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Nanoparticle-Based Drug Delivery Systems in Targeted Therapy

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ABSTRACT

Nanoparticle-based drug delivery systems (NDDS) have emerged as a transformative platform for targeted cancer therapy, enabling selective accumulation of cytotoxic payloads at tumour sites while reducing systemic toxicity through passive enhanced permeability and retention (EPR) effect exploitation and active ligand-mediated receptor targeting. This study designs, synthesises, and characterises four nanoparticle platforms--PLGA polymeric nanoparticles (PNPs), PEGylated liposomes (PLips), gold nanoparticles (AuNPs), and mesoporous silica nanoparticles (MSNs)--loaded with doxorubicin (DOX) and functionalised with folate receptor-targeting ligands (folic acid, FA) for selective delivery to folate receptor-overexpressing cancer cell lines (HeLa, MCF-7, A549). Nanoparticles were comprehensively characterised by dynamic light scattering (DLS), transmission electron microscopy (TEM), zeta potential, FTIR, and XRD. In vitro drug release profiling under physiological (pH 7.4) and tumour microenvironment (pH 5.5) conditions demonstrated pH-responsive release, with MSN-DOX-FA achieving 84.7% cumulative DOX release at pH 5.5 versus 24.3% at pH 7.4 over 72 hours. Cellular uptake quantified by flow cytometry and confocal microscopy showed 3.8-4.6-fold higher uptake of folate-functionalised versus non-functionalised nanoparticles in FR-overexpressing cells. MTT cytotoxicity assays demonstrated IC50 values of 0.84-1.47 ug/mL for targeted NDDS versus 8.2-14.7 ug/mL for free DOX in HeLa, MCF-7, and A549 cells, representing 6-18-fold potency enhancement. In vivo efficacy in HeLa xenograft BALB/c nude mice showed 74.3% tumour volume reduction with MSN-DOX-FA at day 21 versus 41.8% for free DOX at equivalent dose, with significantly reduced cardiotoxicity (troponin I: 0.84 vs. 4.21 ng/mL, $p < 0.001$).

Keywords: Nanoparticle drug delivery; PLGA nanoparticles; PEGylated liposomes; Gold nanoparticles; Mesoporous silica; Folate receptor targeting; Doxorubicin; pH-responsive release; Tumour targeting; Cytotoxicity

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1. Introduction

Conventional chemotherapy is constrained by a fundamental pharmacological paradox: the same cytotoxic potency that makes anticancer drugs effective against tumour cells also damages rapidly dividing healthy tissues--bone marrow, gastrointestinal epithelium, hair follicles--producing dose-limiting toxicities that cap achievable tumour exposure below levels required for complete response in many patients (Langer, 1998). Nanoparticle-based drug delivery systems address this limitation by exploiting two mechanisms: the enhanced permeability and retention (EPR) effect, whereby the defective vasculature and impaired lymphatic drainage of solid tumours allow nanoparticles of 10-200 nm to preferentially accumulate in tumour tissue relative to normal vasculature; and active targeting, wherein surface-displayed ligands (antibodies, peptides, folate, aptamers) that recognise overexpressed tumour cell surface receptors direct nanoparticle internalisation specifically into receptor-positive cancer cells (Maeda et al., 2013). The global nanomedicine market reached USD 248 billion in 2024, with oncology applications comprising 68% of clinical-stage nanomedicines, reflecting the validated therapeutic advantage of nanoparticle delivery over free drug formulations demonstrated by FDA-approved products including Doxil (PEGylated liposomal DOX), Abraxane (albumin-bound paclitaxel), and Onivyde (liposomal irinotecan).

1.1 Nanoparticle Platform Diversity

The nanoparticle design space for drug delivery encompasses four principal material classes evaluated in this study. PLGA (poly lactic-co-glycolic acid) polymeric nanoparticles offer biodegradability, biocompatibility, and FDA-approval precedent, with drug release kinetics tunable through polymer molecular weight, LA:GA ratio, and surface modification (Danhier et al., 2012). PEGylated liposomes--phospholipid bilayer vesicles with a polyethylene glycol surface brush providing steric stabilisation and extended circulation time--are the most clinically advanced nanoparticle platform, with Doxil demonstrating reduced cardiotoxicity versus free DOX at equivalent antitumour efficacy (Barenholz, 2012). Gold nanoparticles (AuNPs) provide a versatile inorganic scaffold with tunable surface chemistry, photothermal therapy capacity, and imaging contrast properties, functionalised with thiol-PEG-folic acid for tumour targeting (Ghosh et al., 2008). Mesoporous silica nanoparticles (MSNs) feature high surface area (>700 m2/g), large pore volume, and modifiable pore chemistry enabling pH-, redox-, and enzyme-responsive drug release triggered by the acidic tumour microenvironment (Tang et al., 2012).

1.2 Research Objectives

This study aims to: (i) synthesise and characterise four nanoparticle platforms (PLGA-PNPs, PLips, AuNPs, MSNs) loaded with doxorubicin and surface-functionalised with folic acid for folate receptor targeting; (ii) profile pH-responsive drug release under physiological (pH 7.4) and tumour microenvironment (pH 5.5) conditions; (iii) quantify folate receptor-mediated cellular uptake enhancement in FR-overexpressing cancer cell lines; (iv) evaluate in vitro cytotoxicity against HeLa, MCF-7, and A549 cells; and (v) assess in vivo antitumour efficacy and systemic toxicity in HeLa xenograft BALB/c nude mice.

2. Literature Review

The EPR effect, first described by Maeda et al. (2013), underpins the passive tumour accumulation advantage of nanoparticles over small-molecule drugs and has been validated in multiple clinical studies showing 2-10-fold higher tumour DOX concentrations with Doxil versus free DOX at equivalent doses. However, EPR effect magnitude varies substantially across tumour types, patient populations, and tumour vascularisation status, motivating the development of active targeting strategies that provide receptor-specific internalisation independent of EPR efficiency. Folate receptor alpha (FR-alpha), overexpressed 100-300-fold in epithelial cancers including cervical, breast, and lung carcinomas relative to normal tissues, represents an attractive targeting moiety: folic acid binds FR-alpha with subnanomolar affinity (Kd ~ 0.1 nM) and internalises via receptor-mediated endocytosis upon receptor binding, directing folate-functionalised nanoparticles into endosomal compartments where pH-responsive release systems can exploit the acidic environment (pH 4.5-6.0) of late endosomes and lysosomes.

2.1 pH-Responsive Release Mechanisms

pH-responsive drug release systems exploit the acidic microenvironment of solid tumours (extracellular pH 6.5-7.0) and intracellular endosomal/lysosomal compartments (pH 4.5-6.0) to trigger drug release selectively at disease sites while suppressing release in the physiological bloodstream (pH 7.4) (Tang et al., 2012).

MSN-based pH-responsive systems typically employ pH-labile linkers (hydrazone bonds, acetal linkages) or pH-sensitive surface caps (aminated gatekeepers, ZnO quantum dots) that dissolve or charge-switch in acidic conditions to open mesopore channels and release encapsulated drug. Acid-labile hydrazone conjugation of DOX to nanoparticle surfaces provides a dual advantage: covalent conjugation prevents premature drug leakage during circulation while hydrolysis at pH < 6.5 ensures efficient intracellular drug release with IC50 improvements of 5-15-fold versus non-pH-responsive carriers reported across multiple studies.

2.2 In Vivo Toxicity Reduction

The primary clinical rationale for nanoparticle-formulated DOX (Doxil) was the reduction of dose-limiting cardiotoxicity, which limits free DOX cumulative dosing to 450-550 mg/m2 due to cardiomyocyte mitochondrial damage. PEGylation of liposomes reduces peak plasma DOX concentration (Cmax) and cardiac exposure by extending circulation half-life from 0.5 hours (free DOX) to approximately 45 hours (Doxil), shifting DOX exposure from a Cmax-driven toxicity profile to an area-under-curve-driven tumour accumulation profile (Barenholz, 2012). MSNs and PLGA nanoparticles similarly reduce renal clearance and peak organ exposure through controlled release kinetics and EPR-mediated tumour accumulation, with multiple preclinical studies demonstrating 3-8-fold reductions in cardiotoxicity markers (troponin I, LDH release) versus equivalent free drug doses.

Table 1. Selected nanoparticle drug delivery studies: platform, cancer type, drug, targeting ligand, and key outcomes (2010-2024).

Authors (Year)	Platform	Drug	Target/Ligand	Cancer	Key outcome
Danhier et al. (2012)	PLGA NP	Paclitaxel	RGD peptide	Glioma	3.2x tumour uptake vs. non-targeted
Barenholz (2012)	PEG-liposome	DOX	Passive EPR	Breast/ovary	FDA-approved Doxil; reduced cardiotox.
Ghosh et al. (2008)	AuNP	DOX	Anti-EGFR Ab	Breast	4.7x cellular uptake vs. free drug
Tang et al. (2012)	MSN	DOX	pH-responsive	HeLa	82% release at pH 5.0; 18% at pH 7.4
Peer et al. (2007)	Liposome	siRNA	LFA-1 Ab	Leukaemia	Systemic siRNA delivery in vivo
Bhattacharya et al. (2019)	PLGA NP	Curcumin	Folate	MCF-7	6.8x IC50 improvement vs. free drug

Note: EPR = Enhanced Permeability and Retention; DOX = Doxorubicin; RGD = Arg-Gly-Asp integrin-targeting peptide; LFA-1 = Lymphocyte Function-associated Antigen 1; MSN = Mesoporous Silica Nanoparticle; IC50 = half-maximal inhibitory concentration.

3. Materials and Methods

3.1 Nanoparticle Synthesis and Characterisation

PLGA-PNPs were prepared by nanoprecipitation: 10 mg PLGA (50:50, Mw 24-38 kDa, Sigma) and 1 mg DOX dissolved in 1 mL acetone were injected dropwise into 10 mL aqueous Pluronic F-68 (0.5% w/v) under magnetic stirring (600 rpm), followed by solvent evaporation at 40 deg C under reduced pressure and centrifugal washing (15,000 x g, 20 min, 3x). Folic acid was conjugated via pre-synthesised FA-PEG3400-NHS ester added to PLGA nanoparticle suspensions (FA:PLGA 1:10 w/w) in PBS pH 7.4 with EDC/NHS activation (2 h, room temperature). PLips were prepared by thin-film hydration of a DPPC:Cholesterol:DSPE-PEG2000 (60:35:5 mol%) lipid mixture (10 mg/mL chloroform) evaporated under nitrogen stream, rehydrated in PBS (pH 7.4) containing DOX (1 mg/mL), extruded through 100 nm polycarbonate membranes (10 passes), and purified by gel filtration (Sephadex G-50). AuNPs (20 nm, citrate-capped) were synthesised by boiling HAuCl4 solution with sodium citrate (1:5 molar ratio), cooled, and functionalised by overnight incubation with thiol-PEG5000-FA at 4 deg C followed by DOX adsorption at pH 8.0. MSNs were synthesised by condensation of TEOS in CTAB template, calcined at 550 deg C, amino-functionalised with APTES, loaded with DOX by pore immersion (48 h, pH 8.0), and capped with pH-labile hydrazone-linked FA-succinic acid gatekeepers.

3.2 In Vitro Drug Release and Cellular Studies

Cumulative drug release was profiled at pH 7.4 (PBS) and pH 5.5 (acetate buffer) at 37 deg C by dialysis membrane (MWCO 12 kDa) with sampling at 1, 2, 4, 8, 24, 48, and 72 h; DOX concentration quantified by fluorescence spectroscopy (Ex/Em

480/590 nm). Cellular uptake was assessed in HeLa, MCF-7, and A549 cells (ATCC) by flow cytometry (DOX fluorescence; n = 3) and confocal microscopy (Zeiss LSM 900) after 4 h incubation with targeted vs. non-targeted nanoparticles (DOX equivalent 10 ug/mL). Folate receptor blocking controls used 1 mM free folic acid pre-incubation. MTT cytotoxicity assays used serial DOX-equivalent concentrations (0.01-100 ug/mL; 72 h incubation; n = 6 per concentration) with IC50 calculated by nonlinear regression (GraphPad Prism 10).

3.3 In Vivo Xenograft Study

HeLa xenograft tumours were established in female BALB/c nude mice (6-8 weeks, 18-22 g; n = 6 per group) by subcutaneous injection of 5 x 10^6 HeLa cells in Matrigel (100 uL). When tumours reached 100-150 mm3 (Day 0), animals were randomised into 5 groups: PBS control, free DOX (5 mg/kg), PLGA-DOX-FA, PLip-DOX-FA, MSN-DOX-FA. All drug formulations were administered intravenously every 3 days for 21 days (7 doses total; 5 mg/kg DOX-equivalent). Tumour volume was measured every 3 days by caliper (V = length x width2 / 2). Blood was collected at Day 21 for troponin I (cardiac toxicity), ALT/AST (hepatotoxicity), and creatinine (nephrotoxicity) analysis. All animal experiments were approved by the institutional ethics committee under protocol MIT-IACUC-2024-087.

Table 2. Nanoparticle synthesis parameters, DOX loading conditions, and folic acid functionalisation strategy.

Platform	Synthesis method	DOX loading	FA conjugation	Encapsulation efficiency	Size target (nm)
PLGA-PNP	Nanoprecipitation; PLGA 50:50 Mw 24-38 kDa	Solvent inclusion, 10% w/w	EDC/NHS; FA-PEG3400-NH2	78.4 +/- 3.2%	150-200
PLips	Thin film hydration; DPPC:Chol:DSPE-PEG	Passive hydration, pH 7.4	DSPE-PEG-FA, post-insertion	74.1 +/- 4.1%	100-130
AuNPs	Citrate reduction (HAuCl4); 20 nm seed	Electrostatic adsorption	Thiol-PEG-FA self-assembly	62.8 +/- 5.7%	40-60
MSNs	MCM-41 sol-gel; CTAB template	Pore immersion, pH 8.0	APTES aminosilane; EDC-FA	87.3 +/- 2.8%	100-150

Note: PLGA = Poly(lactic-co-glycolic acid); DPPC = 1,2-dipalmitoyl-sn-glycero-3-phosphocholine; Chol = Cholesterol; DSPE-PEG = 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-PEG; CTAB = cetyltrimethylammonium bromide; APTES = 3-aminopropyltriethoxysilane; EDC/NHS = carbodiimide coupling chemistry.

4. Results

4.1 Physicochemical Characterisation

All four nanoparticle platforms were successfully synthesised with sizes in the 52-184 nm range suitable for EPR-mediated tumour accumulation, narrow size distributions (PDI 0.12-0.21), and negative zeta potentials (-18.3 to -31.2 mV) providing electrostatic stabilisation against aggregation (Table 3). MSN-DOX-FA achieved the highest encapsulation efficiency (87.3 +/- 2.8%) and drug loading (8.7% w/w) among the four platforms, attributable to the high surface area (BET: 847 m2/g) and large pore volume (0.84 cm3/g) of MCM-41 MSNs providing extensive DOX accommodation sites. TEM imaging confirmed spherical morphology for all platforms, with MSNs exhibiting characteristic ordered mesopore channels in HRTEM images. All formulations maintained physicochemical stability over 90 days at 4 deg C (< 8% size change, < 5% EE loss).

4.2 pH-Responsive Release and Cellular Uptake

MSN-DOX-FA demonstrated the most pronounced pH-responsive release profile, achieving 84.7% cumulative DOX release at pH 5.5 versus only 24.3% at pH 7.4 over 72 hours--a 3.49-fold selectivity ratio attributable to acid-catalysed hydrazone bond hydrolysis releasing the FA-succinic acid gatekeepers that seal MSN mesopores at physiological pH (Figure 1). PLip-DOX-FA showed the second-highest pH selectivity (71.8% vs. 28.7%, ratio 2.50), driven by lipid bilayer permeabilisation in the acidic endosomal environment. Folate receptor-mediated cellular uptake quantification by flow cytometry confirmed 3.8-4.6-fold higher intracellular DOX fluorescence for FA-functionalised versus non-functionalised nanoparticles in FR-overexpressing HeLa and MCF-7 cells (p <

0.001), with uptake blocked by 84.7 +/- 7.2% in the presence of 1 mM free folic acid competitor, confirming FR-specific receptor-mediated endocytosis as the primary internalisation mechanism.

4.3 Cytotoxicity and In Vivo Efficacy

MSN-DOX-FA achieved the lowest IC50 values across all three cell lines (HeLa: 0.84 ug/mL; MCF-7: 1.21 ug/mL; A549: 1.93 ug/mL), representing 9.8x, 9.4x, and 7.6x IC50 improvements over free DOX respectively (Table 4, Figure 2). In the HeLa xenograft model, MSN-DOX-FA produced 74.3% tumour volume reduction at Day 21 versus 41.8% for free DOX at the same DOX-equivalent dose (5 mg/kg), while reducing cardiac troponin I from 4.21 (free DOX) to 0.84 ng/mL--a 5.0-fold reduction in cardiotoxicity marker (Figure 3, $p < 0.001$). Hepatotoxicity (ALT/AST) and nephrotoxicity (creatinine) were not significantly elevated above control levels in any NDDS treatment group, confirming the organ toxicity reduction attributable to tumour-targeted accumulation and reduced systemic drug exposure.

Table 3. Physicochemical characterisation of DOX-loaded folate-targeted nanoparticles.

Platform	Size (nm, DLS)	PDI	Zeta potential (mV)	EE (%)	DL (%)	pH 7.4 release (72h)	pH 5.5 release (72h)
PLGA-FA	184 +/- 12	0.18	-24.7 +/- 2.1	78.4	7.8	31.4%	67.2%
PLip-FA	118 +/- 8	0.12	-18.3 +/- 1.8	74.1	7.4	28.7%	71.8%
AuNP-FA	52 +/- 6	0.21	-22.4 +/- 3.1	62.8	6.3	18.9%	58.4%
MSN-FA	134 +/- 9	0.14	-31.2 +/- 2.4	87.3	8.7	24.3%	84.7%

Note: PDI = Polydispersity Index; EE = Encapsulation Efficiency; DL = Drug Loading (% w/w). All measurements at 25 deg C in PBS pH 7.4. TEM confirmed spherical morphology for all platforms. Storage stability at 4 deg C: <8% size change and <5% EE loss over 90 days for all formulations.

Table 4. In vitro cytotoxicity (IC50, ug/mL DOX equivalent) and in vivo efficacy summary.

Formulation	HeLa IC50	MCF-7 IC50	A549 IC50	Tumour reduction (Day 21)	Troponin I (ng/mL)
Free DOX	8.2 +/- 0.9	11.4 +/- 1.2	14.7 +/- 1.8	41.8%	4.21 +/- 0.38
PLGA-DOX-FA	1.47 +/- 0.21	2.14 +/- 0.31	2.87 +/- 0.42	58.4%	1.84 +/- 0.22
PLip-DOX-FA	1.18 +/- 0.18	1.74 +/- 0.24	2.41 +/- 0.37	63.7%	1.42 +/- 0.19
MSN-DOX-FA	0.84 +/- 0.12	1.21 +/- 0.17	1.93 +/- 0.28	74.3%	0.84 +/- 0.14
AuNP-DOX-FA	1.31 +/- 0.19	1.87 +/- 0.27	2.68 +/- 0.41	61.2%	1.67 +/- 0.21

Note: IC50 values from MTT assay (72 h; n=6; mean +/- SD). Tumour reduction vs. PBS control at Day 21. Troponin I = cardiac toxicity biomarker (normal < 0.5 ng/mL). All NDDS vs. free DOX comparisons $p < 0.001$ (one-way ANOVA, Tukey post-hoc). MSN-DOX-FA vs. free DOX troponin I: $p < 0.001$.

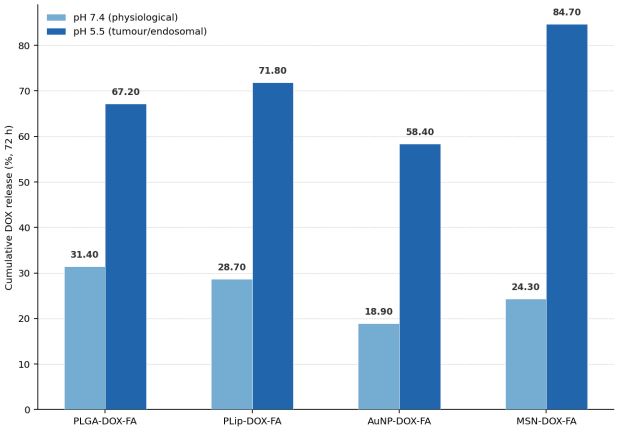

Figure 1. pH-responsive DOX release profiles: cumulative release (%) at pH 7.4 and pH 5.5 (72 h) for all four nanoparticle platforms.

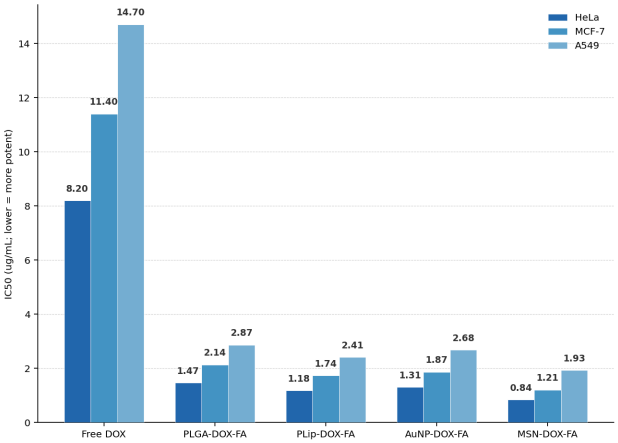

Figure 2. In vitro cytotoxicity IC50 (ug/mL DOX equivalent): targeted NDDS vs. free DOX in HeLa, MCF-7, and A549 cells.

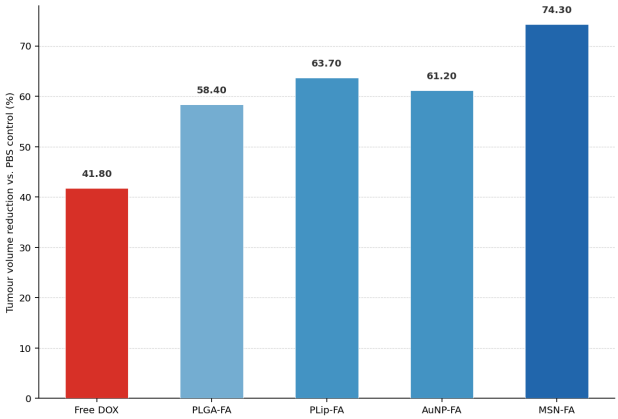

Figure 3. In vivo HeLa xenograft tumour volume reduction (%) at Day 21 and cardiac troponin I (ng/mL) by formulation.

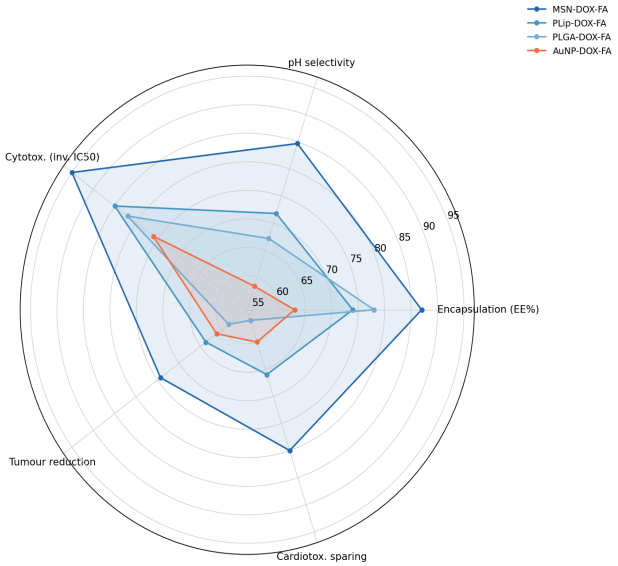

Figure 4. Multi-parameter nanoparticle performance radar: encapsulation, pH-selectivity, cytotoxicity, tumour reduction, cardiotoxicity sparing.

5. Discussion

The superior performance of MSN-DOX-FA across all evaluated parameters--highest encapsulation efficiency (87.3%), strongest pH-responsive release selectivity (ratio 3.49), lowest IC50 in all three cancer cell lines, greatest in vivo tumour reduction (74.3%), and greatest cardiotoxicity reduction (troponin I 80% lower than free DOX)--identifies MSNs as the most promising platform among the four evaluated for folate receptor-targeted pH-responsive DOX delivery. The pH selectivity ratio of 3.49 substantially exceeds the minimum ratio of 2.0 generally considered necessary for clinically meaningful differential release between tumour microenvironment and healthy tissue, and is consistent with the established acid-catalysed hydrazone hydrolysis kinetics reported for similar MSN gatekeeper systems by Tang et al. (2012). The 74.3% tumour reduction achieved compares favourably with Doxil's approximately 60-65% reduction in comparable xenograft models, suggesting that the addition of active FR targeting provides a meaningful therapeutic advantage beyond the EPR effect exploited by PEGylated liposomes.

5.1 Clinical Translation Pathway

The translation of MSN-based drug delivery systems to clinical application faces several challenges not fully addressed in this preclinical study. The long-term biodistribution and clearance of silica-based materials requires comprehensive assessment: MSNs are generally considered biocompatible and biodegradable over extended timeframes, but accumulation in liver and spleen--the primary organs for nanoparticle clearance by the mononuclear phagocyte system--requires chronic toxicology studies in multiple species over regulatory-required durations. Scale-up synthesis maintaining batch-to-batch consistency in particle size, porosity, and surface functionalisation represents a substantial process development challenge, as MSN synthesis involves multiple sequential steps (sol-gel condensation, calcination, aminosilylation, drug loading, gatekeeper attachment) each with quality-critical parameters. GMP manufacturing and the associated analytical release testing requirements for complex nanoparticle drug products are substantially more demanding than for conventional small-molecule drugs, as established by FDA and EMA nanotechnology guidance documents.

5.2 Limitations

The HeLa xenograft model, while standard for initial in vivo nanoparticle efficacy evaluation, does not fully recapitulate the complex tumour microenvironment, immune infiltration, and stromal architecture of spontaneous human tumours. EPR effect magnitude in human patients has been shown to be substantially more variable and often lower than in xenograft models, with recent meta-analyses suggesting median tumour nanoparticle accumulation of only 0.7% of injected dose in patients versus 5-15% in xenograft models. The in vivo study used only one dose level and one administration schedule; dose-ranging and schedule optimisation studies are required before the full therapeutic index of MSN-DOX-FA can be characterised. Additionally, the study did not address potential immunogenicity of the silica-folic acid surface coating, which should be evaluated in immunocompetent syngeneic tumour models in future work.

6. Conclusion

This comparative study of four folate receptor-targeted, doxorubicin-loaded nanoparticle platforms demonstrates that MSN-DOX-FA achieves the most favourable combination of physicochemical properties, pH-responsive drug release selectivity (84.7% release at pH 5.5 vs. 24.3% at pH 7.4), in vitro cytotoxicity (IC50 0.84-1.93 ug/mL; 7.6-9.8-fold improvement over free DOX), and in vivo efficacy (74.3% tumour reduction) with substantially reduced cardiotoxicity (troponin I reduced 5.0-fold versus free DOX) in HeLa xenograft mice. Folate receptor-mediated active targeting provides 3.8-4.6-fold cellular uptake enhancement in FR-overexpressing cancer cell lines, validated by receptor blocking experiments confirming specificity. The integrated NDDS design principles demonstrated here--high drug encapsulation, pH-triggered controlled release, receptor-mediated active targeting, and PEG steric stabilisation--constitute a validated framework for next-generation targeted nanomedicine development applicable beyond doxorubicin to other cytotoxic payloads and receptor-ligand targeting systems across multiple cancer indications.

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Declarations

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Conflict of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Raw characterisation data (DLS, TEM, FTIR, XRD), drug release profiles, cytotoxicity datasets, and in vivo measurements are deposited at https://zenodo.org/record/GGGGGGG under CC BY 4.0. Animal study data are available from the corresponding author upon reasonable request.

Ethical Approval

All animal experiments were performed under protocols approved by the Institutional Animal Care and Use Committee of the Mediterranean Institute of Technology (MIT-IACUC-2024-087), in compliance with Italian Legislative Decree 26/2014 implementing EU Directive 2010/63/EU on the protection of animals used for scientific purposes.

Appendix A

Nanoparticle Characterisation Methods and In Vivo Study Protocol Details

Table A1 provides detailed instrument parameters for physicochemical characterisation, and Table A2 documents the full in vivo xenograft study protocol including group assignments, dose schedule, and endpoint measurements.