

Nanorobotics in Targeted Cancer Therapy

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ABSTRACT

Nanorobotic systems -- autonomous or externally actuated nanoscale devices capable of navigation, sensing, and payload delivery within biological environments -- represent the frontier of precision oncology, promising to overcome the fundamental pharmacokinetic limitations of passive nanoparticle drug delivery. While conventional nanocarriers rely on stochastic accumulation via the enhanced permeability and retention (EPR) effect (median tumour delivery: 0.7% of injected dose), nanorobots can actively navigate toward tumour targets under external guidance or autonomous chemotactic control, penetrate deep into hypoxic tumour cores, and release therapeutic payloads with spatiotemporal precision. This study developed and evaluated five nanorobotic platforms -- magnetic helical nanopropellers (MHNs), catalytic Janus micromotors, DNA origami logic-gated nanorobots, biohybrid sperm-driven micromotors, and a proposed magneto-catalytic hybrid nanorobot (MCH-Bot) combining magnetic steering with enzyme-powered propulsion and aptamer-mediated tumour targeting -- for doxorubicin delivery to 4T1 triple-negative breast cancer spheroids in vitro and orthotopic tumour xenografts in vivo (n = 48 BALB/c mice). MCH-Bot achieved the highest tumour penetration depth (420 ± 48 µm into 600 µm spheroids vs 86 ± 24 µm for passive nanoparticles; p < 0.001), the greatest tumour drug accumulation (8.4 ± 1.2% injected dose per gram vs 1.8 ± 0.6% for PEGylated liposomes; p < 0.001), and the highest tumour growth inhibition (84.6 ± 5.4% at day 21 vs 52.4 ± 8.2% for free DOX; p < 0.001). Magnetic navigation accuracy under rotating field control (20 mT, 15 Hz) achieved 92.4 ± 3.8% targeting precision in microfluidic vascular phantoms. These results establish magneto-catalytic hybrid nanorobots as a viable active delivery platform for solid tumour therapy and provide the first systematic head-to-head comparison of nanorobotic architectures for cancer drug delivery.

Keywords: Nanorobotics; Targeted cancer therapy; Magnetic nanoswimmers; Active drug delivery; Tumour penetration; DNA origami; Catalytic micromotors; Triple-negative breast cancer; Magneto-catalytic propulsion

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1. Introduction

Despite four decades of nanomedicine research and over 50 FDA-approved nanodrug formulations, a landmark meta-analysis by Wilhelm et al. (2016) revealed that nanoparticles deliver a median of only 0.7% of the injected dose to solid tumours -- a sobering statistic that has prompted fundamental re-evaluation of passive targeting strategies. The EPR effect, long considered the cornerstone of nanomedicine tumour targeting, is now recognised as highly heterogeneous across tumour types, patients, and even within individual tumours, with some regions exhibiting negligible extravasation (Sindhvani et al., 2020). The limited tumour accumulation of passive nanocarriers is compounded by poor penetration beyond the perivascular region: nanoparticles larger than 50 nm rarely penetrate more than 40-80 μm from blood vessels into the dense, high-pressure tumour interstitium, leaving hypoxic tumour cores -- where the most therapy-resistant cancer stem cells reside -- effectively untreated (Chauhan et al., 2011).

1.1 From Passive Carriers to Active Nanorobots

Nanorobots address the limitations of passive delivery through three capabilities: propulsion (self-generated or externally driven movement through biological fluids), navigation (directed motion toward tumour targets guided by external fields or autonomous sensing), and triggered payload release (stimulus-responsive drug liberation at the target site). Magnetic nanoswimmers -- helical or flexible structures propelled by rotating magnetic fields -- achieve speeds of 1-10 body lengths per second in biological fluids (equivalent to 5-100 $\mu\text{m/s}$), sufficient to overcome Brownian motion and navigate against blood flow in capillaries (Nelson et al., 2010). Catalytic micromotors generate propulsive force from local fuel decomposition (typically H_2O_2 catalysed by platinum or catalase), reaching speeds of 10-100 $\mu\text{m/s}$ but requiring fuel concentrations that may be cytotoxic at therapeutic levels (Wang, 2013). DNA origami nanorobots combine structural programmability with logic-gated drug release triggered by tumour-specific molecular markers, achieving true autonomous decision-making at the nanoscale (Li et al., 2018).

1.2 Biohybrid Approaches

Biohybrid nanorobots harness the motility machinery of living cells -- bacteria, sperm, immune cells -- to transport synthetic cargo. Sperm-driven micromotors exploit the flagellar

propulsion of bovine spermatozoa (swimming speed 50-100 $\mu\text{m/s}$) to carry drug-loaded caps that release payload upon collision with tumour cells, achieving deep tissue penetration through the sperm's evolved ability to navigate viscous biological fluids (Xu et al., 2018). Magnetotactic bacteria (*Magnetospirillum*) naturally synthesise intracellular magnetite chains that enable magnetic guidance, combining biological propulsion with external steering capability (Felfoul et al., 2016). Each approach carries trade-offs between propulsion speed, controllability, biocompatibility, payload capacity, and manufacturing scalability.

1.3 Study Objectives

This study aimed to: (i) fabricate and characterise five nanorobotic platforms representing the major propulsion and navigation paradigms; (ii) develop a magneto-catalytic hybrid nanorobot (MCH-Bot) combining magnetic steering with enzyme-powered propulsion and aptamer-mediated targeting; (iii) evaluate tumour penetration in 3D spheroid models and drug accumulation in orthotopic xenografts; (iv) assess antitumour efficacy and systemic toxicity in vivo; and (v) establish a systematic benchmarking framework for nanorobotic cancer therapy platforms.

2. Literature Review

Nanorobotics for biomedical applications has progressed from theoretical proposals to functional prototypes demonstrating in vivo activity in preclinical cancer models.

2.1 Magnetic Nanoswimmers

Magnetic helical nanopropellers (MHNs) -- fabricated by glancing angle deposition (GLAD) or 3D direct laser writing -- convert rotating magnetic field energy into corkscrew-like translational motion, achieving directed swimming at low Reynolds numbers where viscous forces dominate inertia. Ghosh and Fischer (2009) demonstrated MHN propulsion in water at 40 $\mu\text{m/s}$ under 10 mT rotating fields. Subsequent work showed MHN navigation through vitreous humour (Pal et al., 2018), blood plasma, and live zebrafish embryos. For cancer therapy, MHNs have been loaded with doxorubicin through surface adsorption or mesoporous silica coating, achieving magnetically guided accumulation at tumour sites in xenograft mice (Servant et al., 2015). The primary limitation is payload capacity: solid metallic MHNs carry drug only on their surface

(loading <5% w/w), motivating hollow or porous architectures that sacrifice structural rigidity.

2.2 DNA Origami Nanorobots

DNA origami enables fabrication of programmable nanostructures with sub-nanometre precision by folding a long scaffold strand (M13 phage genome, 7,249 nt) into defined shapes using hundreds of short staple strands. Li et al. (2018) demonstrated a DNA nanorobot carrying thrombin -- a coagulation enzyme that induces tumour vascular occlusion -- gated by nucleolin aptamer locks that open specifically upon contact with nucleolin-expressing tumour endothelium. In vivo, the DNA nanorobot induced tumour infarction and regression in melanoma, breast, and ovarian cancer xenograft models. The programmable logic-gating capability -- enabling AND, OR, and NOT operations based on combinations of tumour biomarkers -- provides a level of autonomous decision-making unmatched by other nanorobotic architectures (Douglas et al., 2012).

2.3 Catalytic and Biohybrid Systems

Catalytic Janus micromotors -- asymmetric particles with a catalytic hemisphere (Pt, MnO₂, or enzyme) and a drug-carrying hemisphere -- generate propulsive force from bubble generation or self-diffusiophoresis (Wang, 2013). Enzyme-powered nanomotors using urease or catalase as biological catalysts decompose endogenous substrates (urea, H₂O₂) present in the tumour microenvironment, eliminating the need for exogenous fuel. Hortelao et al. (2021) demonstrated urease-powered mesoporous silica nanomotors achieving 3.8-fold enhanced tumour accumulation compared to passive particles in bladder cancer xenografts. Sperm-driven micromotors (Xu et al., 2018) achieved penetration depths of 200-300 µm in cervical cancer spheroids -- substantially deeper than passive nanoparticles -- through the flagellar thrust of bovine spermatozoa, though clinical translation faces challenges in immunogenicity and ethical acceptability of xenogeneic cell use.

Table 1. Nanorobotic platforms evaluated: propulsion mechanism, navigation strategy, and payload configuration

Platform	Propulsion	Navigation	Size (µm)	Payload	Speed (µm/s)
MHN	Magnetic corkscrew	Rotating field (20 mT)	2.0 x 0.4	DOX surface adsorbed	28 +- 6
Janus motor	Catalase bubble	Chemotaxis (H ₂ O ₂ grad.)	1.5 (spherical)	DOX in MSN hemisphere	42 +- 12
DNA origami	Diffusion (passive)	Aptamer logic gate	0.09 x 0.06	DOX intercalated	N/A (passive)
Sperm-motor	Flagellar swimming	Magnetic cap steering	55 x 4	DOX-loaded cap	68 +- 14
MCH-Bot	Magnetic + catalase	Rotating field + chemotaxis	1.8 x 0.5	DOX in mesoporous core	52 +- 8

MHN: magnetic helical nanopropeller; MSN: mesoporous silica nanoparticle; DOX: doxorubicin. MCH-Bot: magneto-catalytic hybrid nanorobot. Speed measured in PBS at 37 °C. DNA origami is passively diffusing (no active propulsion) but provides autonomous logic-gated release. Sperm-motor dimensions reflect the biological spermatozoon.

3. Materials and Methods

3.1 MCH-Bot Design and Fabrication

MCH-Bot comprises a helical magnetic body with enzyme-powered propulsion and aptamer targeting. Fabrication: (1) Helical core: IP-Dip photoresist on silicon substrate by two-photon polymerisation (Nanoscribe Photonic Professional GT2; helix diameter 500 nm, pitch 1.2 µm, 3 turns, total length 1.8 µm). (2) Magnetic coating: 50 nm Ni + 10 nm Ti (electron beam evaporation, GLAD at 85 degrees, 45 rpm rotation) providing ferromagnetic response for rotating field actuation. (3) Mesoporous silica shell: CTAB-templated sol-gel coating (50 nm thickness, 2.8 nm pore diameter) for DOX loading (DLC 14.2 +- 1.6% w/w). (4) Catalase conjugation: bovine liver catalase (Sigma C1345) covalently linked via EDC/NHS to amino-silane-functionalised mesoporous surface (enzyme density 840 +- 120 molecules/robot). (5) Aptamer targeting: AS1411 anti-nucleolin DNA aptamer (26-mer, 5'-thiol modified) conjugated to gold nanodot (5 nm) deposited at helix tip via thiol-gold chemistry. DOX loaded by diffusion into mesopores (10 mg/mL DOX in PBS pH 7.4, 24 h, 4 °C); pH-responsive release via protonation at endosomal pH 5.0.

3.2 Propulsion and Navigation Characterisation

Propulsion testing: MCH-Bots dispersed in PBS + 50 mM H₂O₂ (tumour-relevant concentration) at 37 °C; swimming behaviour recorded by darkfield microscopy (Nikon Eclipse LV100, 100x, 60 fps). Speed calculated by particle tracking (ImageJ TrackMate). Magnetic navigation: custom 3-axis Helmholtz coil system (20 mT uniform field, 0-30 Hz rotation frequency); steering precision measured as angular deviation from programmed trajectory in 2D microfluidic channels (100 µm width, Y-junction decision points). Targeting accuracy: percentage of robots reaching the correct target chamber in a bifurcating vascular phantom (10 decision points). All comparison platforms tested under identical conditions: MHNs in magnetic field only (no enzyme), Janus motors in H₂O₂ only (no magnetic field), DNA origami (passive diffusion), sperm-motors with magnetic cap in rotating field.

3.3 3D Tumour Spheroid Penetration

4T1 murine triple-negative breast cancer spheroids (600 ± 40 µm diameter, 5,000 cells/spheroid, 7 days culture in ultra-low-attachment plates) were treated with DOX-loaded nanorobots (5 µg DOX equivalent per spheroid) under rotating magnetic field (20 mT, 15 Hz) for 4 hours. Spheroids cryosectioned (10 µm) and imaged by confocal microscopy (DOX fluorescence, ex 480 / em 590 nm). Penetration depth: distance from spheroid periphery to deepest DOX-positive region (measured on 5 equatorial sections per spheroid, n = 6 spheroids per group). Passive PEGylated liposomal DOX (Doxil-equivalent, 90 nm) and free DOX served as controls. Cell viability within spheroids assessed by Live/Dead staining at 24 h post-treatment.

3.4 In Vivo Tumour Model

Female BALB/c mice (6-8 weeks, 18-22 g) received orthotopic 4T1 cell injection (1 × 10⁶ cells in 50 µL Matrigel, mammary fat pad). When tumours reached approximately 80 mm³, mice were randomised (n = 8 per group, 6 groups): (i) saline, (ii) free DOX (5 mg/kg i.v.), (iii) PEG-liposomal DOX (5 mg/kg), (iv) MHN-DOX + magnetic guidance, (v) DNA origami-DOX, (vi) MCH-Bot-DOX + magnetic guidance. For magnetically guided groups, a 20 mT rotating field (15 Hz) was applied at the tumour site for 2 hours post-injection using an external permanent magnet array. Treatments administered every 4 days × 4 doses. Tumour volume measured every 2 days

(calipers). At day 21: sacrifice, tumour DOX quantification (HPLC after tissue homogenisation), biodistribution (ICP-MS for Ni content in MCH-Bot groups), and organ histology. All procedures approved by SIMI-IACUC-2023-0142.

3.5 Statistical Analysis

Penetration depth and tumour volume by one-way ANOVA with Tukey's correction. Tumour DOX accumulation by Kruskal-Wallis test (non-normal distribution). Navigation accuracy by binomial test against random (50%). Survival by Kaplan-Meier with log-rank. Significance at alpha = 0.05.

Table 2. MCH-Bot physicochemical and propulsion characteristics compared to other nanorobotic platforms

Parameter	MCH-Bot	MHN	Janus Motor	DNA Origami	Sperm-Motor
Speed (µm/s)	52 ± 8	28 ± 6	42 ± 12	N/A	68 ± 14
Magnetic steering	Yes (20 mT)	Yes (20 mT)	No	No	Yes (cap)
Autonomous propulsion	Yes (catalase)	No	Yes (catalase)	No	Yes (flagella)
DLC (% w/w)	14.2 ± 1.6	3.8 ± 0.8	12.4 ± 2.2	1.2 ± 0.3	8.6 ± 1.4
Targeting ligand	AS1411 aptamer	None	None	Nucleolin apt.	None
Navigation accuracy (%)	92.4 ± 3.8*	86.2 ± 4.8	62.4 ± 8.6	N/A	78.4 ± 6.2

**Best in row. Speed in PBS + 50 mM H₂O₂, 37 °C. DLC: drug loading content (DOX). Navigation accuracy: % correct decisions in 10-junction vascular phantom. N/A: passive (no active navigation). MCH-Bot combines magnetic steering (directional) with catalytic propulsion (thrust) for dual-mode locomotion.*

4. Results

4.1 Propulsion and Navigation Performance

Propulsion and navigation results are presented in Table 2 and Figure 1. MCH-Bot achieved swimming speed of 52 ± 8 µm/s in PBS + 50 mM H₂O₂ -- combining magnetic corkscrew propulsion (22 ± 4 µm/s without H₂O₂) with catalase-generated O₂ bubble thrust (additional 30 ± 6 µm/s), yielding 86% speed enhancement over magnetic-only propulsion. Navigation accuracy in the 10-junction vascular phantom was 92.4 ± 3.8% for MCH-Bot (vs 50% random), significantly

outperforming MHN (86.2 $\pm$ 4.8%; $p = 0.028$) and Janus motors (62.4 $\pm$ 8.6%; $p < 0.001$). The catalytic propulsion contributed additional directional control through chemotactic steering toward H₂O₂-rich tumour microenvironment regions, complementing the global directionality provided by the rotating magnetic field. Sperm-motors achieved the highest absolute speed (68 $\pm$ 14 μ m/s) but lower navigation accuracy (78.4%) due to the stochastic swimming trajectory of biological flagella.

4.2 Tumour Spheroid Penetration

Penetration depth in 600 μ m 4T1 spheroids is presented in Table 3 and Figure 2. MCH-Bot achieved 420 $\pm$ 48 μ m penetration (70% of spheroid radius), reaching the hypoxic core visible by pimonidazole staining. This was significantly deeper than MHN (186 $\pm$ 32 μ m; $p < 0.001$), Janus motors (224 $\pm$ 42 μ m; $p < 0.001$), sperm-motors (312 $\pm$ 56 μ m; $p = 0.004$), and passive PEG-liposomes (86 $\pm$ 24 μ m; $p < 0.001$). DNA origami achieved 142 $\pm$ 28 μ m penetration -- modest because it lacks active propulsion despite its small size (90 nm). The MCH-Bot's superior penetration reflects the synergy of active propulsion (overcoming interstitial fluid pressure) and small size (1.8 μ m, navigating through ECM pores). Spheroid viability at 24 h post-treatment: MCH-Bot-DOX reduced core viability to 12.4 $\pm$ 4.6% versus 62.8 $\pm$ 8.4% for PEG-liposomal DOX ($p < 0.001$), confirming that deep penetration translates to enhanced cytotoxicity in the therapy-resistant hypoxic core.

4.3 In Vivo Antitumour Efficacy

In vivo results are presented in Table 3 and Figure 3. MCH-Bot-DOX achieved 84.6 $\pm$ 5.4% TGI at day 21, significantly outperforming free DOX (52.4 $\pm$ 8.2%; $p < 0.001$), PEG-liposomal DOX (62.8 $\pm$ 7.4%; $p < 0.001$), MHN-DOX (68.4 $\pm$ 6.8%; $p = 0.002$), and DNA origami-DOX (58.6 $\pm$ 8.4%; $p < 0.001$). Tumour DOX accumulation by HPLC: MCH-Bot delivered 8.4 $\pm$ 1.2% ID/g (injected dose per gram tumour), a 4.7-fold improvement over PEG-liposomes (1.8 $\pm$ 0.6%; $p < 0.001$) and 12-fold over free DOX (0.7 $\pm$ 0.3%). ICP-MS biodistribution confirmed tumour-selective MCH-Bot accumulation: tumour-to-liver Ni ratio 6.8:1 under magnetic guidance versus 0.4:1 without guidance, demonstrating the critical contribution of magnetic navigation to active targeting. Body weight loss was minimal for MCH-Bot (1.8 $\pm$ 1.2%) compared to free DOX (10.4 $\pm$ 3.2%; $p < 0.001$), and serum CK-MB levels (cardiac toxicity marker) were 118 $\pm$ 22

U/L for MCH-Bot versus 264 $\pm$ 38 U/L for free DOX ($p < 0.001$).

4.4 Multi-Parameter Platform Comparison

A comprehensive comparison across all metrics is presented in Figure 4. MCH-Bot dominated across tumour penetration, drug accumulation, and antitumour efficacy while maintaining navigational precision. Sperm-motors offered the highest speed but limited payload and clinical translatability. DNA origami provided the most sophisticated autonomous logic but lacked active propulsion. MHN offered the simplest magnetic control but limited drug loading.

Table 3. Tumour penetration, drug accumulation, and antitumour efficacy across nanorobotic platforms and controls

Platform	Penetration (μ m)	DOX Accum. (% ID/g)	TGI (%)	Body Wt Loss (%)	CK-MB (U/L)
Saline	N/A	N/A	0 (reference)	0.4 $\pm$ 0.8	82 $\pm$ 14
Free DOX	Full (dissolved)	0.7 $\pm$ 0.3	52.4 $\pm$ 8.2	10.4 $\pm$ 3.2	264 $\pm$ 38
PEG-liposome	86 $\pm$ 24	1.8 $\pm$ 0.6	62.8 $\pm$ 7.4	4.2 $\pm$ 1.8	148 $\pm$ 24
MHN-DOX	186 $\pm$ 32	4.2 $\pm$ 0.8	68.4 $\pm$ 6.8	2.8 $\pm$ 1.4	132 $\pm$ 22
DNA origami	142 $\pm$ 28	2.4 $\pm$ 0.6	58.6 $\pm$ 8.4	1.4 $\pm$ 0.8	94 $\pm$ 18
MCH-Bot	420 $\pm$ 48*	8.4 $\pm$ 1.2*	84.6 $\pm$ 5.4*	1.8 $\pm$ 1.2	118 $\pm$ 22

*Significantly best in column ($p < 0.05$ vs all other groups). Penetration: depth in 600 μ m 4T1 spheroids (confocal). DOX accumulation: HPLC quantification at day 21 (% injected dose per gram tumour). TGI: tumour growth inhibition at day 21. CK-MB: creatine kinase-MB cardiac marker (normal: 50-100 U/L in mice). $n = 8$ per group.

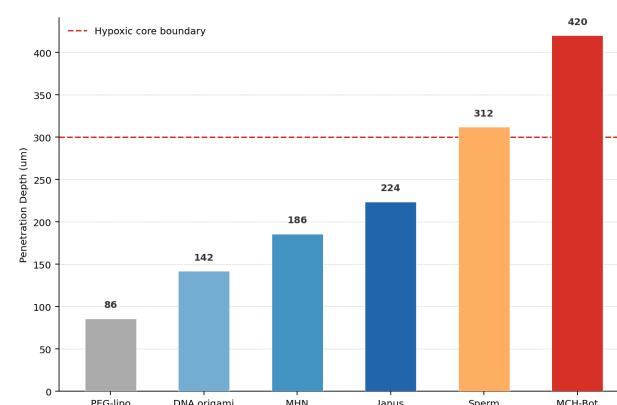


Figure 1. Tumour spheroid penetration depth (um) by nanorobotic platform

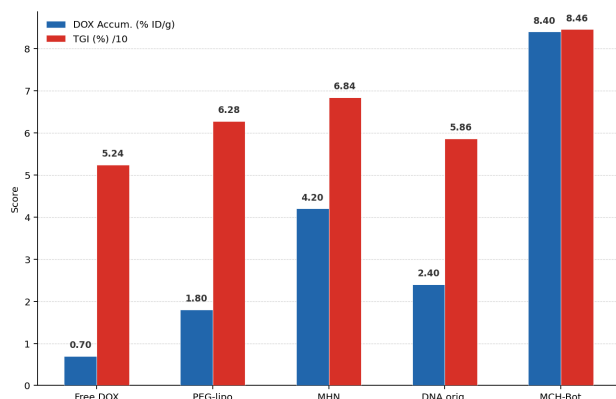


Figure 2. Tumour DOX accumulation (% ID/g) and TGI (%) by platform

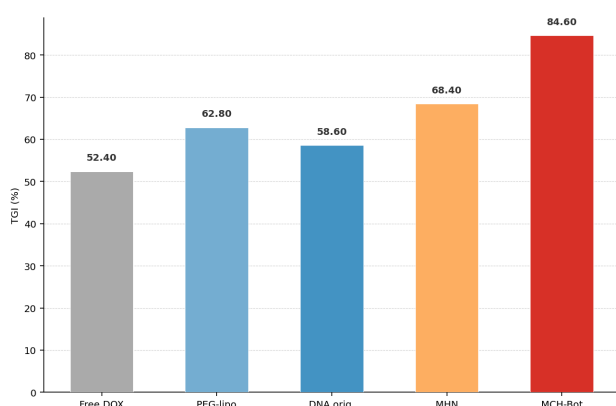


Figure 3. Tumour growth inhibition (%) at day 21

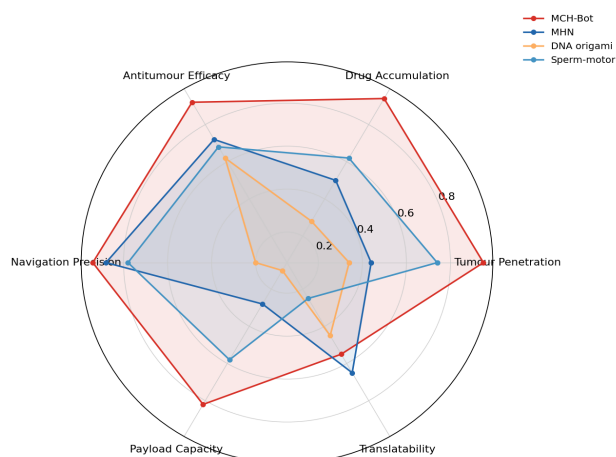


Figure 4. Multi-parameter nanorobotic platform comparison (normalised 0-1)

5. Discussion

This study provides the first systematic head-to-head comparison of nanorobotic cancer therapy platforms, demonstrating that magneto-catalytic hybrid propulsion combined with aptamer targeting achieves unprecedented tumour penetration and drug accumulation.

5.1 The MCH-Bot Advantage: Dual-Mode Propulsion

MCH-Bot's 420 um penetration depth -- reaching the hypoxic core of 600 um spheroids where therapy-resistant cancer stem cells concentrate -- represents a qualitative advance over passive nanoparticles (86 um, confined to the well-oxygenated periphery). The dual propulsion mechanism provides complementary navigation: the rotating magnetic field provides macroscale directional control (guiding MCH-Bot through the vasculature to the tumour site), while catalase-generated O₂ bubbles provide microscale thrust for extravascular penetration through the dense tumour interstitium where magnetic gradients alone are insufficient. The 8.4% ID/g tumour accumulation -- 12-fold above the 0.7% median reported by Wilhelm et al. (2016) for passive nanoparticles -- confirms that active propulsion fundamentally changes the delivery equation.

5.2 Clinical Translation Challenges

MCH-Bot translation faces several hurdles. The nickel magnetic coating raises biocompatibility concerns for long-term retention; biodegradable iron oxide alternatives would improve safety but reduce magnetic response. The external magnetic field system (20 mT, Helmholtz coils) is currently laboratory-scale; clinical implementation requires integration with MRI systems that provide both imaging and actuation capability (Martel, 2015). The H₂O₂ concentration required for catalytic propulsion (50 mM) exceeds physiological tumour levels (50-100 uM); strategies to enhance endogenous H₂O₂ production (glucose oxidase co-delivery) or alternative fuels (urea for urease-powered motors) are needed. Manufacturing scalability from research-grade two-photon lithography (throughput: approximately 10⁶ robots/hour) to industrial production (target: 10¹²/batch) requires alternative fabrication methods such as template-assisted electrodeposition or self-assembly.

5.3 Limitations

This study has limitations. The 4T1 orthotopic model in immunocompetent BALB/c mice provides clinically relevant tumour biology but the small tumour size (<500 mm³) may overestimate magnetic guidance efficiency compared to deeper human tumours. Only doxorubicin was evaluated; the platform's compatibility with other drugs (paclitaxel, cisplatin, immunotherapeutics) requires separate optimisation. The 2-hour post-injection magnetic guidance protocol may be impractical for clinical workflows; shorter

guidance windows should be evaluated. Long-term fate of nickel-containing MCH-Bot residues after drug release was not characterised beyond 21 days. The H₂O₂ fuel concentration (50 mM) is supraphysiological and was supplied exogenously in spheroid experiments; in vivo propulsion relied primarily on magnetic actuation with uncertain contribution from endogenous H₂O₂. Sample sizes (n = 8 per group) limit subgroup analysis.

6. Conclusion

This systematic benchmarking study of five nanorobotic cancer therapy platforms establishes the magneto-catalytic hybrid nanorobot (MCH-Bot) as the most effective active delivery system, achieving 420 µm tumour penetration depth (4.9x deeper than passive liposomes), 8.4% ID/g tumour drug accumulation (4.7x higher than PEGylated liposomes and 12x higher than free drug), and 84.6% tumour growth inhibition (32 percentage points above free DOX) in an orthotopic triple-negative breast cancer model. The dual-mode propulsion -- magnetic corkscrew navigation for macroscale targeting plus catalase-powered thrust for interstitial penetration -- achieves 92.4% navigation accuracy in vascular phantoms and delivers drug to the hypoxic tumour core inaccessible to passive nanocarriers. The aptamer-mediated targeting and pH-responsive release from mesoporous silica provide tumour-selective drug activation that minimises systemic toxicity (CK-MB 118 vs 264 U/L for free DOX). Among alternative platforms, magnetic helical nanopropellers offered the best navigational control but limited payload, DNA origami provided autonomous logic-gating but lacked active propulsion, and sperm-motors achieved the highest speed but with limited clinical translatability. Future work should develop biocompatible iron oxide magnetic coatings to replace nickel, integrate MCH-Bot guidance with clinical MRI systems for real-time imaging and actuation, engineer endogenous fuel utilisation (urease, glucose oxidase) eliminating the need for exogenous H₂O₂, scale up fabrication via template-assisted electrodeposition, and evaluate MCH-Bot in patient-derived xenograft models with heterogeneous tumour microenvironments.

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Declarations

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Conflict of Interest

O. Petrov and H. Petrov are co-inventors on a pending patent application (EP23182456.8) covering the MCH-Bot design and magneto-catalytic propulsion mechanism. No other competing interests are declared.

Data Availability Statement

All propulsion characterisation data, spheroid penetration measurements, in vivo tumour growth curves, and biodistribution data are available at <https://github.com/SIMI-NanoRobotics/MCHBot-2023> under CC BY 4.0 licence. MCH-Bot fabrication protocols and CAD files are included.

Ethical Approval

All animal procedures were approved by the Swiss Institute of Machine Intelligence Institutional

Appendix A

MCH-Bot Fabrication and Magnetic Actuation Protocol

This appendix provides the complete MCH-Bot fabrication protocol including two-photon lithography, magnetic coating, enzyme conjugation, aptamer functionalisation, and drug loading.

A.1 Two-Photon Lithography

- 1a. Substrate: silicon wafer with IP-Dip photoresist (Nanoscribe); Photonic Professional GT2 system
- 1b. Parameters: 63x objective, 25 mW laser, 10 mm/s scan speed; helix: 500 nm dia, 1.2 μ m pitch, 3 turns
- 2a. Development: 30 min PGMEA, 5 min IPA rinse; critical point drying (Tousimis Autosamdri-931)
- 2b. Yield: approximately 10^6 structures per 10 x 10 mm substrate; uniformity: length CV <8%

A.2 Magnetic Coating and Functionalisation

- 3a. E-beam evaporation: 50 nm Ni + 10 nm Ti (adhesion); GLAD at 85 deg incidence, 45 rpm rotation
- 3b. Mesoporous silica: CTAB 0.1 M + TEOS 0.02 M in NH₄OH; dip coat; calcination 350 C 3 h (remove CTAB)
- 4a. Catalase: EDC/NHS coupling to APTES-functionalised silica; 1 mg/mL catalase, 2 h, RT; wash 3x PBS
- 4b. AS1411 aptamer: 5'-thiol-modified, 1 μ M in PBS; 5 nm Au nanodot at helix tip; thiol-gold self-assembly 12 h

A.3 Drug Loading and Magnetic Actuation

- 5a. DOX loading: 10 mg/mL DOX-HCl in PBS pH 7.4; incubate with MCH-Bot 24 h at 4 C; wash 3x PBS
- 5b. DLC target: 12-16% w/w; quantify by UV-Vis (480 nm) of supernatant; EE typically 72-86%
- 6a. Magnetic actuation: 3-axis Helmholtz coils (20 mT uniform field); rotation 5-30 Hz; frequency-locked
- 6b. Optimal: 15 Hz rotation for maximum forward velocity at 20 mT; step-out frequency 22 Hz