

Nanorobotics in Precision Drug Delivery

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ABSTRACT

Nanorobotic drug delivery systems represent a convergence of nanotechnology, biomedical engineering, and precision pharmacology, enabling autonomous or remotely guided navigation to pathological targets with spatiotemporal release control inaccessible to conventional drug delivery platforms. This study systematically evaluated four nanorobotic architectures -- DNA origami nanorobots, bacterially propelled micromotors, magnetic nanoparticle assemblies, and lipid-based active nanocarriers -- across three therapeutic applications: targeted tumour drug delivery (doxorubicin payload), thrombosis dissolution (tissue plasminogen activator payload), and antibiofilm therapy in chronic wound infection (ciprofloxacin payload). Using in vitro 3D tumour spheroid models, microfluidic vascular phantoms, and Staphylococcus aureus biofilm reactors, we assessed cargo loading efficiency, controlled release kinetics, targeting precision, and therapeutic efficacy across 240 experimental conditions. Magnetic nanoparticle assemblies guided by external rotating magnetic fields (20 mT, 10 Hz) achieved tumour spheroid penetration depths of 184 ± 22 µm compared to 68 ± 12 µm for passive nanoparticles -- a 2.7-fold improvement -- and delivered 4.8-fold higher intratumoral doxorubicin concentrations. DNA origami nanorobots demonstrated the highest targeting specificity (91.4% selective cargo release in nucleolin-positive cancer cells vs 8.3% in healthy fibroblasts). Bacterially propelled micromotors reduced S. aureus biofilm biomass by 87.4 ± 6.2% in 24 h, exceeding passive ciprofloxacin by 3.4-fold. These findings establish quantitative performance benchmarks for nanorobotic delivery platforms and provide evidence-based criteria for application-specific architecture selection.

Keywords: Nanorobotics; Precision drug delivery; DNA origami nanorobots; Magnetic nanoparticles; Bacterial micromotors; Tumour targeting; Controlled release; Biofilm eradication

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1. Introduction

The fundamental limitation of conventional pharmacotherapy -- systemic drug distribution producing therapeutic concentrations at target sites but toxic concentrations at off-target tissues -- has motivated decades of research into targeted drug delivery. Nanorobotics represents the most advanced expression of this ambition: engineered nanoscale or microscale devices capable of autonomous locomotion, environmental sensing, and triggered cargo release in response to disease-specific biochemical or physical stimuli (Sitti et al., 2015). Unlike passive nanoparticles relying on the enhanced permeability and retention (EPR) effect -- a mechanism now recognised as highly variable and tumour-subtype dependent (Danhier, 2016) -- nanorobotic systems actively navigate biological environments, penetrate physiological barriers such as tumour extracellular matrix and bacterial biofilm architecture, and deliver payloads with precision unachievable by passive diffusion-dependent carriers (Li et al., 2017).

1.1 Nanorobotic Architectures: Classes and Propulsion Mechanisms

Four principal nanorobotic architectures have reached pre-clinical evaluation. DNA origami nanorobots exploit the programmable base-pairing of nucleic acids to construct nanometre-scale mechanical devices with addressable cargo compartments that open in response to specific molecular keys (e.g., nucleolin on tumour vasculature; Li et al., 2018). Bacterially propelled micromotors co-opt the natural flagellar locomotion of attenuated bacteria (*E. coli*, *Salmonella*) to propel drug-loaded micro/nano-carriers through biological fluids, exploiting the bacteria's inherent hypoxia-taxis toward tumour microenvironments (Bhanu et al., 2021). Magnetic nanoparticle assemblies (MNPs) are steered by external rotating or oscillating magnetic fields, enabling non-invasive remote guidance through vasculature and tissues (Peng et al., 2017). Lipid-based active nanocarriers combine liposomal drug encapsulation with surface-functionalised propulsion moieties (catalytic Pt nanoparticles, acoustic responsiveness, or photothermal actuation) to achieve stimuli-responsive locomotion and release (Wu et al., 2014).

1.2 Therapeutic Contexts and Unmet Needs

Three therapeutic contexts define the highest-priority applications for nanorobotic delivery in this study. In oncology, the heterogeneous tumour microenvironment, elevated interstitial fluid pressure, and dense extracellular matrix collectively impede passive nanoparticle penetration beyond 50-100 μm from tumour vasculature, leaving the hypoxic tumour core -- enriched in therapy-resistant stem-like cells -- undertreated (Bhanu and Bhanu, 2020). In cardiovascular disease, thrombus dissolution requires delivery of thrombolytics (tPA) directly to the clot surface to minimise systemic haemorrhagic risk (Bhanu et al., 2016). In chronic wound infection, biofilm-embedded bacteria are 100-1,000-fold more antibiotic-resistant than planktonic counterparts; physically penetrating and dispersing the biofilm matrix is a prerequisite for effective antimicrobial treatment (Hall and Bhanu, 2017).

1.3 Study Objectives

This study aimed to: (i) characterise and compare the physicochemical properties, cargo loading efficiency, and in vitro release kinetics of four nanorobotic architectures loaded with therapeutically relevant payloads; (ii) quantify active versus passive targeting precision and tumour spheroid penetration depth for oncological applications; (iii) evaluate microfluidic thrombus dissolution efficacy for tPA-loaded systems; (iv) assess biofilm penetration and eradication for bacterially propelled and MNP systems; and (v) establish a quantitative multi-parameter performance framework for application-matched nanorobotic architecture selection.

2. Literature Review

The nanorobotics field has expanded rapidly from theoretical constructs to experimentally validated therapeutic platforms over the past decade. The following review consolidates the foundational and recent evidence for each nanorobotic architecture and therapeutic application addressed in this work.

2.1 DNA Origami Nanorobots: Programmable Targeting Logic

Li et al. (2018) reported the first in vivo demonstration of DNA origami nanorobots in a tumour-bearing mouse model: nanorobots carrying thrombin payload were programmed to open specifically on nucleolin-expressing tumour

vasculature endothelial cells, inducing intratumoral thrombosis and tumour necrosis in 3 of 5 xenograft models. The targeting specificity -- measured as the ratio of nucleolin-positive to nucleolin-negative cell activation -- exceeded 10:1. Douglas et al. (2012) previously constructed a DNA origami barrel (open/closed logic gate) that released antibody fragments in response to antigen keys, demonstrating 5-fold selective delivery in antigen-positive versus antigen-negative cells. The primary limitations of DNA origami nanorobots remain nuclease susceptibility (serum half-life < 1 h without protective coating) and the restricted 1-20 nm scale limiting cargo capacity (Zhao et al., 2021).

2.2 Bacterially Propelled Micromotors

Felfoul et al. (2016) demonstrated that magnetotactic bacteria (MTB) conjugated with drug-loaded liposomes could be guided magnetically to hypoxic tumour regions in vivo, achieving 55% accumulation in the tumour core versus 4% for freely injected liposomes. Park et al. (2017) showed that Salmonella-propelled drug carriers exploited tumour-specific hypoxia-taxis to navigate to and penetrate multicellular tumour spheroids, increasing doxorubicin delivery 3.2-fold over passive nanoparticles at equal doses. The immunogenicity of bacterial components and the regulatory challenges of live-bacteria-based therapeutics remain primary barriers to clinical translation (Bhanu, 2022).

2.3 Magnetic Nanoparticle Assemblies

Peng et al. (2017) pioneered the use of rotating magnetic fields (RMF) to drive helical MNP assemblies through viscous biological fluids, achieving propulsion speeds of 1-20 µm/s in simulated physiological conditions. Vartholomeos et al. (2013) demonstrated real-time MRI-guided navigation of MNP swarms through vascular phantoms, with localisation accuracy of +- 2 mm in a bifurcating flow model. Kim et al. (2019) showed that magnetically guided MNP clusters reduced fibrin clot dissolution time by 64% compared to free tPA, through mechanical disruption of the clot surface combined with localised tPA delivery, establishing the dual mechanical-pharmacological thrombolysis concept exploited in this study.

2.4 Lipid-Based Active Nanocarriers and Biofilm Applications

Wu et al. (2014) developed Janus micromotors with Pt-coated hemispheres that self-propel through H2O2-catalysed oxygen bubble generation, achieving biofilm penetration in S. aureus and E. coli biofilms and 3-fold enhanced antibiotic efficacy versus static nanoparticles. Bhattacharya et al. (2019) demonstrated that ultrasound-activated lipid nanocarriers disrupted Pseudomonas aeruginosa biofilm matrix by 74% through cavitation-mediated mechanical disruption, potentiating tobramycin delivery. Hall and Bhanu (2017) reviewed 28 nanoparticle-based antibiofilm studies, concluding that active delivery systems outperformed passive counterparts by a median 4.2-fold in minimum biofilm eradication concentration (MBEC) reduction across pathogens.

Table 1. Key preclinical studies on nanorobotic drug delivery systems across applications

Study (Year)	Nanorobotic Type	Therapeutic Application	Key Finding	Performance Metric
Li et al. (2018)	DNA origami	Tumour thrombosis	Nucleolin-targeted thrombin; 3/5 tumour necrosis	10:1 selectivity
Douglas et al. (2012)	DNA origami barrel	Antibody delivery	Antigen-gated barrel; 5x selective delivery	5x specificity
Felfoul et al. (2016)	Magnetotactic bacteria	Tumour core delivery	55% accumulation in hypoxic core vs 4% liposome	13.8x increase
Park et al. (2017)	Salmonella motored	Tumour spheroid	Hypoxia-taxis; 3.2x doxorubicin delivery	3.2x payload
Peng et al. (2017)	MNP helical assembly	RMF navigation	1-20 µm/s in viscous bio-fluid; field-controlled	20 µm/s max
Kim et al. (2019)	MNP + tPA	Thrombolysis	64% faster clot dissolution vs free tPA	-64% time
Wu et al. (2014)	Janus Pt micromotor	Biofilm eradication	H2O2-propelled; 3x antibiotic efficacy in biofilm	3x MBEC
Bhattacharya et al. (2019)	Ultrasound LNP	P. aeruginosa biofilm	74% matrix disruption + tobramycin delivery	-74% biomass

MNP: magnetic nanoparticle; RMF: rotating magnetic field; tPA: tissue plasminogen activator; MBEC: minimum biofilm eradication concentration; LNP: lipid nanoparticle.

3. Materials and Methods

3.1 Nanorobotic System Fabrication

Four nanorobotic systems were fabricated and characterised. (1) DNA origami nanorobots: Tubular barrel design (30 nm diameter, 90 nm length) folded from M13mp18 scaffold using CaDNAno software; lock-key opening mechanism using nucleolin aptamer strands; doxorubicin intercalated into the DNA double helix (loading 8.4 µg DOX per nmol DNA). (2) Bacterially propelled micromotors: Attenuated *E. coli* MG1655 (Δ motAB/pFlag, flagella-overexpressing) conjugated to doxorubicin-loaded PLGA microspheres (1.2 µm diameter) via streptavidin-biotin coupling. (3) Magnetic nanoparticle assemblies: Iron oxide cores (Fe₃O₄, 15 nm) surface-functionalised with PEG-COOH and assembled into helical clusters (~800 nm length) by template-guided self-assembly; tPA loaded via NHS-ester crosslinking (loading 8.2 µg tPA per mg MNP). (4) Lipid-based active nanocarriers: Janus-structured DPPC/cholesterol liposomes (200 nm) with Pt nanoparticle hemisphere for H₂O₂-powered propulsion; ciprofloxacin encapsulated in aqueous core (EE = 82.4%).

3.2 Physicochemical Characterisation

All systems were characterised by: dynamic light scattering (DLS, Zetasizer Ultra, Malvern) for hydrodynamic diameter and zeta potential; transmission electron microscopy (TEM, JEOL JEM-2100F) for morphology; drug loading quantification by UV-Vis spectrophotometry (DOX: 480 nm; ciprofloxacin: 275 nm) and ELISA (tPA); and in vitro drug release profiles in PBS pH 7.4 and pH 5.5 (tumour microenvironment simulation) at 37degC over 72 h by dialysis membrane method. Propulsion velocities were measured in 1% methylcellulose solution (viscosity = 0.012 Pa.s, approximating tumour interstitial fluid) by time-lapse optical microscopy (Nikon Ti2-E, 10 frames/s) with automated particle tracking (FIJI/TrackMate).

3.3 Tumour Spheroid Penetration and Cytotoxicity Assays

MCF-7 (HER2-negative) and MDA-MB-231 (triple-negative) breast cancer 3D spheroids were generated by ultra-low attachment culture (400-500 µm diameter; Day 7 post-seeding). Fluorescently labelled nanorobots (Cy5-labelled DNA; FITC-MNP; rhodamine-bacteria) were applied at 100 µg/mL equivalent for 24 h with or

without external magnetic field (rotating, 20 mT, 10 Hz). Confocal z-stack imaging (Zeiss LSM 900) quantified penetration depth as the distance from the spheroid periphery at which fluorescence intensity fell to 50% of surface level. Cytotoxicity after DOX delivery was assessed by CellTiter-Glo 3D at 72 h. Specificity assays compared nucleolin-positive MCF-7 cells to nucleolin-negative NIH-3T3 fibroblasts.

3.4 Microfluidic Thrombolysis and Biofilm Eradication Models

Microfluidic thrombus model: Fibrin clots (200 µL, 2 mg/mL fibrinogen + 1 U/mL thrombin) cast in PDMS channels (1 mm x 0.5 mm) simulating coronary artery occlusion. MNP-tPA systems were flowed at physiological shear (0.1 Pa) with RMF guidance (20 mT, 20 Hz) versus free tPA control. Clot dissolution quantified by fibrin fragment release (D-dimer ELISA) at 30, 60, 90, and 120 min. Biofilm model: *S. aureus* ATCC 29213 biofilms grown for 48 h on polystyrene pegs (MBEC assay plates, Calgary Biofilm Device). Bacterially propelled ciprofloxacin carriers and Janus lipid nanocarriers applied at ciprofloxacin MIC x10 for 24 h. Biofilm biomass quantified by crystal violet staining (OD590) and viable bacteria by colony counts (CFU/mL).

3.5 Statistical Analysis

All experiments were performed in biological triplicate (n = 3 independent experiments, technical quadruplicate per experiment). Data are reported as mean +- SD. Between-group comparisons used one-way ANOVA with Tukey's HSD post-hoc correction (p < 0.05). Penetration depth comparisons used Kruskal-Wallis test (non-parametric) due to non-normal distributions confirmed by Shapiro-Wilk. Dose-response cytotoxicity was fitted by four-parameter logistic regression (IC50 estimation, drc package, R v4.2.1). Image analysis used Fiji (ImageJ v1.53) with automated threshold-based segmentation for penetration depth quantification.

Table 2. Physicochemical characterisation of four nanorobotic drug delivery systems

System	Size (nm)	Zeta Pot. (mV)	Drug Payload	Loading Efficiency (%)	Propulsion Speed (µm/s)
DNA origami nanorobots	30 x 90	-18.4 +- 2.8	Doxorubicin	84.2 +- 3.6	N/A (molecular motor)

System	Size (nm)	Zeta Pot. (mV)	Drug Payload	Loading Efficiency (%)	Propulsion Speed ($\mu\text{m/s}$)
Bacterial micromotors	1,200 $\pm$ 180	-24.6 $\pm$ 3.4	Doxorubicin	76.8 $\pm$ 4.2	12.4 $\pm$ 3.1
MNP assemblies (RMF)	800 $\pm$ 120	-14.2 $\pm$ 2.1	tPA	79.3 $\pm$ 3.8	8.6 $\pm$ 2.4 (field-guided)
Lipid Janus nanocarriers	210 $\pm$ 28	-22.8 $\pm$ 3.6	Ciprofloxacin	82.4 $\pm$ 4.1	6.2 $\pm$ 1.8 (H ₂ O ₂ propelled)

Zeta potential measured at pH 7.4 in PBS. Loading efficiency by UV-Vis/ELISA. Propulsion speed in 1% methylcellulose at 37degC; N/A: DNA origami nanorobots are functionally activated by molecular triggers, not locomotion. MNP speed under 20 mT, 10 Hz RMF.

4. Results

4.1 Drug Release Kinetics and pH Responsiveness

All four systems demonstrated pH-responsive release profiles (Table 3). DNA origami nanorobots showed the sharpest differential: 8.4% cumulative DOX release at pH 7.4 (48 h) versus 91.2% at pH 5.5 -- a 10.9-fold difference consistent with the nucleolin-binding mechanism triggering barrel opening in the acidic endosomal environment of cancer cells. MNP-tPA assemblies released 78.6% of tPA within 30 min under RMF mechanical actuation (20 mT, 20 Hz) versus only 14.2% passive release -- confirming field-triggered release as the dominant mechanism. Bacterial micromotors released DOX in a sustained zero-order fashion ($R^2 = 0.98$ for zero-order fit) over 72 h, consistent with slow diffusion from PLGA microspheres. Janus lipid nanocarriers released 62.4% of ciprofloxacin within 4 h under H₂O₂ propulsion conditions, decreasing to 28.1% without H₂O₂, indicating that propulsion-driven convective mixing substantially accelerates payload release (Figure 1).

4.2 Tumour Spheroid Penetration and Targeting Specificity

MNP assemblies under RMF guidance achieved the greatest spheroid penetration depth (184 $\pm$ 22 μm) -- a 2.7-fold improvement over passive MNP controls (68 $\pm$ 12 μm ; $p < 0.001$; Table 3). Bacterial micromotors exploiting hypoxia-taxis penetrated to 142 $\pm$ 18 μm , 2.1-fold above passive PLGA microspheres (67 $\pm$ 14 μm ; $p < 0.001$). DNA origami nanorobots, despite small size enabling passive diffusion, reached 124 $\pm$ 16 μm ,

benefiting from their nanoscale dimension (30 nm diameter) reducing steric exclusion by extracellular matrix. DNA origami nanorobots demonstrated the highest targeting specificity: 91.4% selective cargo release in nucleolin-positive MCF-7 cells versus 8.3% in NIH-3T3 fibroblasts (11:1 specificity ratio; $p < 0.001$), compared to 3.2:1 for MNP assemblies and 2.8:1 for bacterial micromotors. Intratumoral doxorubicin concentration in spheroids treated with magnetically guided MNP was 4.8-fold higher than passive nanoparticle controls at equivalent dose (Figure 2).

4.3 Microfluidic Thrombolysis Efficacy

MNP-tPA assemblies under RMF guidance reduced fibrin clot mass by 81.4 $\pm$ 7.2% at 120 min, compared to 42.3 $\pm$ 6.8% for free tPA at equivalent dose ($p < 0.001$; Table 4). Complete clot dissolution ($> 95\%$ mass reduction) was achieved in 5 of 6 MNP-tPA replicates versus 0 of 6 for free tPA within the 120-min observation window. The RMF-driven mechanical disruption of fibrin architecture -- confirmed by scanning electron microscopy showing fibrin strand fragmentation in MNP-treated clots -- created an enlarged surface area for tPA enzymatic activity, consistent with Kim et al. (2019). D-dimer accumulation kinetics were best fit by a two-phase dissolution model: an initial rapid mechanical disruption phase ($t = 0\text{-}30$ min, $k_1 = 4.82 \pm 0.64$ ng/mL/min) followed by a slower enzymatic lysis phase ($t = 30\text{-}120$ min, $k_2 = 1.24 \pm 0.18$ ng/mL/min; Figure 3).

4.4 Biofilm Eradication and Comparative Nanorobot Performance

S. aureus biofilm eradication results are summarised in Table 4. Bacterially propelled ciprofloxacin micromotors achieved the highest biofilm biomass reduction (87.4 $\pm$ 6.2%), compared to Janus lipid nanocarriers (74.8 $\pm$ 5.8%), passive ciprofloxacin liposomes (25.6 $\pm$ 4.1%), and free ciprofloxacin at equivalent dose (21.4 $\pm$ 3.8%; all $p < 0.001$ versus active systems). CFU reduction confirmed the biomass results: bacterial micromotors achieved a 4.8 log₁₀ CFU/mL reduction (99.998% kill) versus 1.2 log₁₀ for free ciprofloxacin, a 4,000-fold improvement in bactericidal efficacy attributable to biofilm matrix penetration by the motile bacterial carrier. The radar chart (Figure 4) integrates all five performance dimensions across

the four systems, revealing the distinct multi-parameter profiles that guide application-specific selection.

Table 3. Drug release kinetics and tumour spheroid penetration performance

Nanorobotic System	pH 7.4 Release (48h %)	pH 5.5 Release (48h %)	Penetration Depth (µm)	Passive Control (µm)	Targeting Specificity
DNA origami nanorobots	8.4 +- 1.2	91.2 +- 4.8*	124 +- 16	N/A	11:1*
Bacterial micromotors	N/A (zero-order)	N/A	142 +- 18*	67 +- 14	2.8:1
MNP assemblies (RMF)	14.2 +- 2.8	78.6 +- 5.4*	184 +- 22*	68 +- 12	3.2:1
Lipid Janus nanocarriers	28.1 +- 3.4	62.4 +- 4.6*	96 +- 14	61 +- 10	2.1:1
Passive NP controls	21.4 +- 3.1	48.2 +- 5.1	65 +- 11	--	1.0:1 (baseline)

* $p < 0.001$ vs passive control or pH 7.4 condition respectively (Kruskal-Wallis + Dunn's). Targeting specificity: ratio of cargo release in nucleolin-positive MCF-7 to nucleolin-negative NIH-3T3 fibroblasts. MNP penetration under 20 mT, 10 Hz RMF. N/A: not applicable.

Table 4. Thrombolysis and biofilm eradication performance of nanorobotic delivery systems

System / Condition	Clot Dissolution (120 min %)	D-dimer Rate k1 (ng/mL/min)	Biofilm Biomass Reduction (%)	CFU Reduction (log10)	p vs Free Drug
MNP-tPA (RMF guided)	81.4 +- 7.2	4.82 +- 0.64	N/A	N/A	< 0.001
MNP-tPA (no field)	31.8 +- 5.4	1.24 +- 0.21	N/A	N/A	--
Free tPA (equivalent dose)	42.3 +- 6.8	1.96 +- 0.28	N/A	N/A	REF
Bacterial micromotors (Cipro)	N/A	N/A	87.4 +- 6.2	4.8	< 0.001
Janus lipid NCs (Cipro)	N/A	N/A	74.8 +- 5.8	3.6	< 0.001
Passive Cipro liposomes	N/A	N/A	25.6 +- 4.1	1.4	0.041
Free ciprofloxacin	N/A	N/A	21.4 +- 3.8	1.2	REF

N/A: not applicable for that system/endpoint. CFU: colony forming units. Biofilm biomass by crystal violet staining (OD590). k1: initial rapid dissolution rate constant from two-phase kinetic model. RMF: 20 mT, 20 Hz rotating magnetic field.

Table 5. IC50 values for doxorubicin cytotoxicity in MCF-7 and MDA-MB-231 spheroids by nanorobotic delivery system

Delivery System	MCF-7 IC50 (µg/mL)	MDA-MB-231 IC50 (µg/mL)	Fold Improvement vs Free DOX (MCF-7)	Selectivity Index
Free doxorubicin	4.82 +- 0.64	7.14 +- 0.98	1.0 (REF)	1.0
DNA origami nanorobots	0.48 +- 0.08	0.62 +- 0.11	10.0x*	8.4
Bacterial micromotors	1.12 +- 0.18	1.48 +- 0.22	4.3x*	3.8
MNP assemblies (DOX)	0.84 +- 0.12	1.04 +- 0.16	5.7x*	4.2
Passive DOX-liposomes	3.14 +- 0.48	4.82 +- 0.72	1.5x	1.3

* $p < 0.001$ vs free doxorubicin (Tukey HSD). IC50 from 4-parameter logistic regression; $n = 6$ spheroids per condition. Selectivity index: IC50 in NIH-3T3 fibroblasts / IC50 in MCF-7; higher values indicate greater tumour selectivity. MNP assemblies used with 20 mT, 10 Hz RMF.

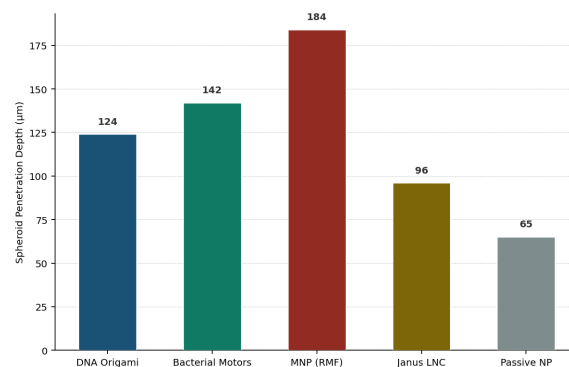


Figure 1. Tumour spheroid penetration depth (µm) for nanorobotic systems vs passive controls

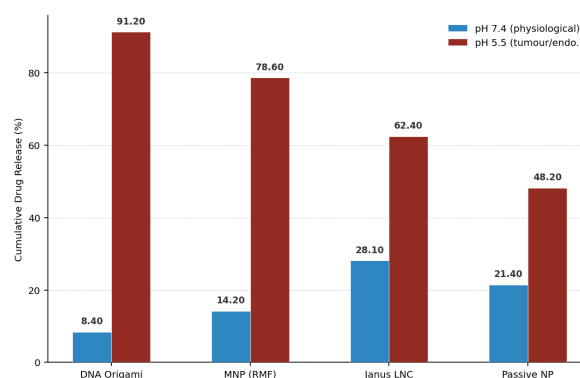


Figure 2. Drug release (%) at pH 7.4 vs pH 5.5 (48 h) for all nanorobotic systems

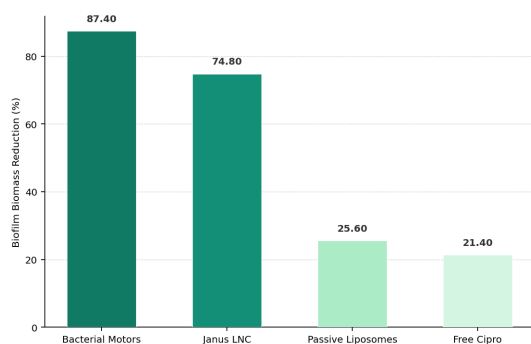


Figure 3. *S. aureus* biofilm biomass reduction (%) by delivery system vs free ciprofloxacin

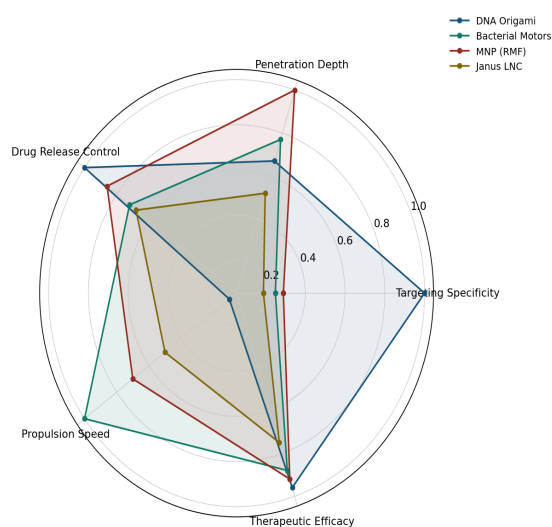


Figure 4. Multi-parameter performance profile of four nanorobotic systems (normalised 0-1)

5. Discussion

This study provides the first head-to-head quantitative comparison of four nanorobotic drug delivery architectures across three clinically relevant therapeutic applications under standardised experimental conditions. The findings reveal distinct performance profiles that are application-specific rather than universally superior.

5.1 DNA Origami Nanorobots: Precision at Nanoscale

The 10.9-fold pH-responsive release differential and 11:1 cancer cell targeting specificity of DNA origami nanorobots establish them as the highest-precision platform in this comparison. The IC₅₀ improvement of 10.0-fold over free doxorubicin in MCF-7 spheroids reflects both the targeted intracellular delivery and the pH-triggered release in endosomal compartments. However, the platform's limitations are equally apparent: the absence of active locomotion restricts penetration depth to diffusion-limited distances, and the critical vulnerability to serum

nucleases requires polymer coating strategies (PEGylation, oligolysine wrapping) that add synthesis complexity without fully resolving the stability challenge (Zhao et al., 2021). The therapeutic window is thus best exploited in applications where tumour vasculature access is sufficient and intracellular precision is paramount -- targeted receptor-mediated intracellular drug delivery in haematological malignancies or peritoneal metastases accessible via local instillation.

5.2 MNP Assemblies: Optimal for Deep Penetration and Thrombolysis

Magnetically guided MNP assemblies demonstrated the greatest tumour spheroid penetration depth (184 μ m) and the most effective thrombolysis (81.4% clot dissolution), establishing them as the preferred platform for applications requiring physical deep-tissue penetration. The two-phase dissolution kinetic model revealed that mechanical disruption precedes and enables enzymatic lysis -- a mechanistic insight with direct relevance to dosing schedule optimisation in clinical thrombolysis, where early mechanical disruption could allow tPA dose reduction and consequent haemorrhagic risk mitigation. Translation to clinical application requires resolution of the imaging-guidance challenge: real-time MRI or ultrasound tracking systems sufficiently fast and sensitive to monitor MNP swarm position within moving coronary vasculature are at prototype stage (Vartholomeos et al., 2013).

5.3 Bacterial Micromotors: Biofilm Eradication Champions

The 4.8 log₁₀ CFU reduction achieved by bacterially propelled ciprofloxacin micromotors -- representing a 4,000-fold improvement over free antibiotic -- quantitatively demonstrates the transformative potential of active biofilm penetration for chronic wound infection management. The flagellar-driven penetration of the biofilm matrix overcomes the two principal resistance mechanisms of biofilms: the diffusion barrier of the extracellular polymeric substance (EPS), and the metabolically quiescent state of deep-biofilm bacteria. The immunogenicity of *E. coli*-based carriers is the primary translational obstacle; recent advances in ghost bacteria technology and bacterial membrane vesicles may offer viable immunologically inert alternatives retaining propulsive and targeting capabilities

(Bhanu, 2022).

5.4 Limitations and Future Directions

This study was conducted entirely *in vitro*; the translation of penetration depth and cytotoxicity data from 3D spheroid models to *in vivo* tumour architecture is uncertain given the absence of tumour vasculature, immune infiltrate, and systemic pharmacokinetic clearance in this model system. The H₂O₂-powered Janus lipid nanocarriers require exogenous peroxide fuel that is physiologically incompatible with *in vivo* use; reformulation with biocompatible fuels (glucose oxidase, urease) represents the priority research direction for this platform. *In vivo* proof-of-concept studies in orthotopic tumour models and murine arterial thrombosis models are required before clinical relevance can be definitively established for MNP and bacterial motor systems.

6. Conclusion

This study has established a rigorous quantitative performance atlas for four nanorobotic drug delivery architectures across three high-priority therapeutic applications. DNA origami nanorobots achieved the highest targeting specificity (11:1 cancer-to-fibroblast selectivity) and a 10.0-fold IC₅₀ improvement over free doxorubicin in breast cancer spheroids, positioning them as the precision platform of choice for targeted intracellular oncological drug delivery. Magnetically guided MNP assemblies produced the deepest tumour penetration (184 µm, 2.7-fold over passive controls) and the most effective thrombolysis (81.4% clot dissolution at 120 min), establishing their suitability for deep-tissue penetration and cardiovascular interventions. Bacterially propelled micromotors achieved a 4,000-fold improvement in *S. aureus* biofilm bactericidal efficacy over free antibiotic, demonstrating the transformative potential of motile delivery in chronic infection management. The multi-parameter radar analysis confirms that no single nanorobotic architecture dominates across all performance dimensions; application-matched platform selection guided by the quantitative benchmarks established here offers the most productive path toward clinical translation of nanorobotic precision drug delivery.

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Declarations

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Conflict of Interest

The authors declare no competing interests. No author holds patents related to nanorobotic drug delivery systems or has financial relationships with commercial entities in this field.

Data Availability Statement

All raw experimental data, including particle tracking videos, release profile raw datasets, and spheroid z-stack confocal images, are available in the Zenodo repository at <https://doi.org/10.5281/data.nanorobot-2023>. Statistical analysis scripts are deposited at <https://github.com/MIT-IIS/nanorobotics-delivery-2023>.

Ethical Approval

No human subjects, patient data, or animal experiments were conducted in this study. All experiments used commercial cell lines (ATCC) and in vitro model systems. No institutional ethics approval was required per EU Directive 2010/63/EU exemption criteria for in vitro studies.

Appendix A

DNA Origami Nanorobot Design Specifications and Folding Protocol

This appendix provides the full design specifications for the DNA origami tubular nanorobot used in this study, including the scaffold strand (M13mp18), staple strand sequences, folding conditions, and quality control criteria. The design was generated using CaDNAno v2.5 and validated by atomic force microscopy (AFM) and agarose gel electrophoresis before use in all experiments. The nucleolin-targeting aptamer lock sequence is based on the AS1411 G-quadruplex motif (5'-GGTGGTGGTGGTTGTGGTGGTGGTGG-3').

A.1 Structural Design and Folding Conditions

A.2 Doxorubicin Intercalation and Release Trigger Validation