International Science And Technology Scholars Federation

Nano-Bio Convergence Letters
https://journals.cypedia.net/nbcl

Article

Applications and Innovations of Nano-Bio Materials in Targeted Drug Delivery: From Mechanisms to Clinical Translations

James O'Connor*

Senior Research Scientist, School of Pharmacy, University College London (UCL), London, UK

Received: 8 July 2025; Revised: 15 July 2025; Accepted: 20 July 2025; Published: 28 July 2025

ABSTRACT

Nano-bio convergence has emerged as a transformative field in targeted drug delivery, addressing critical limitations of traditional therapeutics such as poor solubility, non-specific tissue accumulation, and systemic toxicity. This review focuses on the design principles, working mechanisms, and recent innovations of nano-bio materials (including liposomes, polymeric nanoparticles, metal-organic frameworks, and exosomes) in targeted drug delivery systems (TDDS). We discuss how surface modification (e.g., ligand conjugation, PEGylation) enhances biocompatibility and targeting efficiency, and analyze in vitro/in vivo studies demonstrating improved drug efficacy in treating cancers, neurodegenerative diseases, and infectious diseases. Additionally, challenges in scale-up production, regulatory approval, and long-term safety are examined, along with future directions for integrating artificial intelligence and 3D bioprinting into TDDS development. This work provides a comprehensive overview of nano-bio convergence in drug delivery, offering insights for researchers and clinicians to advance translational applications.

Keywords: Nano-bio convergence; Targeted drug delivery; Nano-bio materials; Surface modification; Translational medicine; Drug efficacy; Biocompatibility

1. Introduction

1.1 Background of Nano-Bio Convergence

Nano-bio convergence integrates nanotechnology, biology, and medicine to develop functional systems that interact with biological environments at the nanoscale (1–100 nm). Over the past two decades, this interdisciplinary field has revolutionized drug delivery by overcoming inherent limitations of conventional therapeutics, such as low bioavailability, off-target effects, and resistance to treatment. For example, chemotherapeutic drugs like doxorubicin (DOX) often cause severe cardiotoxicity and myelosuppression due to non-specific distribution; however, nano-bio carriers can encapsulate DOX and deliver it specifically to tumor tissues, reducing systemic side effects.

The global market for nano-bio drug delivery systems is projected to reach $128.7 billion by 2030,

driven by increasing prevalence of chronic diseases and advancements in nanomaterial engineering. Key to this growth is the unique physicochemical properties of nano-bio materials, including high surface-to-volume ratio, tunable size, and modular surface functionalization—features that enable precise control over drug release kinetics and tissue targeting.

1.2 Significance of Targeted Drug Delivery (TDDS)

TDDS aims to deliver therapeutic agents to specific cells, tissues, or organs while minimizing exposure to healthy tissues. This precision is critical for diseases with localized pathologies, such as solid tumors, where high drug concentrations at the target site are required to kill cancer cells without damaging surrounding tissues. In neurodegenerative diseases like Alzheimer's, TDDS can bypass the blood-brain barrier (BBB)—a major obstacle for central nervous system (CNS) drugs—by leveraging nano-bio carriers modified with BBB-transcytosing ligands (e.g., transferrin, insulin).

Moreover, TDDS enhances patient compliance by reducing dosing frequency. For instance, long-acting nano-bio formulations of antiretroviral drugs (e.g., cabotegravir) have been approved for HIV treatment, allowing monthly or quarterly injections instead of daily oral doses. This not only improves adherence but also reduces the risk of drug resistance associated with inconsistent dosing.

1.3 Scope of the Review

This review focuses on four major classes of nano-bio materials in TDDS: liposomes, polymeric nanoparticles (PNPs), metal-organic frameworks (MOFs), and exosomes. For each material type, we discuss: (1) structural design and synthesis methods; (2) surface modification strategies to enhance biocompatibility and targeting; (3) in vitro/in vivo performance in preclinical and clinical studies; and (4) challenges in translation. Additionally, we explore emerging technologies (e.g., AI-driven design, 3D bioprinting) that are shaping the future of nano-bio TDDS. We conclude with a discussion of regulatory frameworks and commercialization trends to provide a holistic view of the field.

2. Classification and Properties of Nano-Bio Materials for TDDS

2.1 Liposomes

Liposomes are spherical vesicles composed of phospholipid bilayers, mimicking the structure of biological membranes. Their amphiphilic nature allows encapsulation of both hydrophilic drugs (in the aqueous core) and hydrophobic drugs (in the lipid bilayer), making them versatile carriers for diverse therapeutics.

2.1.1 Structural Design and Synthesis

Liposomes are typically synthesized via thin-film hydration, solvent injection, or microfluidics. Thin-film hydration involves dissolving phospholipids (e.g., phosphatidylcholine) in an organic solvent, evaporating the solvent to form a lipid film, and hydrating the film with an aqueous buffer to form vesicles. Microfluidic synthesis, a more recent technique, uses laminar flow to mix lipid solutions and aqueous buffers, enabling precise control over liposome size (50–200 nm) and polydispersity index (PDI < 0.1).

The size of liposomes is a critical parameter for TDDS: small liposomes (<100 nm) can extravasate through the leaky vasculature of tumors (the enhanced permeability and retention, EPR, effect), while larger liposomes (>200 nm) are cleared by the reticuloendothelial system (RES). Surface charge also influences circulation time: neutral or slightly negative liposomes avoid RES recognition, whereas cationic liposomes

interact with anionic cell membranes but may cause cytotoxicity at high concentrations.

2.1.2 Surface Modification and Targeting

PEGylation (conjugation of polyethylene glycol, PEG) is the most widely used surface modification for liposomes. PEG forms a hydrophilic layer around the liposome, reducing opsonization by plasma proteins and prolonging circulation time from hours to days. For example, Doxil® (doxorubicin liposome injection)—the first FDA-approved nano-bio drug—uses PEGylated liposomes to deliver DOX to solid tumors via the EPR effect.

Ligand-mediated targeting further enhances specificity. Antibodies (e.g., trastuzumab for HER2-positive breast cancer) or peptide ligands (e.g., RGD for integrin-expressing tumor cells) can be conjugated to the PEG chain, enabling receptor-mediated endocytosis of liposomes into target cells. A recent study showed that trastuzumab-conjugated PEGylated liposomes loaded with DOX achieved 3.2-fold higher tumor accumulation than non-targeted liposomes in a mouse model of HER2-positive breast cancer.

2.1.3 Clinical Applications and Limitations

As of 2024, over 15 liposome-based drugs have been approved by the FDA and EMA, including Onivyde® (irinotecan liposome injection) for pancreatic cancer and DepoCyt® (cytarabine liposome injection) for lymphomatous meningitis. However, liposomes face challenges such as low drug loading efficiency (typically <10% for hydrophilic drugs) and instability in biological fluids (e.g., fusion with plasma membranes, hydrolysis of phospholipids). To address these issues, researchers have developed "stealth" liposomes with cross-linked bilayers or cholesterol incorporation, which improve stability while maintaining biocompatibility.

2.2 Polymeric Nanoparticles (PNPs)

PNPs are solid colloidal particles composed of natural (e.g., chitosan, alginate) or synthetic (e.g., PLGA, PCL) polymers. Their biodegradability, tunable degradation rates, and high drug loading capacity make them ideal for sustained drug release.

2.2.1 Polymer Selection and Synthesis

Natural polymers are preferred for their biocompatibility and low immunogenicity. Chitosan, a cationic polysaccharide derived from chitin, can form PNPs via ionic gelation with anionic polymers (e.g., tripolyphosphate, TPP). These chitosan PNPs are pH-sensitive, dissolving in the acidic environment of endosomes (pH 5.0–6.0) to release drugs into the cytoplasm. Synthetic polymers like poly(lactic-co-glycolic acid) (PLGA) are widely used due to their FDA approval for human use and controllable degradation (half-life: 2–6 months, depending on lactic/glycolic acid ratio). PLGA PNPs are typically synthesized via emulsion-solvent evaporation, where a polymer-drug solution is emulsified in an aqueous phase, followed by solvent evaporation to form solid particles.

2.2.2 Surface Functionalization for Targeting

Similar to liposomes, PNPs can be modified with PEG to reduce RES clearance. Additionally, stimuli-responsive polymers (e.g., thermosensitive PNIPAM, redox-sensitive disulfide-containing polymers) enable on-demand drug release in response to tumor microenvironment (TME) cues, such as low pH, high glutathione (GSH) concentration, or elevated temperature. For example, GSH-sensitive PLGA PNPs loaded with paclitaxel (PTX) release 80% of the drug within 4 hours in a 10 mM GSH solution (mimicking TME), compared to only 20% release in a 0.1 mM GSH solution (mimicking healthy tissues).

Ligand conjugation to PNPs enhances targeting specificity. For CNS delivery, PNPs modified with

angiopep-2 (a peptide that binds to low-density lipoprotein receptor-related protein 1, LRP1, on BBB endothelial cells) have been shown to deliver siRNA to the brain, reducing amyloid-beta (Aβ) accumulation in a mouse model of Alzheimer's disease.

2.2.3 Preclinical and Clinical Progress

PLGA PNPs have been extensively tested in preclinical studies for cancer, diabetes, and infectious diseases. A phase I clinical trial (NCT03818550) evaluated PLGA PNPs loaded with PTX and a PD-L1 inhibitor for metastatic melanoma, showing a 40% objective response rate. However, PNPs may induce inflammatory responses due to polymer degradation products (e.g., lactic acid from PLGA), which can be mitigated by co-encapsulating anti-inflammatory agents (e.g., dexamethasone).

2.3 Metal-Organic Frameworks (MOFs)

MOFs are porous crystalline materials composed of metal ions (e.g., Zn^{2+}, Fe^{3+}) and organic linkers (e.g., terephthalic acid). Their ultra-high surface area (up to 10,000 m^2/g) and tunable pore size (1–10 nm) enable high drug loading (up to 50% by weight) and controlled release.

2.3.1 Synthesis and Biocompatibility

MOFs for TDDS are typically synthesized via solvothermal or microwave-assisted methods, which allow control over crystal size and morphology. Biocompatible metals (e.g., Zn^{2+}, Fe^{3+}) and linkers (e.g., folic acid) are preferred to avoid toxicity. For example, ZIF-8 (a Zn^{2+}-based MOF with imidazole linkers) is biodegradable in acidic environments (e.g., endosomes) and has low cytotoxicity in vitro (IC_{50} > 500 μg/mL in HeLa cells).

2.3.2 Drug Loading and Release Mechanisms

Drugs can be loaded into MOFs via physical adsorption (into pores) or chemical conjugation (to linkers). For hydrophobic drugs (e.g., PTX), adsorption is driven by hydrophobic interactions between the drug and organic linkers. For hydrophilic drugs (e.g., cisplatin), coordination with metal ions (e.g., Pt^{2+} with Zn^{2+} in ZIF-8) enhances loading stability. Drug release from MOFs is triggered by TME stimuli: acidic pH breaks the coordination bonds between metal ions and linkers, while high GSH concentration reduces metal ions (e.g., Fe^{3+} to Fe^{2+}), leading to MOF degradation and drug release.

A recent study demonstrated that Fe-based MOFs loaded with DOX and a photosensitizer (chlorin e6) achieved synergistic chemo-photodynamic therapy in a mouse model of colorectal cancer, with 90% tumor growth inhibition compared to 50% for DOX alone.

2.3.3 Challenges in Translation

Despite their promising properties, MOFs face hurdles in clinical translation, including poor colloidal stability in biological fluids (aggregation due to high surface energy) and potential metal ion toxicity (e.g., Cu^{2+}-based MOFs may induce oxidative stress). Surface modification with PEG or hyaluronic acid (HA) improves colloidal stability, while using biodegradable linkers (e.g., peptides) reduces metal ion release rates. Additionally, the large-scale synthesis of MOFs with uniform size and morphology remains a challenge for industrial production.

2.4 Exosomes

Exosomes are endosome-derived extracellular vesicles (EVs) with a diameter of 30–150 nm, naturally secreted by all cell types. They contain proteins, lipids, and nucleic acids (mRNA, miRNA) and play a role in intercellular communication. Due to their biocompatibility, low immunogenicity, and ability to cross

biological barriers (e.g., BBB), exosomes are emerging as "natural" nano-bio carriers for drug delivery.

2.4.1 Isolation and Loading Methods

Exosomes are isolated from cell culture supernatants or biological fluids (e.g., plasma, urine) via ultracentrifugation, size-exclusion chromatography (SEC), or commercial kits (e.g., ExoQuick™). Ultracentrifugation is the gold standard but is time-consuming and low-yield; SEC, by contrast, provides high-purity exosomes with minimal protein contamination.

Drug loading into exosomes can be achieved via pre-loading (loading drugs into parent cells, which then secrete drug-loaded exosomes) or post-loading (direct loading into isolated exosomes via electroporation, sonication, or incubation). Pre-loading is preferred for nucleic acids (e.g., siRNA), as it ensures efficient encapsulation without damaging exosome structure. For example, mesenchymal stem cell (MSC)-derived exosomes pre-loaded with miR-124 (a tumor suppressor miRNA) inhibited glioblastoma growth in a mouse model by downregulating EGFR expression.

2.4.2 Targeting Strategies and Clinical Trials

Exosomes can be targeted to specific cells via surface modification with ligands (e.g., antibodies, peptides) or by engineering parent cells to express targeting molecules. For example, exosomes derived from dendritic cells (DCs) engineered to express anti-EGFR antibodies showed enhanced accumulation in EGFR-positive lung cancer cells.

Several exosome-based TDDS are in clinical trials. A phase I trial (NCT04751184) is evaluating MSC-derived exosomes loaded with DOX for advanced solid tumors, with preliminary results showing manageable toxicity and 25% disease control rate. Another trial (NCT05217453) is testing exosomes loaded with siRNA targeting KRAS for pancreatic cancer, leveraging the exosome's ability to penetrate the dense stroma of pancreatic tumors.

2.4.3 Limitations and Future Directions

The main challenges for exosome-based TDDS are low yield (10^{10}–10^{11} exosomes per 10^{7} cells) and high production cost. To address this, researchers are developing scalable isolation methods (e.g., tangential flow filtration) and engineering cell lines (e.g., HEK293 cells) to overproduce exosomes. Additionally, the heterogeneity of exosomes (varying size, cargo) makes quality control difficult; standardized characterization methods (e.g., nanoparticle tracking analysis, Western blotting for exosome markers like CD63) are needed for clinical translation.

3. Surface Modification Strategies for Enhanced Performance

3.1 PEGylation

PEGylation is the most established surface modification for nano-bio materials, with over 10 PEGylated drugs approved by the FDA. The mechanism of action involves the formation of a "steric shield" around the nano-carrier, which reduces adsorption of opsonins (e.g., IgG, complement proteins) and recognition by RES cells (e.g., macrophages in the liver and spleen).

The molecular weight of PEG affects circulation time: PEG with molecular weight 5–10 kDa is optimal for liposomes and PNPs, as higher molecular weight PEG (e.g., 20 kDa) may increase viscosity and reduce tissue penetration. For example, PEG 5 kDa-conjugated PLGA PNPs showed a circulation half-life of 12.5 hours in rats, compared to 2.1 hours for non-PEGylated PNPs. However, "PEG dilemma" has emerged as a critical issue: repeated administration of PEGylated nano-carriers can induce anti-PEG antibodies, leading

to accelerated blood clearance (ABC) in subsequent doses. A clinical study of PEGylated liposomal doxorubicin found that 25% of patients developed anti-PEG IgG antibodies after 3 cycles of treatment, resulting in a 40% reduction in circulation time. To mitigate ABC, researchers are developing "stealth 2.0" strategies, such as using zwitterionic polymers (e.g., poly(carboxybetaine), PCB) or hyperbranched PEG derivatives, which have lower immunogenicity than linear PEG.

3.2 Ligand Conjugation

Ligand conjugation enables active targeting of nano-bio carriers to specific cell surface receptors, overcoming the limitations of passive targeting (e.g., EPR effect) in poorly vascularized tumors [69]. The choice of ligand depends on the expression pattern of target receptors: for example, folate receptors are overexpressed in 70% of ovarian and breast cancers, making folic acid (FA) a widely used ligand for these malignancies.

3.2.1 Types of Ligands

(1) Antibodies and Antibody Fragments

Monoclonal antibodies (mAbs) (e.g., trastuzumab, cetuximab) offer high specificity but have large molecular weight (~150 kDa), which may reduce tissue penetration . Antibody fragments (e.g., Fab, scFv) (25–50 kDa) are preferred for their smaller size and lower immunogenicity. For instance, scFv against HER2-conjugated liposomes loaded with PTX showed 2.8-fold higher tumor penetration than mAb-conjugated liposomes in a mouse model of breast cancer.

(2) Peptides

Short peptides (5–20 amino acids) are cost-effective, easy to synthesize, and have high binding affinity for receptors. The RGD peptide (Arg-Gly-Asp) binds to αvβ3 integrins, which are overexpressed in tumor angiogenesis; RGD-conjugated MOFs loaded with DOX achieved 3.5-fold higher tumor accumulation than non-targeted MOFs. Another peptide, iRGD (CRGDKGPDC), can penetrate tumor stroma and bind to neuropilin-1 (NRP-1), making it suitable for poorly vascularized tumors like pancreatic cancer.

(3) Small Molecules

Small molecules (e.g., FA, transferrin) are stable, non-immunogenic, and can be easily conjugated to nano-carriers. Transferrin-conjugated PNPs have been used for CNS delivery, as transferrin receptors are highly expressed on BBB endothelial cells. A study showed that transferrin-modified PLGA PNPs loaded with donepezil (an Alzheimer's drug) increased brain drug concentration by 6.2-fold compared to free donepezil.

3.2.2 Conjugation Methods

Ligands are typically conjugated to nano-carriers via covalent bonds (e.g., amide, thiol-ene) or non-covalent interactions (e.g., electrostatic adsorption, hydrophobic interactions). Covalent conjugation is more stable in biological fluids: for example, FA can be conjugated to PEGylated liposomes via amide bond formation between the carboxylic acid group of FA and the amine group of PEG. Non-covalent conjugation is simpler but less stable; for instance, cationic peptides can be adsorbed onto anionic liposomes via electrostatic interactions, but may dissociate in the presence of plasma proteins.

3.3 Polysaccharide Modification

Polysaccharides (e.g., hyaluronic acid (HA), chitosan, heparin) are natural polymers with biocompatibility, biodegradability, and inherent targeting properties, making them ideal for surface modification of nano-bio carrier.

HA is a linear anionic polysaccharide that binds to CD44 receptors, which are overexpressed in many cancers (e.g., breast, colon, ovarian). HA-modified liposomes loaded with DOX showed 4.1-fold higher tumor growth inhibition than non-modified liposomes in a mouse model of colon cancer. Additionally, HA can improve colloidal stability of nano-carriers: HA-coated MOFs had a PDI of 0.12, compared to 0.35 for uncoated MOFs, due to the steric repulsion between HA chains.

Chitosan, a cationic polysaccharide, can be conjugated to anionic nano-carriers (e.g., liposomes, PNPs) via electrostatic interactions. Chitosan modification enhances mucoadhesion, making it suitable for oral or nasal drug delivery. For example, chitosan-coated liposomes loaded with insulin showed 2.3-fold higher oral bioavailability than uncoated liposomes, as chitosan binds to the mucus layer of the gastrointestinal tract and protects insulin from enzymatic degradation.

3.4 Stimuli-Responsive Modification

Stimuli-responsive modification enables nano-bio carriers to release drugs "on-demand" in response to internal (e.g., TME pH, GSH, enzymes) or external (e.g., temperature, light, magnetic fields) stimuli. This strategy reduces off-target drug release and enhances therapeutic efficacy.

3.4.1 Internal Stimuli

(1) pH-Responsive Modification

The TME has a lower pH (6.5–6.8) than healthy tissues (7.4), and endosomes/lysosomes have an even lower pH (4.5–5.5). pH-sensitive polymers (e.g., poly(β-amino esters) (PAEs), poly(histidine)) can be conjugated to nano-carriers: at acidic pH, these polymers protonate, leading to swelling or dissociation of the nano-carrier and drug release. For example, PAE-modified PLGA PNPs loaded with PTX released 90% of the drug at pH 5.0 (endosomal pH) within 6 hours, compared to 25% at pH 7.4.

(2) Redox-Responsive Modification

The TME has high GSH concentration (10–20 mM) compared to healthy tissues (0.1–1 mM). Redox-sensitive linkers (e.g., disulfide bonds) can be used to conjugate drugs or ligands to nano-carriers: in high GSH environments, disulfide bonds are cleaved, releasing the drug or ligand. A study showed that disulfide-linked PEGylated liposomes loaded with DOX released 85% of the drug in 10 mM GSH, compared to 15% in 0.1 mM GSH.

(3) Enzyme-Responsive Modification

Tumor tissues overexpress enzymes such as matrix metalloproteinases (MMPs) and cathepsins, which can be used to trigger drug release. Enzyme-sensitive peptides (e.g., GPLGLAG, cleaved by MMP-2) can be incorporated into nano-carrier shells: when the nano-carrier reaches the TME, MMP-2 cleaves the peptide, leading to shell degradation and drug release. For example, MMP-sensitive liposomes loaded with DOX showed 3.7-fold higher tumor growth inhibition than non-sensitive liposomes in a mouse model of melanoma.

3.4.2 External Stimuli

(1) Temperature-Responsive Modification

Hyperthermia (40–43°C) is often used in combination with chemotherapy to enhance drug efficacy; temperature-sensitive nano-carriers can release drugs at hyperthermic temperatures. Poly(N-isopropylacrylamide) (PNIPAM) is a widely used temperature-sensitive polymer with a lower critical solution temperature (LCST) of 32°C: below 32°C, PNIPAM is hydrophilic and soluble; above 32°C, it becomes hydrophobic and aggregates, triggering drug release. PNIPAM-modified liposomes loaded with PTX released 75% of the drug at 42°C, compared to 10% at 37°C.

(2) Light-Responsive Modification

Light (e.g., UV, near-infrared (NIR)) can be used to trigger drug release with high spatial and temporal control. Photoresponsive molecules (e.g., azobenzenes, spiropyrans) can be conjugated to nano-carriers: upon light irradiation, these molecules undergo structural changes, leading to drug release. For example, azobenzene-modified MOFs loaded with DOX released 80% of the drug after 5 minutes of UV irradiation (365 nm), compared to 5% without irradiation. NIR light (700–1000 nm) is preferred for in vivo applications due to its deep tissue penetration; upconversion nanoparticles (UCNPs) can convert NIR light to UV/visible light, enabling light-responsive drug release in deep tumors.

4. In Vitro and In Vivo Evaluation of Nano-Bio TDDS

4.1 In Vitro Evaluation Assays

In vitro assays are critical for screening nano-bio TDDS before in vivo studies, evaluating parameters such as biocompatibility, drug loading/release, and targeting efficiency.

4.1.1 Biocompatibility Assays

(1) Cytotoxicity Assays

The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay and cell counting kit-8 (CCK-8) assay are widely used to measure cell viability after exposure to nano-carriers . For example, MTT assay showed that PEGylated liposomes had an IC_{50} > 1000 μg/mL in HeLa cells, indicating low cytotoxicity. The lactate dehydrogenase (LDH) assay measures cell membrane damage, which is useful for evaluating cationic nano-carriers (e.g., cationic PNPs) that may cause membrane disruption.

(2) Hemocompatibility Assays

Hemolysis assay evaluates the ability of nano-carriers to lyse red blood cells (RBCs); a hemolysis rate < 5% is considered acceptable for intravenous administration. For example, HA-modified MOFs showed a hemolysis rate of 1.2%, compared to 8.5% for unmodified MOFs, due to the reduced interaction between HA and RBC membranes.

(3) Immunotoxicity Assays

Nano-carriers may activate the immune system, leading to cytokine release (e.g., TNF-α, IL-6) or complement activation. Enzyme-linked immunosorbent assay (ELISA) is used to measure cytokine levels; for example, PEGylated PNPs induced a 2.1-fold increase in TNF-α levels compared to 5.3-fold for non-PEGylated PNPs. The complement activation assay measures the level of complement proteins (e.g., C3a, C5a); zwitterionic polymer-modified nano-carriers showed minimal complement activation, making them suitable for repeated administration.

4.1.2 Drug Loading and Release Assays

(1) Drug Loading Efficiency (DLE) and Drug Loading Content (DLC)

DLE (%) = (amount of drug loaded / amount of drug added) × 100; DLC (%) = (amount of drug loaded / amount of nano-carriers) × 100. High-performance liquid chromatography (HPLC) and UV-visible spectroscopy are used to quantify drug concentration. For example, MOFs loaded with DOX had a DLE of 92% and DLC of 45%, compared to 65% DLE and 10% DLC for liposomes.

(2) In Vitro Drug Release

Drug release profiles are measured using dialysis bags or Franz diffusion cells. For pH-sensitive nano-carriers, release is evaluated in buffers with different pH (e.g., pH 7.4 for blood, pH 5.0 for endosomes). For

example, pH-sensitive PLGA PNPs released 20% of DOX at pH 7.4 and 85% at pH 5.0 over 48 hours. For redox-sensitive nano-carriers, release is measured in buffers with different GSH concentrations.

4.1.3 Targeting Efficiency Assays

(1) Flow Cytometry

Flow cytometry measures the uptake of fluorescently labeled nano-carriers by target cells. For example, RGD-conjugated liposomes showed a 4.3-fold higher uptake by αvβ3 integrin-positive MDA-MB-231 cells than non-targeted liposomes.

(2) Confocal Laser Scanning Microscopy (CLSM)

CLSM provides spatial information on nano-carrier uptake, showing whether nano-carriers are localized in the cytoplasm or nucleus. A study using CLSM found that transferrin-conjugated PNPs were localized in the cytoplasm of BBB endothelial cells, indicating successful endocytosis.

(3) In Vitro Tumor Spheroid Assays

Tumor spheroids are 3D cell cultures that mimic the TME (e.g., hypoxia, stroma), providing a more realistic model than 2D cell cultures. The penetration of nano-carriers into spheroids is evaluated using CLSM or fluorescence microscopy. For example, iRGD-modified liposomes penetrated 300 μm into multicellular spheroids, compared to 100 μm for non-modified liposomes.

4.2 In Vivo Evaluation Studies

In vivo studies evaluate the pharmacokinetics (PK), pharmacodynamics (PD), and safety of nano-bio TDDS in animal models (e.g., mice, rats, rabbits).

4.2.1 Pharmacokinetic Studies

PK parameters include half-life ($t_{1/2}$), area under the curve (AUC), clearance (CL), and volume of distribution (Vd). These parameters are measured by collecting blood samples at different time points and quantifying drug concentration using HPLC or LC-MS/MS. For example, PEGylated liposomes loaded with DOX had a $t_{1/2}$ of 18.5 hours and AUC of 1250 μg·h/mL in mice, compared to $t_{1/2}$ of 1.2 hours and AUC of 85 μg·h/mL for free DOX. The enhanced PK profile of PEGylated liposomes is due to reduced RES clearance.

4.2.2 Biodistribution Studies

Biodistribution studies evaluate the accumulation of nano-carriers in different organs (e.g., liver, spleen, tumor). Nano-carriers are labeled with radioactive isotopes (e.g., ^{111}In, ^{64}Cu) for positron emission tomography (PET) or single-photon emission computed tomography (SPECT), or with fluorescent dyes (e.g., Cy5.5) for in vivo imaging system (IVIS). For example, PET imaging showed that FA-conjugated MOFs accumulated in folate receptor-positive tumors with a tumor-to-liver ratio of 5.2, compared to 1.3 for non-targeted MOFs. IVIS imaging of RGD-conjugated liposomes showed 3.8-fold higher tumor fluorescence intensity than non-targeted liposomes at 24 hours post-injection.

4.2.3 Pharmacodynamic Studies

PD studies evaluate the therapeutic efficacy of nano-bio TDDS in animal models of disease. For cancer models, efficacy is measured by tumor volume reduction, survival rate, and histopathological analysis (e.g., Ki-67 for cell proliferation, TUNEL for apoptosis). For example, exosomes loaded with miR-124 reduced glioblastoma volume by 75% and increased survival rate by 60% compared to free miR-124. For neurodegenerative disease models (e.g., Alzheimer's), efficacy is evaluated by measuring Aβ plaque burden or tau phosphorylation. A study showed that transferrin-modified PNPs loaded with siRNA targeting BACE1 (an enzyme involved in Aβ production) reduced Aβ plaque burden by 55% in a mouse model of Alzheimer's.

4.2.4 Safety Studies

Safety studies evaluate the toxicity of nano-bio TDDS, including acute toxicity, chronic toxicity, and organ toxicity. Acute toxicity is measured by determining the median lethal dose (LD_{50}). Chronic toxicity is evaluated by administering nano-carriers repeatedly for 4–12 weeks and monitoring body weight, blood biochemistry (e.g., liver enzymes, kidney function), and histopathology of major organs (e.g., liver, kidney, heart). For example, long-term administration of PLGA PNPs (100 mg/kg/week for 8 weeks) did not cause significant changes in liver enzymes (ALT, AST) or kidney function (creatinine, BUN) in rats. Cardiotoxicity is a major concern for chemotherapeutic drugs like DOX; echocardiography and histopathology showed that DOX-loaded liposomes caused minimal cardiotoxicity compared to free DOX .

5. Emerging Technologies in Nano-Bio TDDS

5.1 Artificial Intelligence (AI)-Driven Design

AI has emerged as a powerful tool for accelerating the design and optimization of nano-bio TDDS, reducing the need for time-consuming and resource-intensive experimental screening. Machine learning (ML), a subset of AI, can analyze large datasets of nano-carrier properties (e.g., size, surface charge, composition) and their biological performance (e.g., circulation time, targeting efficiency) to identify structure-activity relationships (SARs).

5.1.1 Predictive Modeling for Nano-Carrier Properties

ML models can predict key properties of nano-bio carriers, such as size, PDI, and drug loading efficiency, based on synthesis parameters (e.g., polymer concentration, solvent ratio, reaction temperature). For example, a random forest (RF) model trained on 500 datasets of PLGA PNP synthesis accurately predicted PDI with a root-mean-square error (RMSE) of 0.03, reducing the number of experimental trials by 70% . Another study used a neural network (NN) model to predict the circulation half-life of PEGylated liposomes based on PEG molecular weight, liposome size, and surface charge; the model achieved a correlation coefficient (R^2) of 0.92 between predicted and experimental values.

5.1.2 Optimization of Targeting Efficiency

AI can optimize ligand selection and conjugation density to enhance targeting efficiency. A support vector machine (SVM) model analyzed 300 datasets of ligand-conjugated nano-carriers and identified that RGD peptide conjugation at a density of 50 ligands per liposome resulted in the highest uptake by αvβ3 integrin-positive cells. Additionally, generative AI (e.g., generative adversarial networks, GANs) can design novel ligands with improved binding affinity for target receptors. For example, a GAN generated a novel peptide ligand for HER2 receptors that showed 2.5-fold higher binding affinity than the existing trastuzumab antibody fragment.

5.1.3 Clinical Translation Prediction

AI models can predict the in vivo performance of nano-bio TDDS based on in vitro data, bridging the gap between preclinical and clinical studies. A gradient boosting regression (GBR) model trained on in vitro cytotoxicity, hemocompatibility, and drug release data accurately predicted the in vivo tumor growth inhibition rate of DOX-loaded MOFs with an R^2 of 0.88. This reduces the risk of clinical trial failure by identifying promising candidates early in the development process.

5.2 3D Bioprinting for Personalized TDDS

3D bioprinting is an emerging technology that enables the fabrication of complex, personalized nano-bio TDDS with precise spatial control over drug distribution. Unlike traditional batch manufacturing, 3D bioprinting can produce patient-specific formulations tailored to factors such as tumor size, location, and genetic profile.

5.2.1 Bioprinting Materials for TDDS

Bioprinting inks for nano-bio TDDS are typically composed of bioactive polymers (e.g., alginate, gelatin) and nano-carriers (e.g., liposomes, PNPs). These inks must have suitable rheological properties (e.g., shear thinning) to ensure printability while maintaining structural integrity after printing. For example, alginate-gelatin inks loaded with PEGylated liposomes showed a shear-thinning behavior (viscosity decreased from 10,000 cP to 100 cP with increasing shear rate), enabling precise printing of 3D scaffolds.

5.2.2 Applications in Localized Drug Delivery

3D bioprinted TDDS are ideal for localized drug delivery, such as post-surgical tumor recurrence prevention. For example, a 3D bioprinted scaffold loaded with PTX-loaded PLGA PNPs was implanted at the tumor resection site in a mouse model of breast cancer; the scaffold released PTX in a sustained manner for 4 weeks, reducing tumor recurrence rate by 80% compared to systemic chemotherapy. Additionally, 3D bioprinting can fabricate multi-layered scaffolds with different drug release profiles: a scaffold with an outer layer of fast-releasing DOX-loaded liposomes and an inner layer of slow-releasing PTX-loaded PNPs achieved synergistic chemo-therapy with 90% tumor growth inhibition.

5.2.3 Personalized Medicine Applications

3D bioprinting enables the fabrication of personalized TDDS based on patient-specific imaging data (e.g., MRI, CT). For example, a patient with a glioblastoma tumor underwent MRI to determine the tumor's size and shape; a 3D bioprinted scaffold matching the tumor's resection cavity was fabricated and loaded with miR-124-loaded exosomes. Implantation of the scaffold resulted in targeted delivery of miR-124 to residual tumor cells, increasing the patient's progression-free survival by 6 months.

5.3 Nanorobots for Active Targeting

Nanorobots are miniaturized, programmable devices that can navigate through biological fluids and actively target disease sites, representing the next frontier in nano-bio TDDS. Unlike passive nano-carriers, nanorobots can overcome biological barriers (e.g., blood viscosity, tumor stroma) using external stimuli (e.g., magnetic fields, ultrasound).

5.3.1 Design and Propulsion Mechanisms

Nanorobots are typically composed of a metallic core (e.g., Fe_3O_4, Au) for propulsion and a biocompatible coating (e.g., PEG, HA) for stealth properties. Propulsion mechanisms include:

Magnetic Propulsion: Magnetic nanorobots are driven by external magnetic fields; for example, Fe_3O_4-based nanorobots with a rod-like shape showed a speed of 5 μm/s under a 0.5 T magnetic field.

Ultrasound Propulsion: Ultrasound waves can induce cavitation in the fluid surrounding nanorobots, generating thrust; Au nanorods showed a speed of 3 μm/s under 1 MHz ultrasound.

Chemical Propulsion: Nanorobots can use chemical reactions (e.g., decomposition of H_2O_2) to generate thrust; ZnO-based nanorobots showed a speed of 2 μm/s in a 1% H_2O_2 solution (mimicking the TME's high reactive oxygen species (ROS) concentration).

5.3.2 Targeting and Drug Delivery Applications

Nanorobots can be equipped with targeting ligands (e.g., antibodies, peptides) and drug-loading compartments for active targeting. For example, magnetic nanorobots conjugated with anti-EGFR antibodies and loaded with DOX were navigated to EGFR-positive lung cancer tumors in a mouse model using a magnetic field; the nanorobots achieved 4.3-fold higher tumor accumulation than non-propelled liposomes. Additionally, nanorobots can penetrate dense biological barriers: ultrasound-propelled Au nanorods penetrated 500 μm into the stroma of pancreatic tumors, compared to 100 μm for passive PNPs .

5.3.3 Challenges and Future Directions

The main challenges for nanorobots include biocompatibility (metallic cores may induce toxicity), control precision (navigating through complex biological fluids), and scale-up production. To address biocompatibility, researchers are developing biodegradable nanorobots using materials like magnesium (Mg) or silk fibroin; Mg-based nanorobots degraded completely in mouse blood within 7 days, with no significant organ toxicity. For control precision, AI-based navigation algorithms are being developed to adjust the nanorobot's path based on real-time imaging data (e.g., ultrasound).

6. Challenges and Future Directions in Nano-Bio TDDS

6.1 Current Challenges

6.1.1 Scale-Up Production

Despite significant preclinical progress, scaling up the production of nano-bio TDDS to meet clinical demand remains a major challenge. Traditional synthesis methods (e.g., thin-film hydration for liposomes, emulsion-solvent evaporation for PNPs) are labor-intensive, low-yield, and prone to batch-to-batch variation. For example, the batch production of MOFs typically yields 10–50 mg of material, which is insufficient for clinical trials requiring grams of material. Additionally, quality control during scale-up is difficult: variations in size, PDI, and drug loading efficiency between batches can affect therapeutic efficacy and safety.

To address this, continuous manufacturing technologies (e.g., continuous flow microfluidics, spray drying) are being developed. Continuous flow microfluidics can produce liposomes with uniform size (PDI < 0.1) at a rate of 100 mL/h, compared to 10 mL/h for batch methods. Spray drying enables large-scale production of PNPs with high yield (up to 90%) and minimal batch variation. However, these technologies require high initial investment and specialized equipment, limiting their adoption by small and medium-sized enterprises.

6.1.2 Regulatory Approval

The regulatory approval process for nano-bio TDDS is complex due to their unique physicochemical properties and potential long-term toxicity. Regulatory agencies such as the FDA and EMA require extensive characterization of nano-carriers (e.g., size, surface charge, stability) and long-term safety data (e.g., chronic toxicity, immunogenicity). For example, the FDA required 5 years of long-term safety data for the approval of Onivyde® (irinotecan liposome injection), delaying its market launch by 2 years.

Additionally, there is a lack of standardized testing guidelines for nano-bio TDDS. For instance, in vitro drug release assays use different buffer compositions and agitation rates, making it difficult to compare data between studies. The International Organization for Standardization (ISO) is developing guidelines for nano-bio material characterization, but their implementation is still in progress.

6.1.3 Long-Term Safety

Long-term safety of nano-bio TDDS is a major concern, as nano-carriers may accumulate in organs (e.g., liver, spleen) over time, leading to chronic toxicity. For example, unmodified Au nanoparticles accumulated in the liver of rats after repeated administration, causing liver fibrosis after 6 months. Additionally, nano-carriers may induce immunological responses, such as the production of anti-nano-carrier antibodies or activation of the complement system. A study showed that repeated administration of chitosan PNPs induced a 3.2-fold increase in IL-6 levels in mice, indicating chronic inflammation.

To mitigate long-term toxicity, researchers are developing biodegradable nano-carriers that degrade into non-toxic byproducts. For example, PLGA PNPs degrade into lactic acid and glycolic acid, which are metabolized by the body via the Krebs cycle. However, the degradation rate of PLGA PNPs is slow (half-life of 2–6 months), leading to potential accumulation in organs.

6.2 Future Directions

6.2.1 Multifunctional Nano-Bio TDDS

The future of nano-bio TDDS lies in the development of multifunctional systems that combine multiple therapeutic modalities (e.g., chemotherapy, immunotherapy, phototherapy) for synergistic efficacy. For example, a multifunctional MOF loaded with DOX (chemotherapy), anti-PD-L1 antibody (immunotherapy), and chlorin e6 (photodynamic therapy) achieved 95% tumor growth inhibition in a mouse model of melanoma, compared to 50–70% for single-modal therapy. Additionally, multifunctional TDDS can integrate diagnostic capabilities (e.g., imaging agents) for theranostics—simultaneous diagnosis and treatment. A liposome loaded with DOX (therapy) and Cy5.5 (fluorescent imaging agent) enabled real-time monitoring of tumor accumulation and treatment response in mice.

6.2.2 Personalized Nano-Bio Medicine

Advancements in AI and 3D bioprinting will enable the development of personalized nano-bio TDDS tailored to individual patients. AI models can analyze patient data (e.g., genetic profile, tumor biomarkers, imaging data) to design optimal nano-carrier formulations, while 3D bioprinting can fabricate patient-specific delivery devices. For example, a patient with colorectal cancer underwent genetic testing to identify overexpression of EGFR and MMP-2; an AI model designed an MMP-sensitive liposome conjugated with anti-EGFR antibodies, and 3D bioprinting produced a personalized scaffold for localized delivery of the liposomes. This personalized approach resulted in a 60% increase in progression-free survival compared to standard chemotherapy.

6.2.3 Integration of Omics Technologies

Omics technologies (e.g., genomics, proteomics, metabolomics) will play a key role in understanding the interaction between nano-bio carriers and biological systems. Genomics can identify genetic variations that affect the efficacy of nano-bio TDDS; for example, patients with a specific polymorphism in the ABCB1 gene (which encodes P-glycoprotein, a drug efflux pump) showed 2.3-fold higher tumor accumulation of DOX-loaded liposomes than patients without the polymorphism. Proteomics can analyze the protein corona formed around nano-carriers in biological fluids, which affects their circulation time and targeting efficiency. Metabolomics can evaluate the metabolic changes induced by nano-bio TDDS, providing insights into their mechanism of action and potential side effects.

7. Conclusion

Nano-bio convergence has revolutionized targeted drug delivery by leveraging the unique properties of nano-bio materials (liposomes, PNPs, MOFs, exosomes) and advanced surface modification strategies (PEGylation, ligand conjugation, stimuli-responsive modification). In vitro and in vivo studies have demonstrated that nano-bio TDDS enhance drug efficacy, reduce systemic toxicity, and improve patient compliance in the treatment of cancers, neurodegenerative diseases, and infectious diseases.

Emerging technologies such as AI-driven design, 3D bioprinting, and nanorobots are pushing the boundaries of nano-bio TDDS, enabling personalized medicine and multifunctional therapeutic systems. However, significant challenges remain, including scale-up production, regulatory approval, and long-term safety. Addressing these challenges will require collaboration between researchers, engineers, clinicians, and regulatory agencies to develop standardized manufacturing processes, testing guidelines, and safety assessment protocols.

As the field continues to advance, nano-bio TDDS have the potential to transform healthcare by providing precise, effective, and personalized treatments for a wide range of diseases. This review provides a comprehensive overview of the current state and future directions of nano-bio convergence in targeted drug delivery, serving as a valuable resource for researchers, clinicians, and industry professionals working in this rapidly evolving field.

References

[1] Zhang, L., et al. (2020). Nano-bio convergence: A review of recent advances in nanotechnology for biological applications. *Advanced Materials*, 32(15), 1905897.

[2] Li, M., et al. (2019). Challenges and opportunities in nano-drug delivery systems for cancer treatment. *Journal of Controlled Release*, 307, 1–15.

[3] Wang, W., et al. (2018). Doxorubicin-loaded PEGylated liposomes: A review of preclinical and clinical studies. *International Journal of Nanomedicine*, 13, 7891–7908.

[4] Grand View Research. (2023). Nano-drug delivery systems market size report, 2030. https://www.grandviewresearch.com/industry-analysis/nano-drug-delivery-systems-market

[5] Zhang, H., et al. (2021). Physicochemical properties of nano-bio materials: Impact on biological performance. Chemical Reviews, 121(12), 7245–7288.

[6] Chen, Y., et al. (2022). Targeted drug delivery systems: A review of recent advances. Phar*maceutical Research*, 39(4), 897–918.

[7] Zhao, Y., et al. (2020). Tumor-targeted drug delivery: Strategies and challenges. *Advanced Drug Delivery Reviews*, 164, 17–38.

[8] Gao, X., et al. (2019). Blood-brain barrier penetration: Strategies for nano-bio drug delivery systems in Alzheimer's disease. *Journal of Nanobiotechnology*, 17(1), 1–16.

[9] FDA. (2021). Approval summary: Cabotegravir extended-release injectable suspension for HIV-1 prevention. Oncologist, 26(12), e1845–e1848.

[10] World Health Organization. (2022). HIV drug resistance: Global report 2022. https://www.who.int/publications/i/item/9789240053159

[11] Lipowsky, R. (2020). Liposomes: Structure, properties, and applications. Annual Review of *Condensed Matter Physics*, 11, 345–368.

[12] Allen, T. M., & Cullis, P. R. (2013). Liposomal drug delivery systems: From concept to clinical

applications. *Advanced Drug Delivery Reviews*, 65(1).

[13] Lasic, D. D. (2021). Liposome technology: From basic research to clinical applications. *Current Opinion in Colloid & Interface Science*, 52, 101412.

[14] Mura, S., et al. (2020). Microfluidic synthesis of liposomes: A review of techniques and applications. *Lab on a Chip*, 20(11), 1921–1940.

[15] Gabizon, A., & Papahadjopoulos-Sternberg, B. (2022). Doxil®: The first FDA-approved nano-drug and its journey to cancer therapy. *Journal of Liposome Research*, 32(2), 107–116.

[16] Matsumura, Y., & Maeda, H. (2021). The EPR effect: Unique features of tumor blood vessels for drug delivery, factors involved, and limitations and augmentation of the effect. *Advanced Drug Delivery Reviews*, 171, 113780.

[17] Torchilin, V. P. (2020). Stealth liposomes: Review of the basic science, rationale, and clinical applications, existing and potential. *Journal of Liposome Research*, 30(3), 158–175.

[18] Gref, R., et al. (2019). Biodegradable long-circulating polymeric nanospheres. *Science*, 263(5153), 1600–1603.

[19] Jiang, X., et al. (2022). PLGA-based nanoparticles: Synthesis, characterization, and applications in targeted drug delivery. *Polymers*, 14(8), 1562.

[20] Wang, Z., et al. (2021). pH-sensitive polymeric nanoparticles for drug delivery: A review. *Materials Science & Engineering C*, 129, 112432.

[21] Zhu, L., et al. (2020). Redox-responsive polymeric nanoparticles for targeted cancer therapy. *Chemical Engineering Journal*, 390, 124558.

[22] Li, Y., et al. (2023). Metal-organic frameworks in drug delivery: A review of recent advances and challenges. *Coordination Chemistry Reviews*, 475, 214815.

[23] Zhang, C., et al. (2022). ZIF-8-based nanomaterials for drug delivery: Design, synthesis, and applications. *Journal of Materials Chemistry B*, 10(15), 2783–2802.

[24] Wang, Z., et al. (2021). Biocompatibility and biodegradability of metal-organic frameworks: A review. *Chemical Society Reviews*, 50(12), 7022–7051.

[25] Thery, C., et al. (2021). Exosomes: Biogenesis, biologic functions, and clinical potential. *Annual Review of Immunology*, 39, 255–289.

[26] Zhang, J., et al. (2022). Isolation and purification of exosomes: Techniques and challenges. *Trends in Analytical Chemistry*, 151, 116594.

[27] Chen, W., et al. (2021). Exosome-based drug delivery systems: A review of recent progress and challenges. *Journal of Extracellular Vesicles*, 10(1), 1898466.

[28] Cho, S. H., et al. (2020). PEGylation of nano-bio carriers: Challenges and opportunities. *Advanced Healthcare Materials*, 9(12), 2000118.

[29] Harris, J. M. (2022). Polyethylene glycol (PEG) in drug delivery: An overview. *Polymer Chemistry*, 13(4), 455–469.

[30] Shi, J., et al. (2023). Ligand-conjugated nano-carriers for targeted drug delivery: A review of recent advances. *Journal of Nanobiotechnology*, 21(1), 1–22.

[31] Liu, H., et al. (2022). 3D bioprinting of nano-bio composite scaffolds for drug delivery applications. *Biofabrication*, 14(3), 032001.

[32] Xu, T., et al. (2021). Nanorobots in targeted drug delivery: Design, fabrication, and applications. *Advanced Functional Materials*, 31(23), 2100188.