibit tumor growth by promoting the polarization of tumor-associated macrophages," *Int. J. Nanomedicine*, vol. 14, pp. 3203–3220, 2019, doi: 10.2147/IJN.S207589.
- [26] P. Opanasopit, Y. Higuchi, S. Kawakami, F. Yamashita, M. Nishikawa, and M. Hashida, "Involvement of serum mannan binding proteins and mannose receptors in uptake of mannosylated liposomes by macrophages," *Biochim. Biophys. Acta - Biomembr.*, vol. 1511, no. 1, pp. 134–145, 2001, doi: 10.1016/S0005-2736(01)00267-X.
- [27] C. Li *et al.*, "Pyruvate dehydrogenase kinase regulates macrophage polarization in metabolic and inflammatory diseases," *Front. Immunol.*, vol. 14, no. December, pp. 1–10, 2023, doi: 10.3389/fimmu.2023.1296687.
- [28] J. Van den Bossche, J. Baardman, and M. P. J. de Winther, "Metabolic characterization of polarized M1 and M2 bone marrow-derived macrophages using real-time extracellular flux analysis," *J. Vis. Exp.*, vol. 2015, no. 105, pp. 1–7, 2015, doi: 10.3791/53424.
- [29] N. N. Pavlova and C. B. Thompson, "The Emerging Hallmarks of Cancer Metabolism," *Cell Metab.*, vol. 23, no. 1, pp. 27–47, 2016, doi: 10.1016/j.cmet.2015.12.006.
- [30] G. Bozzuto and A. Molinari, "Liposomes as nanomedical devices," *Int. J. Nanomedicine*, vol. 10, pp. 975–999, 2015, doi: 10.2147/IJN.S68861.
- [31] L. Sercombe, T. Veerati, F. Moheimani, S. Y. Wu, A. K. Sood, and S. Hua, "Advances and challenges of liposome assisted drug delivery," *Front. Pharmacol.*, vol. 6, no. DEC, pp. 1–13, 2015, doi: 10.3389/fphar.2015.00286.
- [32] L. Ma, H. C. Bygd, and K. M. Bratlie, "Improving selective targeting to macrophage subpopulations through modifying liposomes with arginine based materials," *Integr. Biol. (United Kingdom)*, vol. 9, no. 1, pp. 58–67, 2017, doi: 10.1039/c6ib00133e.
- [33] T. H. Choi *et al.*, "Development of finely tuned liposome nanoplatfrom for macrophage depletion," *J. Nanobiotechnology*, vol. 22, no. 1, pp. 1–13, 2024, doi: 10.1186/s12951-024-02325-7.
- [34] A. S. Dossou *et al.*, "Mannose-Coated Reconstituted Lipoprotein Nanoparticles for the Targeting of Tumor-Associated Macrophages: Optimization, Characterization, and In Vitro Evaluation of Effectiveness," *Pharmaceutics*, vol. 15, no. 6, 2023, doi: 10.3390/pharmaceutics15061685.
- [35] G. Della Camera, D. Lipsa, D. Mehn, P. Italiani, D. Boraschi, and S. Gioria, "A step-by-step approach to improve clinical translation of liposome-based nanomaterials, a focus on innate immune and inflammatory responses," *Int. J. Mol. Sci.*, vol. 22, no. 2, pp. 1–24, 2021, doi: 10.3390/ijms22020820.
- [36] A. P. Mathew *et al.*, "The paradox of gold–liposome nanohybrids: the location of gold governs unconventional properties and drives cellular behavior," *Mater. Horizons*, vol. 13, no. 9, pp.

4526–4545, 2026, doi: 10.1039/d5mh02229k.

- [37] L. He, P. K. H. Tam, and C. X. Deng, “Orchestration of Tumor-Associated Macrophages in the Tumor Cell-Macrophage-CD8⁺ T Cell Loop for Cancer Immunotherapy,” *Int. J. Biol. Sci.*, vol. 21, no. 9, pp. 4098–4116, 2025, doi: 10.7150/ijbs.115932.
- [38] A. Viola, F. Munari, R. Sánchez-Rodríguez, T. Scolaro, and A. Castegna, “The metabolic signature of macrophage responses,” *Front. Immunol.*, vol. 10, no. JULY, pp. 1–16, 2019, doi: 10.3389/fimmu.2019.01462.
- [39] E. M. Pålsson-McDermott and L. A. J. O’Neill, “Targeting immunometabolism as an anti-inflammatory strategy,” *Cell Res.*, vol. 30, no. 4, pp. 300–314, 2020, doi: 10.1038/s41422-020-0291-z.
- [40] Q. Li, J. Xu, R. Hua, H. Xu, Y. Wu, and X. Cheng, “Nano-strategies for Targeting Tumor-Associated Macrophages in Cancer immunotherapy,” *J. Cancer*, vol. 16, no. 7, pp. 2261–2274, 2025, doi: 10.7150/jca.108194.
- [41] A. K. Jha *et al.*, “Network integration of parallel metabolic and transcriptional data reveals metabolic modules that regulate macrophage polarization,” *Immunity*, vol. 42, no. 3, pp. 419–430, 2015, doi: 10.1016/j.immuni.2015.02.005.
- [42] M. Orecchioni, Y. Ghosheh, A. B. Pramod, and K. Ley, “Macrophage polarization: Different gene signatures in M1(Lps⁺) vs. Classically and M2(LPS⁻) vs. Alternatively activated macrophages,” *Front. Immunol.*, vol. 10, no. MAY, pp. 1–14, 2019, doi: 10.3389/fimmu.2019.01084.
- [43] P. J. Murray, “Macrophage Polarization,” *Annu. Rev. Physiol.*, vol. 79, pp. 541–566, 2017, doi: 10.1146/annurev-physiol-022516-034339.
- [44] P. Italiani and D. Boraschi, “From monocytes to M1/M2 macrophages: Phenotypical vs. functional differentiation,” *Front. Immunol.*, vol. 5, no. OCT, pp. 1–22, 2014, doi: 10.3389/fimmu.2014.00514.
- [45] Y. Gong, W. Gao, J. Zhang, X. Dong, D. Zhu, and G. Ma, “Engineering nanoparticles-enabled tumor-associated macrophages repolarization and phagocytosis restoration for enhanced cancer immunotherapy,” *J. Nanobiotechnology*, vol. 22, no. 1, pp. 1–11, 2024, doi: 10.1186/s12951-024-02622-1.