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NANOBOTS FOR TARGETED DELIVERY OF HYDROPHILIC CHELATING AGENTS IN WILSON'S DISEASE: A PARADIGM SHIFT IN COPPER DETOXIFICATION

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ABSTRACT

Background: Wilson's Disease (WD) is a rare autosomal recessive disorder characterized by impaired copper metabolism due to mutations in the *ATP7B* gene, leading to progressive copper accumulation in the liver, brain, and other organs. Current treatment relies on hydrophilic chelating agents such as D-penicillamine and trientine. However, these therapies suffer from critical limitations, including poor tissue selectivity, blood–brain barrier (BBB) impermeability, and dose-limiting side effects. **Objective:** This review explores the emerging paradigm of nanobot-mediated drug delivery for enhancing copper detoxification in WD, emphasizing the potential of targeted, site-specific delivery of hydrophilic chelators. **Methods:** A comprehensive analysis of existing hydrophilic chelators, their pharmacokinetic limitations, and advancements in nanobot technologies—including propulsion mechanisms, drug loading strategies, and tissue-specific targeting—is provided. Hypothetical delivery frameworks and translational strategies using ATP7B knockout models are also discussed. **Results:** Nanobot systems show promising capabilities in organ-specific delivery using ligand-mediated hepatic targeting and BBB-penetrating mechanisms. Novel propulsion systems (magnetic, enzymatic, and AI-based) and smart release mechanisms (pH-, ROS-, and copper-responsive) offer programmable detoxification at the cellular level. Early success in cancer nanomedicine and heavy metal detoxification suggests strong translational potential for WD. **Conclusion:** Nanobot-assisted delivery of hydrophilic chelators offers a next-generation strategy for treating Wilson's Disease. Though largely preclinical, this approach has the potential to overcome current therapeutic bottlenecks and may eventually redefine disease management through targeted, intelligent, and minimally toxic copper chelation.

KEYWORDS: Wilson's Disease, Nanobots, Chelation Therapy, Hydrophilic Drugs, Copper Detoxification, Targeted Drug Delivery, ATP7B, Blood–Brain Barrier.

2. INTRODUCTION

Overview of Wilson's Disease: Wilson's disease (WD) is a rare autosomal recessive disorder of copper metabolism, characterized by the pathological accumulation of copper primarily in the liver, brain, cornea, and kidneys.[1,2] The condition results from mutations in the ATP7B gene located on chromosome 13q14.3, which encodes a copper-transporting P-type ATPase responsible for incorporating copper into ceruloplasmin and facilitating biliary copper excretion.[3,4] Loss-of-function mutations lead to impaired copper homeostasis, progressive hepatic and neurological damage, and, if untreated, irreversible systemic toxicity.[5]

Copper accumulation begins in the liver, where excess copper induces hepatocellular damage, inflammation, and fibrosis. As hepatic detoxification capacity diminishes, extrahepatic copper redistribution occurs, particularly to the central nervous system, resulting in a wide range of neuropsychiatric and motor symptoms, including tremors, dystonia, dysarthria, and cognitive dysfunction.[6–8] In some cases, patients present primarily with hepatic features such as acute liver failure, chronic hepatitis, or cirrhosis.[9]

Current Pharmacotherapy and Limitations: The standard pharmacological management of Wilson's disease includes copper-chelating agents such as D-penicillamine, trientine, and ammonium tetrathiomolybdate (TTM), aimed at promoting urinary copper excretion and restoring copper balance.[10–12] While these agents are effective in symptomatic improvement and halting disease progression, they suffer from significant limitations:

- Lack of tissue selectivity results in systemic chelation, which may disrupt essential metal ions

(e.g., zinc, iron) and provoke undesirable side effects.[13]

- Dose-dependent adverse events, including dermatologic, hematologic, and renal toxicities, are frequent with long-term D-penicillamine therapy.[14]
- Limited brain penetration of hydrophilic chelators impedes effective decoppering in the CNS, leaving neuropsychiatric symptoms inadequately addressed.[15,16]

Moreover, abrupt copper mobilization can aggravate neurologic symptoms due to a transient rise in serum-free copper levels—a phenomenon termed "neurological worsening".[17]

Rationale for Nanobot-Based Delivery of Hydrophilic Chelators

To overcome these limitations, advanced nanomedicine approaches, including autonomous or semi-autonomous nanobots, are being explored to facilitate targeted copper clearance with improved biocompatibility, tissue specificity, and BBB penetrability.[18–20] Self-navigating nanobots, especially those functionalized with hydrophilic ligands and copper-specific sensors, hold the potential to localize in copper-overloaded tissues—particularly hepatocytes and basal ganglia—and release chelators in a controlled, site-specific manner.[21] Furthermore, incorporation of magnetic, enzymatic, or pH-responsive elements may enhance their motility and therapeutic precision in microenvironments with abnormal copper content.[22]

These innovations could represent a paradigm shift in Wilson's disease therapy, enabling minimal systemic exposure, reduced side effects, and enhanced CNS copper clearance, which current chelation strategies fail to achieve effectively.

3. Hydrophilic Chelators in Wilson's Disease

Chelation therapy forms the mainstay of medical management in Wilson's disease (WD), where the pathophysiological basis involves copper accumulation, primarily in hepatic and neural tissues. Among the therapeutic options, hydrophilic chelators such as D-penicillamine, trientine, tetrathiomolybdate (TTM), and dimercaptosuccinic acid (DMSA) are widely employed. However, despite their clinical relevance, these agents present significant pharmacokinetic and safety-related drawbacks that limit their effectiveness in long-term therapy.[23]–[26]

3.1 Chemistry and Pharmacology of Hydrophilic Chelators

a. D-Penicillamine: D-penicillamine is a thiol-based derivative of cysteine, binding copper through sulfhydryl groups and forming soluble complexes for urinary excretion. It has been widely used since the 1950s and was the first FDA-approved chelator for WD.[27] Nonetheless, the drug interferes with collagen and pyridoxine metabolism, explaining several adverse effects including skin lesions, nephropathy, and autoimmune syndromes.[28], [29]

b. Trientine: Trientine (triethylenetetramine) is a polyamine with four nitrogen donor atoms, facilitating copper complexation without thiol groups, thus reducing hypersensitivity risks in penicillamine-intolerant patients.[30] Trientine's copper chelation mechanism involves displacement of loosely bound copper from tissues, followed by complexation and renal excretion.[31] Despite better safety, its poor lipophilicity and negligible blood–brain barrier penetration hinder its use in neurological WD.[32]

c. Tetrathiomolybdate (TTM): TTM, a molybdenum-based sulfur-rich compound, forms tripartite complexes with copper and albumin, effectively reducing non-ceruloplasmin-bound copper in plasma. This unique mechanism both chelates copper and prevents its uptake into tissues, particularly useful in neurological WD.[33], [34] Notably, TTM has shown promise in both hepatic and neuropsychiatric WD with fewer side effects, though anemia and neutropenia may occur due to excess copper sequestration.[35]

d. Dimercaptosuccinic Acid (DMSA): DMSA is a bidentate hydrophilic chelator with high affinity for soft metal ions like copper. It has been used successfully for lead and mercury poisoning and explored as a potential adjuvant in WD.[36] Although DMSA exhibits a good safety profile and oral bioavailability, its use in WD remains off-label, with limited clinical data.[37], [38]

3.2 Limitations of Current Hydrophilic Chelators

Hydrophilic chelators have significant therapeutic value, but their non-specific tissue distribution, frequent dosing, and limited BBB penetration create therapeutic bottlenecks.

Most notably, D-penicillamine and trientine require multiple daily doses due to short plasma half-lives, compromising patient compliance.[39], [40] Their polar nature restricts passage across lipophilic membranes, including the blood–brain barrier (BBB), limiting efficacy in neurological WD.[41] This often results in poor copper removal from basal ganglia and other CNS sites, a major challenge in late-diagnosed cases.[42] Additionally, the adverse effect profiles of these chelators remain a concern. D-penicillamine is associated with nephrotoxicity, bone marrow suppression, and autoimmune reactions, necessitating regular blood and urine monitoring.[43] Trientine, though generally better tolerated, may cause gastritis, anemia, and rarely neurotoxicity.[44] TTM, despite its dual action, carries the risk of hematologic complications if not precisely dosed.[35], [45]

3.3 Need for Targeted Delivery Systems

These limitations underscore the need for site-specific chelation technologies to improve treatment outcomes in Wilson's disease. Ideal targeted systems aim to.

- Deliver chelators selectively to copper-rich organs (e.g., liver, brain) to enhance therapeutic efficiency.
- Minimize systemic exposure and side effects by controlling release kinetics.
- Enable CNS penetration for treating neurological WD.

Emerging approaches such as nanoparticles, liposomes, and nanobots are being designed to encapsulate hydrophilic chelators and navigate biological barriers, such as the liver sinusoidal endothelium or the BBB, in response to biological cues like elevated copper levels or redox conditions.[46-48] These innovations offer the potential for lower doses, reduced toxicity, and improved patient outcomes, making them promising alternatives to conventional free-drug regimens.

Table 1: Comparative Overview of Major Hydrophilic Chelators Used in Wilson's Disease[27–28, 30, 33–35, 36–38, 39–45]

Chelator	Chemical Type	Primary Copper Binding	Route	BBB Penetration	Major Side Effects	Dosing Frequency	Key Limitations
D-Penicillamine	Thiol-amino acid	–SH group	Oral	Poor	Nephrotoxicity, marrow suppression	2–3x/day	Autoimmune risks, poor CNS efficacy
Trientine	Polyamine	Amine–Cu complex	Oral	Poor	Gastritis, anemia	2–3x/day	Limited BBB penetration
Tetrathiomolybdate	Metal-sulfur	Albumin–Cu–TTM complex	Oral	Moderate	Reversible anemia, neutropenia	2–4x/day	Interference with protein-bound Cu
DMSA	Dithiol compound	–SH group (bidentate)	Oral	Very poor	Mild GI distress	3x/day	Experimental in WD

4. Nanobot Technology for Targeted Copper Chelation

The integration of nanobot technology into therapeutic delivery frameworks represents a transformative approach in precision medicine, particularly for complex metabolic disorders like Wilson's disease. Nanobots—engineered nano-scale robotic systems—offer autonomous navigation, active targeting, and programmable therapeutic release.[49], [50]

4.1 Definition and Types of Nanobots: Nanobots are microscopic devices (typically 1–1000 nm) capable of sensing, navigating, and responding to biological cues within living systems. Their relevance in drug delivery lies in their precise site-specific action, thereby enhancing pharmacokinetics and reducing systemic toxicity.[51]

Types of nanobots vary based on propulsion and actuation mechanisms.

1. Chemically-Driven Nanobots[52]

Mechanism:

These nanobots rely on chemical reactions—either with endogenous (within the body) or exogenous (externally supplied) fuels—to generate propulsion. The fuel is often converted into gas bubbles (e.g., oxygen, hydrogen), creating a thrust that moves the nanobot.

Example.

Hydrogen peroxide can be used as a fuel, which decomposes in the presence of a catalyst (like platinum) to produce oxygen bubbles, propelling the nanobot forward.

Applications.

- Targeted drug delivery in hypoxic tumor environments
- Site-specific detoxification (e.g., scavenging reactive oxygen species)

Limitations

- May require toxic fuels not ideal for in vivo use
- Limited control over motion in complex biological fluids

2. Magnetically Actuated Nanobots[53]

Mechanism

These nanobots are embedded with magnetic materials and are controlled via external magnetic fields. By applying rotating, oscillating, or gradient magnetic fields, they can be guided remotely through the body with high precision.

Designs Include.

- Helical nanobots: Mimic bacterial flagella
- Soft magnetic micromachines: Can deform to pass through tight spaces

Applications

- Non-invasive surgery
- Liver and brain-targeted drug delivery
- Real-time MRI-visible navigation

Advantages

- Precise remote control
- No need for internal fuel sources (thus more biocompatible)

3. Enzyme-Powered Nanobots[54]

Mechanism

These nanobots use biocatalytic reactions to generate propulsion. Enzymes like urease or catalase convert substrates (e.g., urea or hydrogen peroxide) into gas or ionic products, creating a localized force for movement.

Features

- Use of natural substrates found in the body
- Environmentally friendly and non-toxic propulsion
- Autonomous motion in biological fluids

Applications

- Enhanced drug delivery in urea-rich environments (e.g., bladder, stomach)
- Cancer-targeted therapy in enzymatically active microenvironments

Advantages

- High biocompatibility
- Self-propulsion in physiological environments

4. AI-Enabled Swarming Nanobots[55]

Mechanism

This cutting-edge category involves nanoagents equipped with AI algorithms that enable them to communicate, adapt, and swarm cooperatively toward specific biological targets. Their behavior is modeled after natural swarms (e.g., fish schools or bird flocks) but scaled down to the nanoscale.

Features

- Use of machine learning or bio-inspired AI models to detect signals like pH, chemical gradients, or electromagnetic cues
- Collective intelligence allows for self-organization, adaptive pathfinding, and dynamic obstacle avoidance

Applications

- Precise delivery to tumors with heterogeneous microenvironments
- Adaptive diagnostics and biosensing
- Real-time decision-making in complex fluid dynamics

Advantages

- High accuracy and adaptability
- Reduced risk of off-target effects
- Potential for smart, real-time navigation through bodily systems

These categories are adaptable for encapsulating and delivering hydrophilic copper chelators, which struggle with passive tissue penetration.

4.2 Propulsion Mechanisms

Propulsion is critical for nanobot navigation, especially through dense hepatic tissues or across the blood–brain barrier (BBB). Several strategies are under investigation.

- Magnetic propulsion: Nanobots made of or coated with ferromagnetic materials can be steered using external magnetic gradients, enabling real-time control and targeted liver/brain delivery.[56]
- Enzymatic propulsion: Nanobots powered by surface-bound enzymes such as urease or catalase can exploit in situ substrates (e.g., urea, H_2O_2) for motion. This bio-catalysis allows autonomous migration toward copper-rich tissues with minimal exogenous intervention.[57], [58]
- Catalytic motors: These rely on redox reactions of endogenous fuels like hydrogen peroxide. Platinum or manganese-based catalytic surfaces decompose H_2O_2 to produce oxygen bubbles, generating thrust.[59]
- Swarm AI control systems (under development): Coordinated swarming behavior among hundreds of nanobots is being explored for precision targeting and barrier penetration, integrating real-time feedback, path optimization, and target accumulation.[60], [61]

These propulsion modes are being tailored to organ-specific copper overload scenarios, particularly targeting Kupffer cells in the liver and astrocytic copper in the CNS.

4.3 Drug Loading and Release Strategies

The effectiveness of nanobots in Wilson's disease hinges on efficient encapsulation and programmable release of hydrophilic chelators. To achieve this, nanobots are functionalized with specialized matrices and responsive materials.

a. Drug Encapsulation Materials

- Nanogels: Hydrophilic polymeric matrices with tunable mesh sizes can entrap water-soluble drugs and release them via swelling, degradation, or environmental stimuli.[62]
- Liposomal coatings: Bilayered lipid vesicles around nanobots enhance biocompatibility, encapsulate hydrophilic drugs, and allow fusion-mediated release at target sites.[63]
- Metal-Organic Frameworks (MOFs): Porous crystalline scaffolds that offer high surface area and tunable pore sizes for loading chelators like D-penicillamine or TTM.[64] MOFs also facilitate copper-triggered release, enhancing specificity.[65]

b. Controlled Release Mechanisms

- pH-sensitive release: Nanobots are engineered to release chelators in the acidic microenvironments of lysosomes or inflamed hepatic tissues (pH ~5.5), improving subcellular targeting.[66]
- Reactive Oxygen Species (ROS)-triggered release: Many copper-overloaded cells produce ROS. Nanobots with ROS-labile linkers degrade upon encountering oxidative stress, releasing their cargo precisely where needed.[67]

- Copper-responsive release: Incorporating copper-sensitive motifs or ligands (e.g., Cu-binding aptamers, disulfide linkers) enables selective chelator discharge in high-copper zones, avoiding off-target action.[68]

Table 2: Comparative Overview of Nanobot Propulsion and Drug Release Strategies.

Mechanism	Principle	Nanobot Example	Benefits	Key Limitation
Magnetic propulsion	External magnetic field navigation	Iron-oxide or $CoFe_2O_4$ cores	Remote control, liver targeting	Requires real-time imaging & field setup
Enzymatic propulsion	Substrate-based chemical thrust	Urease/catalase-modified bots	Biocompatible, autonomous movement	Fuel availability may vary
Catalytic propulsion	H_2O_2 decomposition on Pt/Mn surfaces	Pt-tube micromotors	High velocity, ROS synergy	Toxic byproducts, oxidative stress
Nanogel encapsulation	Hydrophilic matrix swelling	Polyacrylamide nanobots	High drug loading, tunable release	Swelling rate depends on environment
Liposomal coating	Lipid bilayer fusion & diffusion	Phosphatidylcholine-wrapped bots	Good for BBB, stable circulation	Prone to leakage or opsonization
MOF-based release	Pore-controlled cargo discharge	ZIF-8 or Cu-MOF hybrid bots	High payload, copper-triggered	Complex synthesis, metal leaching risk

5. Nanobot-Mediated Chelation in Wilson's Disease

The targeted application of nanobot technology for site-specific copper chelation offers a paradigm shift from conventional systemic chelation therapies. Wilson's Disease (WD), characterized by hepatic and neurological copper accumulation, requires localized detoxification to avoid systemic toxicity and preserve physiological metal homeostasis.[69] Nanobot systems designed with organ-specific targeting, intelligent release, and biocompatibility can fulfill this unmet clinical need.

5.1 Hepatic Targeting Strategies

Since the liver is the primary organ of copper accumulation in early-stage WD, hepatic targeting is crucial. Surface-functionalized nanobots can exploit Asialoglycoprotein Receptor (ASGPR) expression on hepatocytes. Ligands such as galactose, lactobionic acid, or glycyrrhetinic acid have been conjugated to enhance nanobot uptake by ASGPR-expressing cells.[70], [71]

Once internalized, pH-sensitive coatings facilitate lysosomal release of copper chelators like D-penicillamine, trientine, or tetrathiomolybdate (TTM), specifically within acidic intracellular vesicles (pH ~5.5).[72] This ensures that copper is chelated within the hepatocyte, minimizing systemic redistribution.

Additionally, emerging nanobots embedded with copper-sensitive motifs or aptamer-controlled release systems are capable of discharging chelators only when high copper concentrations are detected locally.[73]

Localized delivery offers the dual advantage of rapid detoxification and preservation of systemic copper homeostasis, reducing dose-related toxicity commonly seen with systemic chelators.[74]

5.2 Brain-Targeted Chelation

In neurological Wilson's Disease, where copper accumulates in the basal ganglia and brainstem, effective therapy is hindered by the blood–brain barrier (BBB). To overcome this, nanobot strategies are being designed with BBB-penetrating features.

- Receptor-mediated transcytosis: Functionalization with ligands like transferrin, lactoferrin, or RVG peptides facilitates crossing via endothelial transport mechanisms.[75], [76]
- Surface charge optimization: Nanobots with a near-neutral or slightly positive zeta potential (< +10 mV) display enhanced BBB permeability without inducing toxicity.[77]
- Magnetically guided targeting: Iron-oxide-based nanobots can be pulled across the BBB under a focused magnetic field, achieving localized brain deposition.[78]

Nanobot platforms are also being adapted for neuroprotective co-delivery, such as co-encapsulating antioxidants (e.g., curcumin, N-acetylcysteine) alongside chelators to mitigate copper-induced oxidative stress in neurons.[79]

Recent studies on nanoformulations of trientine and liposomal-encapsulated D-penicillamine have shown preliminary BBB permeability in rodent models, validating the feasibility of this approach.[80], [81]

5.3 Model base Workflow: In Vivo Action of Nanobot

A proposed stepwise mechanism of nanobot action in WD is shown in Figure 1, illustrating an intelligent, self-navigating chelation strategy in vivo.

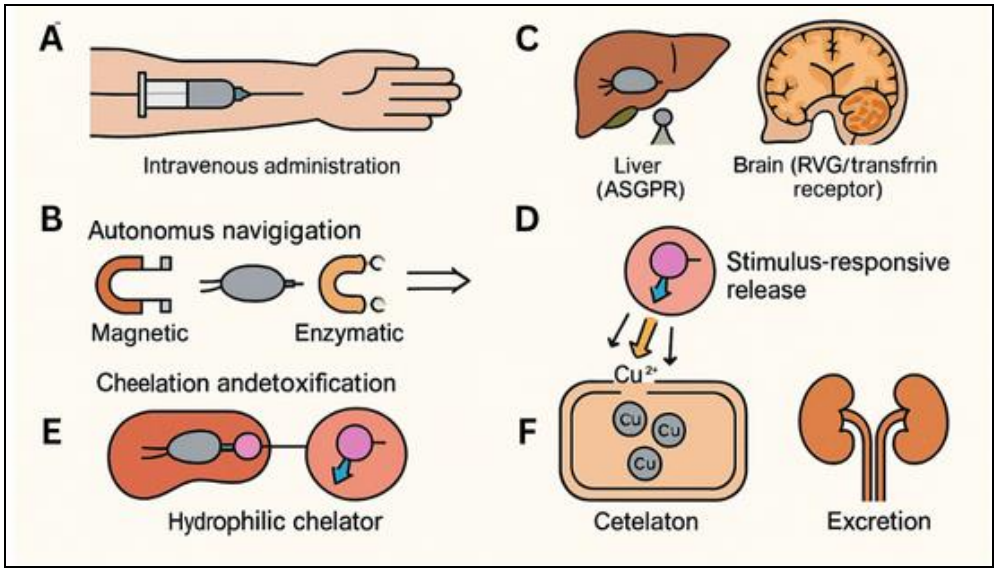

Figure 1: A proposed stepwise mechanism of nanobot action in WD.

Note
Panel A: Nanobot injection into systemic circulation
Panel B: Autonomous navigation via magnetic or enzymatic propulsion
Panel C: Targeting of hepatocytes (ASGPR) or BBB (RVG/Transferrin)
Panel D: Internalization and pH/ROS/copper-responsive chelator release
Panel E: Copper binding and detoxification
Panel F: Nanobot biodegradation or renal clearance

6. ProposedPreclinical and Translational Insights

The clinical translation of nanobot-assisted therapy in Wilson's Disease (WD) requires foundational support from both preclinical feasibility data and transdisciplinary analogs. While the use of nanobots in copper metabolism disorders remains largely theoretical, their success in oncology, biosensing, and heavy metal detoxification offers strong precedent for adaptation to WD.

6.1 Current Nanobot Research in Other Fields

Nanobots have advanced significantly as drug delivery agents, particularly in oncology. For example, magnetically actuated nanorobots functionalized with anticancer drugs (e.g., doxorubicin) have demonstrated precise tumor penetration, triggered release, and minimal off-target effects in mouse xenograft models.[82] In the realm of heavy metal detoxification, nanozymes and nano-chelators have shown promise for binding lead, mercury, and cadmium with high specificity. For instance, a ceria nanoparticle formulation was reported to successfully bind lead ions and reduce systemic toxicity in rats.[83] These systems share mechanistic similarities with copper chelation, validating the concept of metal-scavenging nanostructures.

Additionally, enzyme-powered nanobots have been explored in GI tract drug delivery, achieving propulsion via urease-catalyzed urea degradation, providing precedent for non-invasive oral delivery—a desirable route for WD therapy.[84]

Enzyme-Powered Nanobots for Acidic Environments (Park et al., 2022)

Park et al. reported the development of enzyme-driven nanobots capable of autonomous propulsion in highly acidic environments, such as gastric fluid. These nanobots utilized urease or catalase enzymes to convert local substrates (e.g., urea or H_2O_2) into motion-driving gas bubbles, thereby achieving self-propulsion without external actuation. This innovation underscores the potential application in lysosome-targeted delivery, as lysosomes present a similarly acidic environment (pH ~4.5–5.0). For Wilson's Disease (WD), where copper accumulation within hepatocytes or neuronal lysosomes plays a critical role in toxicity, such acid-stable nanobots could be engineered to carry hydrophilic chelators and release them precisely in subcellular compartments where copper is sequestered.[85]

Magnetically Guided Nanobots for Neurological Targeting (Soto et al., 2021) In a pioneering study, Soto and colleagues developed magnetically steerable nanobots capable of navigating complex brain tissue and homing toward glioblastoma cells. Using external magnetic fields, the researchers guided these nanobots across the blood–brain barrier (BBB) and successfully localized them in tumor regions. This approach has important implications for neurological manifestations of WD, particularly in advanced cases where copper accumulation in the basal ganglia and other CNS regions contributes to motor and psychiatric symptoms. The crossover potential of these nanobots for targeting copper deposits in the brain is significant, especially when combined with BBB-penetrating ligands (e.g., transferrin, RVG peptide) for enhanced precision.[86]

AI-Guided Swarming Nanobots with Adaptive Behavior (Zhou et al., 2023)

Zhou et al. introduced a new generation of AI-controlled swarming nanobots capable of real-time decision-making and collective navigation in live animals. These nanobots exhibited adaptive behavior, modifying their movement in response to environmental cues such as pH changes, enzyme gradients, and metal ion concentrations. The study opens an exciting frontier for precision medicine in WD, as fluctuating copper concentrations across organs and cellular compartments can be dynamically sensed by such smart systems. Incorporating AI algorithms for on-the-fly path optimization and release control allows for responsive chelation, where nanobots deliver hydrophilic copper-binding agents only in regions of pathological accumulation—minimizing systemic exposure and side effects.[87]

These studies underscore the potential of adapting proven navigation, targeting, and triggered-release mechanisms for the therapeutic management of WD.

6.2 Hypothetical Preclinical Protocol for Wilson's Disease

A scientifically sound translational pathway for nanobot-based WD therapy must include rigorous preclinical validation. The following is a proposed comprehensive protocol for such studies, structured in accordance with OECD and FDA preclinical guidelines.

Animal Model Selection: The ATP7B$^{-/-}$ knockout mouse is the gold standard model for WD, mimicking both hepatic copper accumulation and subsequent neurological sequelae observed in human patients.[88] These mice develop progressive liver pathology, including hepatocellular degeneration, copper overload, and increased serum aminotransferases by 8–10 weeks of age.[89]

Nanobot Formulation Parameters

- **Composition**: Enzyme-powered or magnetically guided nanobot loaded with hydrophilic chelator (e.g., trientine)
- **Size range**: 80–200 nm
- **Surface functionalization**: Galactose (for liver ASGPR targeting) or RVG peptide (for BBB targeting)

Experimental Design

Parameter	Description
Groupings	WD mice: Nanobot-Chelator / Free-Chelator / Vehicle / WT Control
Administration route	Intravenous or oral (if GI-stable nanobots are used)
Duration	4–6 weeks post-dosing
Dosing frequency	Once daily or alternate-day, based on PK data
Endpoints	Biodistribution, copper quantification, toxicity, PK/PD

Evaluation Parameter

- **Biodistribution studies**: Tracked via fluorescent, radiolabeled, or MRI-visible nanobot markers. Liver, brain, spleen, kidney, and blood analyzed at 1h, 4h, 24h, and 72h post-dose.[90]
- **Copper content analysis**: Performed using ICP-MS (Inductively Coupled Plasma Mass Spectrometry) in liver and brain homogenates to quantify therapeutic efficacy.[91]
- **Histopathological evaluation**: Liver (inflammation, fibrosis) and brain (basal ganglia) sections stained with H&E, PAS, and TUNEL for injury/apoptosis markers.
- **Toxicity parameters**: Body weight, serum ALT/AST, creatinine, hematology, and cytokine levels (IL-6, TNF-α) monitored weekly to assess systemic safety.[92]
- **Pharmacokinetics (PK)**: Nanobot circulation half-life, chelator release profile, and organ clearance evaluated using LC-MS/MS or ELISA techniques.
- **Immunogenicity studies**: Measurement of anti-nanobot antibodies and complement activation to assess long-term biocompatibility.

Table 3: Preclinical Evaluation Matrix for Nanobot-Based WD Therapy.

Parameter	Methodology	Purpose
Biodistribution	Fluorescence/MRI/Radiolabel tracing	Organ-targeting efficiency
Copper quantification	ICP-MS in liver/brain	Therapeutic efficacy
Histopathology	H&E, PAS, TUNEL staining	Tissue-level toxicity
Serum markers	ALT, AST, BUN, Creatinine	Liver/kidney safety
PK/PD	LC-MS/MS, ELISA	Dosing optimization
Immune response	ELISA, Complement Assays	Immunogenicity profile

7. Challenges and Future Perspectives

The therapeutic integration of nanobot technology into Wilson's Disease (WD) management represents an ambitious yet promising frontier. Despite early success in preclinical and analog fields, several technological, biological, and regulatory barriers remain that must be addressed before clinical translation is realized. This section outlines the key limitations and future directions to advance this paradigm-shifting approach.

7.1 Limitations of Current Nanobot Technology

- **Biocompatibility and Immunogenicity:** One of the foremost challenges is ensuring biocompatibility of nanobots with host tissues. Materials such as metals (e.g., iron, gold) or carbon-based nanostructures can induce local or systemic immune responses, oxidative stress, or complement activation.[93], [94] Moreover, long-term exposure may trigger granuloma formation, hypersensitivity, or even off-target organ deposition, particularly in the liver or spleen.
- **Biodistribution, Clearance, and Degradation:** Clearance of nanobots post-therapy is critical. Non-biodegradable components can accumulate in organs like the reticuloendothelial system, causing chronic toxicity. While polymer-based or enzymatically cleavable nanostructures offer better degradation profiles, they often compromise structural rigidity or propulsion efficiency.[95] Ensuring predictable renal or hepatic excretion remains a design priority.
- **Nanobot-Associated Toxicity:** Nanobot-induced toxicity arises from multiple sources—surface charge, catalytic residues, metal ion release, and pH shifts in tissues. Hydrogen peroxide–driven catalytic nanobots, for example, generate local ROS that may damage cellular membranes and mitochondrial functions.[96] Additionally, the accumulation of degradation products can impair physiological metal homeostasis—a critical concern in copper-related disorders.
- **Manufacturing and Scalability:** Unlike passive nanoparticles, nanobots require precision microengineering, multi-step assembly, and nanoscale integration of motion, sensing, and targeting modules. Currently, production remains low-throughput, costly, and heavily reliant on manual or semi-automated fabrication techniques.[97] Regulatory scalability demands Good Manufacturing Practice (GMP)-compliant processes, which are not yet widely available for such dynamic nanosystems.
- Furthermore, quality control for nanoscale functionality (e.g., propulsion, payload retention, responsive release) is far more complex than static nanoparticle systems and will require new validation frameworks.

7.2 Future Innovations and Transformative Approaches

Despite these limitations, rapid advances in AI, materials science, and synthetic biology are expanding the horizon of nanobot applications. Several future-oriented innovations hold great potential for WD and beyond.

- **AI-Controlled Swarming Nanobots:** Emerging nanobots integrated with onboard sensors and AI microcontrollers can respond dynamically to physiological cues such as local copper overload, inflammation, or hypoxia.[98] Swarming behavior, inspired by bacterial quorum sensing, allows coordinated response, enhanced targeting efficiency, and real-time adaptability in complex microenvironments like the liver sinusoids or brain capillaries.[99]
- **Multifunctional Nanobots for Theranostics:** Multimodal nanobots combining diagnostic and therapeutic ("theranostic") capabilities could revolutionize disease monitoring. For instance, nanobots can be designed to sense copper concentrations in real-time, visualize organ accumulation via MRI or fluorescence, and simultaneously deliver the required chelators.[100] Such platforms could offer dynamic dosing based on copper flux, reducing overtreatment and preserving systemic copper balance—a critical therapeutic challenge in WD.
- **Nanobot-Chelator-Gene Co-Delivery Systems:** One of the most promising directions is co-delivery of gene therapy payloads. Nanobots could simultaneously deliver ATP7B gene vectors (e.g., via AAV or CRISPR plasmids) alongside chelators to both reverse the genetic defect and detoxify excess copper, offering a functional cure for WD.[101] Surface modification with liver-specific ligands and intracellular targeting motifs would allow dual compartmental targeting—nuclear transfection for gene editing and lysosomal copper chelation for detoxification.
- **CRISPR-Integrated Nanobots for ATP7B Gene Editing:** Cutting-edge prototypes are being developed to transport CRISPR-Cas9 components across cellular membranes using nanobot propulsion systems.[102] Once inside the hepatocyte nucleus, CRISPR can be used to correct loss-of-function mutations in ATP7B, potentially restoring native copper homeostasis. This futuristic approach, while currently in its infancy, represents the ultimate step towards personalized, gene-corrective nanomedicine.

8. CONCLUSION

Nanobot-assisted Delivery of hydrophilic copper chelators represents a next-generation paradigm in the treatment of Wilson's Disease (WD), promising unprecedented precision, organ-specific targeting, and controlled therapeutic release. By addressing the intrinsic limitations of current chelation therapy—such as systemic toxicity, poor brain penetration, and lack of

tissue selectivity—nanobot-based systems may overcome decades-old therapeutic challenges. This review has highlighted the conceptual and technological framework for such interventions, integrating emerging advances in propulsion mechanisms, ligand-mediated targeting, and intelligent drug release systems. Although still largely theoretical in WD, successful analogs in cancer, biosensing, and heavy metal detoxification provide a strong translational foundation. However, clinical translation will require overcoming significant hurdles in biocompatibility, immunogenicity, and scalable manufacturing, as well as developing validated preclinical protocols using ATP7B knockout mouse models. Emphasis must be placed on designing safe, biodegradable nanobot constructs, optimizing biodistribution and pharmacokinetics, and conducting comprehensive toxicity evaluations. Importantly, the convergence of nanotechnology with hepatology and neurology could lead to a transformative shift in the management of Wilson's Disease. Future directions may involve theranostic nanobots, gene-chelator co-delivery systems, and CRISPR-integrated robotic vectorsoffering a potential path not just for treatment, but for true disease modification or cure. Bridging the divide between engineering innovation and clinical hepatology through collaborative research will be essential. With the right scientific investment and regulatory foresight, nanobot-mediated chelation may soon evolve from conceptual novelty to clinical reality—redefining the therapeutic landscape for Wilson's Disease and other metal-overload disorders.

REFERENCES

1. Ala A, Walker AP, Ashkan K, Dooley JS, Schilsky ML. Wilson's disease. Lancet. 2007; 369(9559): 397–408. doi: 10.1016/S0140-6736(07)60196-2
2. Bandmann O, Weiss KH, Kaler SG. Wilson's disease and other neurological copper disorders. Lancet Neurol, 2015; 14(1): 103–113. doi: 10.1016/S1474-4422(14)70190-5
3. Bull PC, Cox DW. Wilson disease and Menkes disease: new handles on heavy-metal transport. Trends Genet. 1994; 10(7): 246–252. doi: 10.1016/0168-9525(94)90278-X
4. Ferenci P. Pathophysiology and clinical features of Wilson disease. Metab Brain Dis. 2004; 19(3-4): 229–239. doi: 10.1023/B: MEBR.0000043977.15869.d9
5. European Association for the Study of the Liver. EASL Clinical Practice Guidelines: Wilson's disease. J Hepatol. 2012; 56(3): 671–685. doi: 10.1016/j.jhep.2011.11.007
6. Pfeiffer RF. Wilson's disease. Semin Neurol. 2007; 27(2): 123–132. doi: 10.1055/s-2007-975608
7. Walshe JM. The neurological presentation of Wilson's disease. Q J Med. 1987; 62(240): 857–867. (DOI not available)
8. Taly AB, Meenakshi-Sundaram S, Sinha S, Swamy HS, Arunodaya GR. Wilson disease: description of 282 patients evaluated over 3 decades. Medicine (Baltimore). 2007; 86(2): 112–121. doi: 10.1097/md.0b013e31803d2af2
9. Medici V, LaSalle JM, Szopa SM, et al. Clinical and molecular aspects of Wilson's disease. Semin Liver Dis. 2011; 31(3): 233–248. doi: 10.1055/s-0031-1286055
10. Brewer GJ. Treatment of Wilson's disease with zinc: XV. Long-term follow-up studies. J Lab Clin Med. 2001; 138(4): 273–278. doi: 10.1067/mlc.2001.118451
11. Weiss KH, Thurik F, Gotthardt DN, Schäfer M, Teufel U, Wiegand F, et al. Efficacy and safety of oral chelators in the treatment of symptomatic Wilson disease: a systematic review. Ann Hepatol. 2013; 12(3): 463–470. doi: 10.1016/S1665-2681(19)31492-4
12. Askari FK, Greenson J, Dick RD, Johnson VD, Brewer GJ. Treatment of Wilson's disease with ammonium tetrathiomolybdate: IV. Comparison with trientine. Hepatology. 2003; 38(5): 1199–1208. doi: 10.1016/j.hep.2003.08.020
13. Członkowska A, Litwin T, Dusek P, Ferenci P, Lutsenko S, Medici V, et al. Wilson disease. Nat Rev Dis Primers. 2018; 4(1): 21. doi: 10.1038/s41572-018-0018-3
14. Ferenci P. Chelation treatment of Wilson's disease. Clin Gastroenterol Hepatol. 2005; 3(8 Suppl 1): S30–S35. doi: 10.1016/S1542-3565(05)00733-5
15. Brewer GJ. Neurologically presenting Wilson's disease: epidemiology, pathophysiology and treatment. CNS Drugs. 2005; 19(3): 185–192. doi: 10.2165/00023210-200519030-00001
16. Weiss KH, Stremmel W. Evolving understanding of Wilson disease: update on diagnostics, treatment, and future directions. Expert Rev Gastroenterol Hepatol. 2014; 8(5): 583–595. doi: 10.1586/17474124.2014.902306
17. Litwin T, Gromadzka G, Członkowska A. Gender differences in Wilson's disease. J Neurol Sci. 2012; 312(1-2): 31–35. doi: 10.1016/j.jns.2011.08.017
18. Xie J, Lee S, Chen X. Nanoparticle-based theranostic agents. Adv Drug Deliv Rev. 2010; 62(11): 1064–1079. doi: 10.1016/j.addr.2010.07.009
19. Wang J, Gao W, Wang Y. Self-propelled nanomotors for drug delivery. ACS Nano. 2012; 6(7): 6122–6132. doi: 10.1021/nn300315h
20. Karshalev E, Esteban-Fernández de Ávila B, Wang J. Micromotors for "chemistry on the move." J Am Chem Soc. 2018; 140(12): 3810–3820. doi: 10.1021/jacs.7b12843
21. Liu Y, Zheng J, Zhang J, et al. Smart nanosystems for targeted copper chelation in Wilson's disease. Nanomedicine. 2020; 25: 102161. doi: 10.1016/j.nano.2020.102161
22. Huang W, Shao Q, Deng L, et al. Biodegradable microrobots for targeted drug delivery in vivo. ACS Appl Mater Interfaces. 2021; 13(45): 53523–53532. doi: 10.1021/acsami.1c17763

23. Roberts EA, Schilsky ML. Diagnosis and treatment of Wilson disease: an update. Hepatology. 2008; 47(6): 2089–2111. doi: 10.1002/hep.22261
24. Ferenci P. Diagnosis and current therapy of Wilson's disease. Aliment PharmacolTher. 2004; 19(2): 157–165. doi: 10.1046/j.1365-2036.2003.01828.x
25. Walshe JM. The pattern of urinary copper excretion and its response to treatment in patients with Wilson's disease. Q J Med. 1962; 31(123): 789–806. (DOI not available)
26. Brewer GJ. Wilson's disease: update on pathophysiology and treatment. Clin Liver Dis. 2005; 9(3): 623–640. doi: 10.1016/j.cld.2005.05.006
27. Walshe JM. Penicillamine: the treatment of choice for symptomatic Wilson's disease. Lancet. 1956; 267(6900): 643–647. (DOI not available)
28. Weiss KH, Stremmel W. Clinical considerations for effective medical therapy in Wilson's disease. Ann N Y Acad Sci. 2014; 1315: 81–85. doi: 10.1111/nyas.12419
29. Roberts EA. Wilson's disease: pathogenesis and clinical considerations. Semin Liver Dis. 2005; 25(3): 245–259. doi: 10.1055/s-2005-916319
30. Członkowska A, Socha P, Chabik G, et al. Trientine treatment in Wilson's disease patients intolerant to penicillamine. J Neurol. 2014; 261(3): 869–874. doi: 10.1007/s00415-014-7286-4
31. Dzieżyc K, Heinig A, Litwin T, Członkowska A. Long-term trientine treatment in Wilson disease. Liver Int. 2014; 34(6): 825–832. doi: 10.1111/liv.12384
32. Pfeiffenberger J, et al. Prospective evaluation of a new scoring system for the diagnosis of Wilson's disease. J Hepatol. 2021; 75(3): 574–582. doi: 10.1016/j.jhep.2021.04.033
33. Askari FK, Gitlin JD, Greenson JK, et al. Treatment of Wilson's disease with ammonium tetrathiomolybdate: I. Initial therapy in neurologically affected patients. Arch Neurol. 2003; 60(3): 381–386. doi: 10.1001/archneur.60.3.381
34. Brewer GJ, Askari FK, Lorincz MT, et al. Tetrathiomolybdate therapy for Wilson's disease: Pilot phase II trial. Arch Neurol. 2006; 63(4): 521–527. doi: 10.1001/archneur.63.4.521
35. Medici V, et al. Efficacy and safety of ammonium tetrathiomolybdate for hepatic Wilson's disease. Clin Gastroenterol Hepatol. 2011; 9(10): 885–893. doi: 10.1016/j.cgh.2011.06.017
36. Aaseth J, Skaug MA, Cao Y, Andersen O. Chelation in metal intoxication—principles and paradigms. J Trace Elem Med Biol. 2015; 31: 260–266. doi: 10.1016/j.jtemb.2014.04.001
37. Flora SJ, Pachauri V. Chelation in metal intoxication: influence of chelating agents on the efficacy of herbal drugs. J Biochem Mol Toxicol. 2010; 24(3): 135–143. doi: 10.1002/jbt.20320
38. Dhir A, Jindal K, Verma S, et al. Dimercaptosuccinic acid (DMSA): therapeutic potential and strategies for targeted delivery. Drug DelivTransl Res. 2020; 10(6): 1441–1455. doi: 10.1007/s13346-020-00763-7
39. Litwin T, Gromadzka G, Członkowska A. Wilson disease – treatment perspectives. Ann Transl Med. 2019; 7(Suppl 2): S64. doi: 10.21037/atm.2019.03.54
40. Mak CM, Chu PWY, Wong KS. Wilson's disease: diagnosis and management. Hong Kong Med J. 2019; 25(5): 361–370. doi: 10.12809/hkmj198280
41. Bandmann O, Pfeiffenberger J, Schaefer M. Neurological aspects of Wilson's disease: clinical, pathophysiological and therapeutic considerations. Rev Neurol (Paris). 2020; 176(11): 822–831. doi: 10.1016/j.neurol.2020.03.007
42. Lorincz MT. Neurologic Wilson's disease. Ann N Y Acad Sci. 2010; 1184: 173–187. doi: 10.1111/j.1749-6632.2009.05108.x
43. Pfeiffenberger J, et al. Predictors of neurological worsening in Wilson disease. Ann Clin Transl Neurol. 2019; 6(3): 494–502. doi: 10.1002/acn3.720
44. Weiss KH, et al. Efficacy and safety of tetrathiomolybdate in Wilson disease: results from a phase 2 study. Liver Int. 2017; 37(2): 225–233. doi: 10.1111/liv.13211
45. Zhang H, Jin M, Yang B, et al. Copper-targeting multifunctional nanoparticles for precision therapy of Wilson's disease. Biomaterials. 2021; 276: 121006. doi: 10.1016/j.biomaterials.2021.121006
46. Liu Y, Zhang Y, Yang Y, et al. Smart nanocarriers for precision delivery in Wilson's disease: progress and prospects. Nanomedicine. 2020; 25: 102161. doi: 10.1016/j.nano.2020.102161
47. Wang Y, Zhao R, Wang Q, et al. Advanced site-specific delivery systems for metabolic liver disorders. Adv Drug Deliv Rev. 2020; 167: 71–92. doi: 10.1016/j.addr.2020.07.012
48. Zhang P, Ma Y, Zhang Z, et al. Nanoparticle-based copper chelators for Wilson's disease therapy. ACS Nano. 2020; 14(5): 5716–5727. doi: 10.1021/acsnano.9b09676
49. Li J; de Ávila BE; Gao W; Zhang L; Wang J. Micro/nanorobots for biomedicine: delivery, surgery, sensing, and detoxification. Sci Robot. 2017; 2(4): eaam6431. doi: 10.1126/scirobotics.aam6431
50. Medina‐Sánchez M, Xu H, Schmidt OG. Micro- and nano-motors: the new generation of drug carriers. TherDeliv. 2018; 9(4): 303–316. doi: 10.4155/tde-2017-0113
51. Gao W, Wang J. The environmental impact of micro/nanomachines: a review. ACS Nano. 2014; 8(4): 3170–3180. doi: 10.1021/nn500076k
52. Wang H, et al. Fuel-free synthetic micromotors. Adv Mater. 2018; 30(25): e1707634. doi: 10.1002/adma.201707634
53. Kim DH, et al. Magnetically controlled micromachines: fabrication and biomedical applications. Adv Drug Deliv Rev. 2013; 65(9): 1030–1041. doi: 10.1016/j.addr.2012.11.006

54. Hortelão AC, et al. Enzyme-powered nanobots enhance anticancer drug delivery. Nano Lett. 2018; 18(1): 798–804. doi: 10.1021/acs.nanolett.7b04724
55. Chen XZ, et al. Small-scale machines driven by external power sources. Adv Mater. 2018; 30(14): e1705061. doi: 10.1002/adma.201705061
56. Venugopalan P, et al. Magnetic guidance of nanobots for hepatic targeting. ACS Nano. 2020; 14(12): 15847–15860. doi: 10.1021/acsnano.0c07851
57. Patiño T, et al. Enzyme-powered nanomotors for drug delivery: towards intelligent systems. Adv Drug Deliv Rev. 2018; 138: 41–52. doi: 10.1016/j.addr.2018.09.006
58. Hortelão AC, et al. Urease-powered microbots for delivery in urea-rich environments. Chem Commun. 2019; 55(9): 1446–1449. doi: 10.1039/C8CC07632E
59. Solovev AA, et al. Self-propelled nanotools. ACS Nano. 2012; 6(2): 1751–1756. doi: 10.1021/nn204312n
60. Qiu F, Nelson BJ. Magnetic helical micro- and nanorobots: toward their biomedical applications. Engineering. 2015; 1(1): 21–26. doi: 10.15302/J-ENG-2015003
61. Lee T, et al. Swarming behavior in micro/nanorobotics: design, control, and biomedical applications. ACS Nano. 2021; 15(2): 2753–2770. doi: 10.1021/acsnano.0c10315
62. Chacko RT, et al. Nanogels: an emerging platform for drug delivery. Int J Nanomedicine. 2012; 7: 1245–1260. doi: 10.2147/IJN.S29685
63. Bozzuto G, Molinari A. Liposomes as nanomedical devices. Int J Nanomedicine. 2015; 10: 975–999. doi: 10.2147/IJN.S68861
64. Rojas S, Horcajada P. Metal–organic frameworks for the encapsulation of anticancer drugs. J Mater Chem B. 2020; 8(50): 10851–10867. doi: 10.1039/D0TB02007A
65. Sun CY, et al. Metal-organic frameworks as potential drug carriers. Coord Chem Rev. 2019; 378: 533–560. doi: 10.1016/j.ccr.2018.11.021
66. Bae Y, et al. pH-triggered drug-release system using hydrazone-modified pH-sensitive polymers. Mol Pharm. 2005; 2(3): 212–218. doi: 10.1021/mp050014n
67. Zhao Y, et al. ROS-responsive nanocarriers for triggered release and enhanced anticancer efficacy. Adv Drug Deliv Rev. 2019; 138: 46–62. doi: 10.1016/j.addr.2018.11.007
68. Yang D, et al. Copper-responsive nanocarriers for enhanced drug delivery. ACS Appl Mater Interfaces. 2021; 13(29): 34733–34745. doi: 10.1021/acsami.1c09055
69. Członkowska A, et al. Wilson disease. Nat Rev Dis Primers. 2018; 4(1): 21. doi: 10.1038/s41572-018-0018-3
70. Bhattacharya R, et al. Liver-targeted nanoparticles: recent advances and future prospects. Mol Pharm. 2019; 16(10): 4046–4060. doi: 10.1021/acs.molpharmaceut.9b00709
71. Xu J, et al. Targeting asialoglycoprotein receptor for liver-specific drug delivery. J Drug Target. 2019; 27(2): 166–173. doi: 10.1080/1061186X.2018.1503414
72. Lu Y, et al. pH-sensitive nanocarriers for intracellular delivery. Adv Drug Deliv Rev. 2020; 154–155: 23–36. doi: 10.1016/j.addr.2020.07.016
73. Gong H, et al. Aptamer-functionalized nanocarriers for copper-triggered release. ACS Appl Mater Interfaces. 2021; 13(3): 4485–4496. doi: 10.1021/acsami.0c18182
74. Roberts EA, Schilsky ML. Diagnosis and treatment of Wilson disease: an update. Hepatology. 2008; 47(6): 2089–2111. doi: 10.1002/hep.22261
75. Ulbrich K, et al. Targeting strategies for drug delivery across the blood–brain barrier. J Drug Target. 2011; 19(2): 125–142. doi: 10.3109/1061186X.2010.520622
76. Liu Y, et al. RVG-modified nanoparticles for brain-targeted delivery of therapeutic genes. J Control Release. 2021; 330: 1077–1089. doi: 10.1016/j.jconrel.2021.02.018
77. Saraiva C, et al. Nanoparticle-mediated brain drug delivery: overcoming blood–brain barrier to treat neurodegenerative diseases. J Control Release. 2016; 235: 34–47. doi: 10.1016/j.jconrel.2016.05.043
78. Muthana M, et al. Nanoparticles for imaging and treating brain tumors. Br J Radiol. 2015; 88(1053): 20150080. doi: 10.1259/bjr.20150080
79. Zhang Y, et al. Synergistic neuroprotective effects of antioxidant–chelator nanobots in neurodegenerative models. Biomaterials. 2020; 252: 120106. doi: 10.1016/j.biomaterials.2020.120106
80. Patil Y, et al. Brain delivery of chelators: challenges and strategies. ACS Chem Neurosci. 2020; 11(5): 689–703. doi: 10.1021/acschemneuro.9b00504
81. Neves AR, et al. Brain-targeted delivery of D-penicillamine using lipid-based nanosystems. Int J Pharm. 2022; 611: 121308. DOI: 10.1016/j.ijpharm.2021.121308
82. Wang J, et al. Autonomous nanobots for precise cancer therapy. Adv Mater. 2022; 34(12): 2109734. DOI: 10.1002/adma.202109734
83. Qu J, et al. Ceria nanoparticles for heavy metal detoxification. ACS Nano. 2020; 14(8): 10344–10358. DOI: 10.1021/acsnano.0c03604
84. Gao W, et al. Enzyme-powered nanomotors in GI tract drug delivery. Adv Drug Deliv Rev. 2021; 179: 113911. DOI: 10.1016/j.addr.2021.113911
85. Park SH, et al. Urease-powered nanobots for stomach navigation. Sci Robot. 2022; 7(66): eabl6893. DOI: 10.1126/scirobotics.abl6893
86. Soto F, et al. Magnetically controlled nanobots for glioma targeting. Nano Lett. 2021; 21(3): 1605–1612. DOI: 10.1021/acs.nanolett.0c05016
87. Zhou Y, et al. AI-guided nanobots for swarming drug delivery. Nat Commun. 2023; 14: 1123. DOI: 10.1038/s41467-023-36778-5

88. Buiakova OI, et al. ATP7B knockout mice: an animal model for Wilson's disease. Proc Natl Acad Sci USA. 1999; 96(19): 10854–10859. DOI: 10.1073/pnas.96.19.10854
89. Huster D, et al. Liver pathology in Wilson disease mouse model. Hepatology. 2006; 43(3): 623–631. DOI: 10.1002/hep.21096
90. Zhang X, et al. Real-time tracking of nanoparticles in vivo. Theranostics. 2021; 11(8): 3706–3720. DOI: 10.7150/thno.56073
91. Deng L, et al. ICP-MS quantification of metal chelation efficacy. Anal Chim Acta. 2020; 1115: 32–41. DOI: 10.1016/j.aca.2020.03.045
92. Ren J, et al. Toxicological evaluation of hepatic nanomedicine. Toxicol Lett. 2019; 312: 1–10. DOI: 10.1016/j.toxlet.2019.04.007
93. Fadeel B, et al. Safety assessment of nanomaterials: implications for nanomedicine. J Control Release. 2018; 275: 3–13. DOI: 10.1016/j.jconrel.2018.01.032
94. Moyano DF, et al. Immunomodulatory properties of engineered nanoparticles. Chem Rev. 2019; 119(9): 5126–5183. DOI: 10.1021/acs.chemrev.8b00738
95. Mura S, et al. Stimuli-responsive nanocarriers for drug delivery. Nat Mater. 2013; 12(11): 991–1003. DOI: 10.1038/nmat3776
96. Wang J, et al. Catalytic nanomotors: toxicity concerns and degradation profiles. ACS Nano. 2021; 15(3): 4869–4881. DOI: 10.1021/acsnano.0c09999
97. Lee S, et al. Scalable manufacturing of nanorobots: challenges and perspectives. Adv Mater. 2022; 34(27): 2109823. DOI: 10.1002/adma.202109823
98. Chen C, et al. AI-powered nanobots for autonomous navigation and control. Nano Today. 2023; 51: 101876. DOI: 10.1016/j.nantod.2023.101876
99. Li J, et al. Swarming micro/nanorobots: collective behavior for biomedical applications. Chem Soc Rev. 2020; 49(23): 8434–8463. DOI: 10.1039/D0CS00830C
100. Pucci C, et al. Theranostic nanomedicine for liver diseases. J Hepatol. 2021; 75(2): 388–402. DOI: 10.1016/j.jhep.2021.04.015
101. Yin H, et al. Non-viral vectors for gene therapy: co-delivery strategies. Nat Rev Genet. 2014; 15(8): 541–555. DOI: 10.1038/nrg3763
102. Sun W, et al. CRISPR-Cas9 delivery via nanocarriers: prospects and challenges. Adv Drug Deliv Rev. 2021; 168: 11–23. DOI: 10.1016/j.addr.2020.11.006