

Hydrogel-Mediated Nanocarrier Delivery of Methotrexate Derivatives for Advanced Neurodegenerative Disorders

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Abstract

Neurodegenerative disorders such as Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, and multiple sclerosis are characterized by progressive neuronal dysfunction, chronic neuroinflammation, oxidative stress, mitochondrial impairment, synaptic dysfunction, and blood-brain barrier (BBB) disruption. Persistent activation of microglia and astrocytes contributes substantially to the progression of neuronal injury through excessive production of pro-inflammatory cytokines, reactive oxygen species, nitric oxide, and other neurotoxic mediators. Methotrexate (MTX), an established antifolate and immunomodulatory drug, possesses potential anti-inflammatory activity; however, its clinical application in neurological disorders is limited by poor central nervous system exposure, restricted BBB permeability, systemic toxicity, and rapid disposition. This review explores hydrogel-mediated nanocarrier systems as an integrated strategy for improving the delivery of MTX derivatives to diseased neural tissues. Various nanocarriers, including lipid-based, polymeric, inorganic, and hybrid systems, can enhance drug protection, cellular uptake, targeting, and controlled release. Incorporation of these nanocarriers into biocompatible hydrogels provides localized drug retention, reduced burst release, prolonged therapeutic exposure, and stimulus-responsive drug liberation. pH-, ROS-, thermo-, and enzyme-responsive hydrogels may further enable site-specific release in pathological microenvironments. Intranasal, intrathecal, epidural, and post-surgical administration routes offer complementary approaches for improving CNS exposure while potentially reducing peripheral toxicity. Preclinical evaluation using BBB-on-chip platforms, brain organoids, disease-relevant animal models, histological assessments, and behavioral studies can provide evidence of therapeutic efficacy and safety.

Keywords: Methotrexate; Nanocarriers; Hydrogels; Neuroinflammation; Blood-Brain Barrier (BBB).

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Introduction

Neurodegenerative disorders, including Alzheimer's disease (AD), Parkinson's disease (PD), amyotrophic lateral sclerosis (ALS), and multiple sclerosis (MS), constitute a rapidly expanding global health challenge because population ageing, prolonged survival, and limited disease-modifying therapies have increased their clinical, social, and economic consequences (Wichmann et al., 2022). These disorders involve progressive neuronal dysfunction and loss, but their pathogenesis also

encompasses protein misfolding, mitochondrial impairment, oxidative stress, synaptic failure, excitotoxicity, and disruption of neurovascular homeostasis (Sakka et al., 2011). Neuroinflammation represents a convergent mechanism connecting these pathological events. In particular, microglia, the resident innate immune cells of the central nervous system, continuously survey the neural microenvironment and respond to damaged neurons, misfolded proteins, myelin

debris, and pathogen-associated or damage-associated molecular patterns (Zhao et al., 2022). Persistent stimulation transforms homeostatic microglia into disease-associated, pro-inflammatory phenotypes characterized by activation of Toll-like receptor 4, nuclear factor- κ B, NLRP3 inflammasome, and mitogen-activated protein kinase signalling (Prager et al., 2014). Consequently, excessive secretion of tumour necrosis factor- α , interleukin-1 β , interleukin-6, reactive oxygen species, nitric oxide, and glutamate amplifies synaptic injury, axonal degeneration, blood–brain barrier (BBB) permeability, and neuronal apoptosis (Hablitz et al., 2020). Although microglia can also promote phagocytosis, repair, and resolution, chronic or dysregulated activation converts a protective response into a self-perpetuating neurotoxic cycle across AD, PD, ALS, and MS. Recent literature identifies microglia-driven neuroinflammation as a major contributor to disease progression rather than merely a secondary consequence of neuronal injury (Hornkjøl et al., 2022). Methotrexate (MTX), a folate-antagonist and dihydrofolate-reductase inhibitor traditionally used in oncology, has subsequently become an established immunomodulatory agent in rheumatoid arthritis and other inflammatory diseases (Stielow et al., 2023). At low, intermittent doses, MTX increases extracellular adenosine, suppresses T-cell activation, reduces leukocyte proliferation, and downregulates inflammatory cytokines, thereby providing a mechanistic rationale for investigating MTX and chemically optimized MTX derivatives in neurological inflammation (Yamada et al., 2016). However, conventional MTX administration is constrained by poor and variable CNS exposure, active efflux transport, rapid systemic disposition, and the restrictive BBB, whose endothelial tight junctions, enzymatic barrier, basement membrane, and pericyte–astrocyte interactions severely limit paracellular and transcellular drug penetration (Burman et al., 2023). Moreover, free MTX may cause myelosuppression, hepatotoxicity, nephrotoxicity, gastrointestinal injury, mucositis, and immunosuppression, while excessive CNS exposure could potentially produce neurotoxicity. Therefore, a dual-platform delivery system combining nanocarriers with hydrogels offers a rational solution (Li et al., 2022). Polymeric nanoparticles, lipid nanoparticles, nanogels, dendrimers, or exosome-mimetic vesicles can encapsulate MTX derivatives, protect them from premature degradation, improve aqueous dispersion, facilitate receptor-mediated transcytosis, and enhance endocytosis by activated microglia or infiltrating immune cells (Hay et al., 2022). Surface ligands such as transferrin, lactoferrin, apolipoprotein-derived peptides, glucose analogues, or antibodies may further improve BBB interaction and cellular selectivity. Hydrogel matrices based on

chitosan, hyaluronic acid, alginate, gelatin, polyethylene glycol, or injectable self-assembling peptides can then provide a hydrated, biocompatible depot that minimizes burst release and sustains local drug availability through diffusion, swelling, enzymatic degradation, or stimulus-responsive cleavage (Rawal et al., 1985). Intranasal, intrathecal, or intracerebral administration could increase regional exposure while reducing peripheral distribution (Bhatt, Singh, et al., 2025). The integrated system therefore couples nanocarrier-mediated targeting and intracellular uptake with hydrogel-mediated retention and controlled pharmacokinetics, potentially reducing dosing frequency and systemic toxicity (Dupoirion et al., 2023). This review aims to examine the molecular basis of MTX-mediated immunomodulation, the design and characterization of MTX-derivative nanocarriers embedded within therapeutic hydrogels, their capacity to regulate microglial activation and BBB dysfunction, and the translational challenges involving biodegradability, immunogenicity, manufacturing reproducibility, long-term safety, and clinical validation (Qiu et al., 2022). Figure 1. Illustration of various nanocarrier systems for brain-targeted drug delivery across the blood–brain barrier. Different nanocarriers facilitate the transport of therapeutic agents across the blood–brain barrier and their delivery to neuronal tissue. This targeted approach may enhance brain delivery and help reduce Alzheimer’s disease–associated neuronal degeneration.

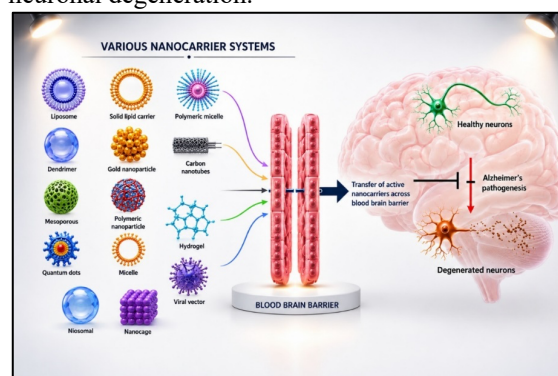


Figure 1: Various Nanocarrier Systems for Targeted Delivery Across the Blood–Brain Barrier in Alzheimer’s Disease

Pathological Landscape and the MTX Rationale

Advanced neurodegeneration is characterized by a self-amplifying neuroinflammatory microenvironment in which injured neurons, activated microglia, reactive astrocytes, infiltrating immune cells, and dysfunctional endothelial cells interact through cytokine, oxidative, and extracellular-matrix signalling networks (Bolen et al., 2025). Persistent activation of microglia and astrocytes promotes the release of tumor-necrosis factor- α (TNF- α), interleukin-1 β (IL-1 β), IL-6, chemokines, nitric oxide (NO), and reactive oxygen species (ROS), thereby impairing synaptic function,

mitochondrial respiration, axonal transport, and neuronal viability (Xie et al., 2022). ROS and reactive nitrogen species oxidize lipids, proteins, and nucleic acids, while activating redox-sensitive transcription factors such as nuclear factor- κ B (NF- κ B), which further increases inducible nitric-oxide synthase, cyclooxygenase-2, and pro-inflammatory cytokine expression (Zhang et al., 2023). This feed-forward relationship between oxidative stress and inflammation also compromises the blood–brain barrier, facilitating peripheral immune-cell recruitment and aggravating central nervous-system injury (Wang et al., 2015). During chronic disease or tissue injury, astrocytes undergo reactive astrogliosis and contribute to glial-scar formation. Although the scar initially restricts lesion expansion and isolates damaged tissue, its dense extracellular matrix, chondroitin-sulfate proteoglycans, and persistent inflammatory signalling can inhibit axonal regeneration, synaptic remodeling, and neuronal network reconstruction (Liddelow et al., 2017). Methotrexate (MTX), a classical antifolate, provides a pharmacological rationale for interrupting these processes through complementary mechanisms (Escartin et al., 2021). MTX competitively inhibits dihydrofolate reductase and, at pharmacologically relevant concentrations, targets thymidylate synthase and enzymes involved in de-novo purine biosynthesis, producing intracellular accumulation of 5-aminoimidazole-4-carboxamide ribonucleotide (AICAR) (Leng et al., 2021). AICAR inhibits adenosine deaminase and adenosine monophosphate deaminase, increasing extracellular adenosine, which activates A2A and A3 adenosine receptors on immune and glial cells (Tian et al., 2025). Adenosinergic signaling suppresses NF- κ B activity, cytokine production, leukocyte adhesion, microglial activation, and oxidative mediator release; experimental evidence indicates that adenosine-receptor antagonism can reverse MTX-mediated anti-inflammatory effects (Vilella et al., 2024). Nevertheless, conventional MTX has limited suitability for selective CNS therapy because its hydrophilic, ionizable structure restricts passive blood–brain-barrier penetration, while systemic exposure may cause hepatotoxicity, myelosuppression, mucosal injury, and dose-dependent neurotoxicity (Bhatt et al., 2025). CNS toxicity has been associated with folate depletion, altered homocysteine and S-adenosylmethionine metabolism, excitatory amino-acid imbalance, defective myelination, and disturbed adenosine homeostasis (Endresen et al., 2001). Therefore, MTX derivatives are being investigated to optimize lipophilicity, enhance membrane permeability, prolong circulation, and enable receptor-, transporter-, or nanoparticle-mediated delivery to activated microglia, astrocytes, or diseased brain regions. Structural modification or conjugation with lipids, polymers, peptides, antibodies, or targeting

ligands may improve pharmacokinetics and reduce nonspecific hepatic accumulation and neurotoxic concentrations (Sperling et al., 1992). Conceptually, a signalling diagram should depict MTX derivatives entering the CNS, accumulating within activated glia, inhibiting folate-dependent purine metabolism, increasing AICAR and adenosine, stimulating A2A/A3 receptors, and suppressing NF- κ B, TNF- α , IL-1 β , ROS, NO, astrogliosis, and glial-scarring pathways, thereby preserving neuronal integrity (Surette et al., 1999).

Table 1: Emerging Gene- and Nucleic Acid–Based Therapeutic Strategies for Alzheimer’s Disease

Therapeutic Class	Target / Mechanism	Lead Candidate	Phase	Key Findings / Status	Reference
Tau-ASO	MAPT mRNA degradation $\rightarrow$ $\downarrow$ tau synthesis	BIIB080	Phase 1b/2	$\downarrow$ CSF total and p-tau; well tolerated; Phase 2 ongoing.	(Rosenhal et al., 2013)
APP-ASO	APP mRNA suppression $\rightarrow$ $\downarrow$ A β 42	Undisclosed	Preclinical/Early clinical	Reduced A β 42 with targeted CNS delivery.	(Bao et al., 2004)
Anti-Aβ mAb	A β binding and clearance	Investigational mAb	Phase 1	Intrathecal delivery improves CNS exposure; studies ongoing.	(Vilella et al., 2024)
Neurotrophic Gene	NGF/BDNF	AAV-NGF/BDNF	Preclinical/Early	Promotes neuronal	(Zhang et al.,

Therapy	expression → neuronal support		clinical	survival and cognitive function.	2023)
Amyloid/Tau Gene Therapy	Aβ degradation or tau suppression	AAV-neprilysin/RNAi	Preclinical	Reduced pathology and improved cognition in animal models.	(Qiu et al., 2022)
Synaptic/Metabolic Gene Therapy	PGC-1α, PSD95, Homer1a modulation	AAV vectors	Preclinical	Improves mitochondrial function and synaptic resilience.	(Hay et al., 2022)

Types of Nanocarriers for MTX Derivatives

Lipid-Based Nanocarriers

Lipid-based nanocarriers provide biocompatible vesicular or matrix systems capable of improving methotrexate (MTX) solubilization, protecting chemically labile derivatives, and modifying pharmacokinetic behaviour (Iglesias et al., 2008). Liposomes are spherical phospholipid bilayer vesicles containing an aqueous core and hydrophobic membrane domain. Hydrophilic MTX can be entrapped within the aqueous compartment, whereas more lipophilic MTX derivatives may be incorporated into the lipid bilayer (Baldwin et al., 2004). Surface modification with polyethylene glycol (PEG), transferrin, antibodies, peptides, or folate ligands can prolong systemic circulation and facilitate receptor-mediated uptake by tumour cells or activated glia. However, conventional liposomes may undergo premature leakage, opsonization, and reticuloendothelial-system clearance (Dahl et al., 2014). Solid lipid nanoparticles (SLNs) consist of physiologically tolerated solid lipids stabilized by surfactants. Their rigid lipid matrix can provide controlled MTX release, improve chemical stability, and reduce nonspecific tissue exposure (Adamson et

al., 2014). SLNs may also support brain delivery through enhanced endothelial interaction and lipid-mediated transcytosis, although drug expulsion during storage and limited loading of hydrophilic MTX remain important challenges. Lipid nanocarriers have been investigated among several systems intended to overcome the therapeutic limitations of free MTX (Alberto et al., 2013).

Polymer-Based Nanocarriers

Polymeric systems offer adjustable particle size, surface charge, degradation kinetics, and ligand density. Poly (lactic-co-glycolic acid) (PLGA) nanoparticles are biodegradable carriers composed of lactic-acid and glycolic-acid units, which hydrolyse into metabolically acceptable products (Ambrosi et al., 2010). MTX may be physically encapsulated or covalently conjugated to PLGA, enabling sustained release through polymer erosion and diffusion. PLGA nanoparticles can improve biodistribution and facilitate co-delivery with other therapeutic agents; their surfaces may be coated with PEG or functionalized with brain-targeting ligands (Anselmi et al., 2008). Dendrimers, particularly polyamidoamine (PAMAM) dendrimers, possess highly branched, monodisperse architectures with internal cavities and numerous peripheral amine groups. These structural features permit MTX encapsulation, electrostatic complexation, or covalent conjugation, while terminal-group modification can reduce cytotoxicity and introduce targeting moieties (Adamson et al., 2014). Nevertheless, cationic dendrimers may disrupt cellular membranes and require careful control of surface charge. Polymeric micelles are self-assembled core-shell structures formed from amphiphilic block copolymers (Alberto et al., 2013). Their hydrophobic core can solubilize lipophilic MTX derivatives, whereas the hydrophilic corona enhances colloidal stability and circulation time. Stimuli-responsive micelles incorporating pH-, redox-, or enzyme-sensitive linkages may release MTX selectively within acidic endosomes, lysosomes, or inflamed tissue (Ambrosi et al., 2010).

Inorganic and Hybrid Nanocarriers

Mesoporous silica nanoparticles (MSNs) contain ordered nanoscale pores, high surface area, and chemically modifiable silanol groups. MTX can be adsorbed within mesopores, while pore capping, polymer grafting, or peptide functionalization enables controlled and stimuli-responsive release (Beckel et al., 2014). TAT-functionalized MSNs have demonstrated improved brain accumulation and MTX delivery compared with free drug, suggesting potential utility for blood-brain-barrier-oriented formulations. Gold nanoparticles (AuNPs) offer tunable size, surface chemistry, optical properties, and photothermal activity (Geborek et al., 1989). MTX can be attached through thiol, amine, or linker chemistry, and AuNPs can be integrated with silica shells to create hybrid

reservoirs. Near-infrared irradiation may generate localized hyperthermia and accelerate drug release, producing chemo-photothermal synergy (Iwamoto et al., 2010).

Table 2: Single-Stimulus-Responsive Hydrogels for Tumour-Targeted Drug Delivery

No.	Hydrogel/System	Stimulus	Key Response/Mechanism	Tumour Model	Ref.
1	aP/IR@FMKB	Enzyme (MMP-2)	MMP-2-triggered degradation enables drug release; photothermal activity enhances tumour suppression.	4T1 breast tumour	(Kofied et al., 1986)
2	UCNJ	ATP	Intracellular ATP induces hydrogel breakdown and DOX release.	HeLa cells	(Rosenthal et al., 2013)
3	NHS-SS-NHS chitosan nanohydrogel	GSH	GSH-sensitive disulfide bonds promote rapid DOX release under reducing conditions.	A549 cells	(Kofied et al., 1986)
4	DH-G3-CPT	pH	pH-dependent ester cleavage enables sustained CPT release.	Mouse head & neck cancer	(Baldwin et al., 2004)
5	AHB Gel	ATP	ATP recognition triggers rapid fluorescence, enabling tumour-	Tumour model	(Sprling et al., 1992)

			region imaging.		
6	PN-DATKC2/PGNR-C3	ROS	ROS responsiveness supports localized PDT/PTT and controlled drug delivery.	HeLa cells	(Leng et al., 2021)
7	Au NBPSPt NCs@DOX gel	Light	NIR irradiation regulates DOX release and photothermal therapy; Pt NCs support catalytic ROS generation.	4T1 tumour	(Bolen et al., 2025)
8	TNP/DOX/ZnPC	Thermal	Heat-responsive nanoparticles provide enhanced anticancer activity through combined drug delivery.	5637 cells; nude mice	(Hay et al., 2022)
9	HiROSponse DOX/PTX	ROS	ROS-responsive system enables co-delivery of DOX/PTX and suppresses tumour progression.	Melanoma-bearing mice	(Hablitz et al., 2020)

Hydrogels as Macroscopic Delivery Depots for the CNS

Hydrogels are three-dimensional, water-swollen polymeric networks that can function as macroscopic depots for sustained and localized delivery of methotrexate (MTX) derivatives within the central nervous system (CNS) (Iwamoto et al., 2010). Their interconnected aqueous pores permit

diffusion of therapeutic molecules, nutrients, and metabolites while their extracellular-matrix-like architecture can fill irregular lesions and reduce rapid drug dispersion (Wan et al., 2022). An ideal CNS-compatible hydrogel should exhibit high cytocompatibility, low immunogenicity, minimal neuroinflammation, and absence of toxic degradation products. Its elastic modulus should approximate the softness of native neural tissue, generally within the sub-kilopascal range, thereby minimizing mechanical mismatch, compression, and glial activation; reported brain stiffness commonly lies approximately between 0.1 and 1.0 kPa, depending on anatomical region and measurement method (Cao et al., 2022). The hydrogel should also possess suitable porosity, water retention, injectability, and tunable degradation, allowing drug release to remain synchronized with tissue repair while eventual resorption prevents chronic foreign-body reactions (Shalaby et al., 2022). Natural polymers such as hyaluronic acid, chitosan, gelatin, and alginate provide biological recognition, whereas synthetic polymers offer reproducible crosslinking and degradation control. Stimuli-responsive or “smart” hydrogels are particularly valuable because neurodegenerative and injured CNS tissues display pathological changes in acidity, redox status, temperature, and enzymatic activity (Koliqi et al., 2025). pH-responsive hydrogels incorporate ionizable groups that undergo protonation or deprotonation, causing changes in swelling, mesh size, and drug–matrix interactions. Since inflamed microdomains may become relatively acidic, these systems can accelerate MTX-derivative release at diseased sites while limiting liberation in physiological tissue (Girija et al., 2022). ROS-responsive hydrogels contain oxidation-labile bonds or ROS-scavenging components, including boronic-esters, thioketals, selenium-containing linkages, or antioxidant moieties (Hano & Abbasi, 2021). Elevated hydrogen peroxide and related oxidants can cleave these chemical switches, trigger localized drug release, and simultaneously attenuate oxidative stress, mitochondrial injury, and microglial activation (Ijaz et al., 2020). Such responsiveness may be combined with pH sensitivity to obtain sequential or synergistic release behaviour. Thermo-responsive hydrogels are liquid at room temperature, enabling syringe administration, but undergo sol–gel transition near physiological temperature after injection (Wang et al., 2017). Polymers such as poloxamers, chitosan-based formulations, and poly(N-isopropylacrylamide) derivatives can provide in-situ gelation, conformal lesion filling, and prolonged depot retention; however, residual monomers, nondegradable segments, and excessive inflammatory responses require careful evaluation (Sood et al., 2023). Shear-thinning hydrogels exhibit decreased viscosity under applied shear during

needle passage and rapidly recover their network after injection. This property supports minimally invasive delivery through narrow cannulas, reduces injection force and tissue disruption, and permits homogeneous distribution around the lesion. Hyaluronan–methylcellulose systems exemplify injectable, shear-thinning matrices (Hamed et al., 2018).

Hydrogel-Nanocarrier Composite Systems

Hydrogel–nanocarrier composite systems represent an advanced drug-delivery strategy in which methotrexate (MTX) or an MTX derivative is encapsulated within nanoscale carriers and subsequently dispersed throughout a three-dimensional, water-swollen hydrogel network (Bhubhanil et al., 2021). This hierarchical architecture combines the high drug-loading capacity, cellular internalization, and physicochemical protection of nanocarriers with the biodegradability, injectability, tissue adhesiveness, and site-retentive properties of hydrogels (Kamar et al., 2019). The composite concept is therefore synergistic rather than merely additive: the hydrogel functions as a localized depot, whereas the nanocarrier regulates intracellular trafficking, protects MTX from premature degradation, and may improve tumour accumulation through enhanced permeability, receptor-mediated uptake, or stimulus-responsive interactions (Tao et al., 2019). In pharmacokinetic terms, the system can generate sequential, two-stage release. During primary release, nanocarriers gradually diffuse through hydrogel pores or are liberated as the matrix swells, erodes, or undergoes stimulus-induced disassembly (Xue et al., 2019). This stage is governed by mesh size, cross-linking density, polymer relaxation, hydrogel degradation, nanoparticle diameter, surface charge, and interfacial affinity between the carrier and matrix (Gupta et al., 2020). During secondary release, MTX escapes from the nanocarrier through molecular diffusion, polymer erosion, desorption, or cleavage of hydrolysable, redox-sensitive, enzymatically degradable, or pH-labile linkages (Karri et al., 2016). Consequently, the overall release profile may exhibit an initial lag phase, reduced burst release, sustained diffusion, and tumour-microenvironment-triggered acceleration. Mathematical interpretation may involve Higuchi diffusion, zero-order kinetics, first-order degradation, or Korsmeyer–Peppas behaviour, although model selection should be supported by mechanistic data rather than correlation coefficients alone (Zhao et al., 2024). Acidic extracellular tumour regions, elevated glutathione concentrations, reactive oxygen species, and tumour-associated enzymes can be exploited to destabilize the nanocarrier or hydrogel selectively. Importantly, hydrogel immobilization reduces rapid systemic redistribution, renal filtration, enzymatic metabolism, and reticuloendothelial-system

sequestration of MTX-loaded nanoparticles (Tao et al., 2019). It also limits premature clearance from the administration site and maintains a higher local concentration gradient for prolonged periods. By moderating drug liberation, the composite can extend the therapeutic window, reduce peak-related systemic toxicity, and sustain pharmacologically relevant MTX exposure while minimizing repeated dosing (Majie et al., 2024). Nevertheless, translation requires rigorous evaluation of rheological behaviour, injectability, sterility, biodegradation products, nanoparticle stability, dose uniformity, burst release, immunogenicity, and in-vivo pharmacokinetics. Thus, hydrogel–nanocarrier composites provide a rational platform for spatiotemporally controlled MTX delivery, integrating local retention with nanoscale transport and offering improved therapeutic precision over free drug or either carrier alone (Girija et al., 2022).

Routes of Administration for the Composite Platform

The composite platform may integrate a methotrexate (MTX) derivative with nanocarriers and stimuli-responsive hydrogels to improve central nervous system (CNS) exposure, prolong residence time, and reduce peripheral toxicity (Bhubhanil et al., 2021). Because the blood–brain barrier (BBB) restricts the entry of most systemically administered therapeutics, route selection is a critical determinant of pharmacokinetics, biodistribution, therapeutic efficacy, and safety. Intranasal, intrathecal or epidural, and post-surgical implantation routes provide complementary strategies for achieving either widespread neuraxial exposure or highly localized drug deposition (Kamar et al., 2019).

Intranasal Delivery

Intranasal administration is a non-invasive strategy for direct nose-to-brain transport (Majie et al., 2024). Following deposition in the upper nasal cavity, the MTX derivative can interact with the olfactory epithelium and access the CNS through olfactory neuronal pathways. A second route involves branches of the trigeminal nerve, which connect the nasal mucosa with the brainstem, cerebellum, and other regions of the central nervous system (Fu et al., 2024). These pathways can partially circumvent the BBB and reduce dependence on systemic circulation. However, intranasal delivery should be described as BBB-bypassing rather than simply “crossing” the BBB, because drug transport may also involve extracellular diffusion, perivascular movement, and limited systemic absorption (Karri et al., 2016). Recent reviews identify olfactory and trigeminal transport as the principal anatomical mechanisms of nose-to-brain delivery. A mucoadhesive hydrogel can substantially improve this route. Polymers such as chitosan, carbopol, hyaluronic acid, hydroxypropyl methylcellulose, and poloxamer-based systems increase formulation viscosity and

adhesive interaction with mucin (Li et al., 2022). Consequently, the hydrogel resists mucociliary clearance, prolongs contact with the olfactory mucosa, and supports sustained release of the MTX derivative (Rawal et al., 1985). Chitosan is particularly attractive because its cationic charge promotes electrostatic interaction with negatively charged mucosal glycoproteins and may transiently modulate epithelial tight junctions. Thermoresponsive formulations can be administered as low-viscosity liquids and subsequently undergo sol–gel transition at nasal temperature, improving retention and dosing reproducibility (Bolen et al., 2025).

Intrathecal and Epidural Injections

Intrathecal administration introduces the therapeutic directly into the cerebrospinal fluid (CSF), usually through the lumbar subarachnoid space, cisterna magna, or ventricular system. This approach bypasses the BBB and enables distribution along the neuraxis, including the spinal cord, meninges, periventricular compartments, and selected cortical regions (Wang et al., 2015). Pulsatile CSF movement generated by cardiac and respiratory cycles contributes to rostral dispersion. Nevertheless, lumbar administration generally produces greater exposure near the injection site, whereas cranial or cisternal administration may provide more efficient delivery to hindbrain and forebrain regions (Escartin et al., 2021). A shear-thinning injectable hydrogel is suitable for intrathecal or epidural delivery because it can flow through a fine-gauge needle under applied stress and rapidly recover its three-dimensional network after injection (Vilella et al., 2024). This property permits minimally invasive administration while producing a conformable depot within the CSF-accessible or epidural space. Reversible supramolecular interactions, dynamic covalent bonds, or physically cross-linked polymer networks can generate shear-thinning behavior. After placement, the hydrogel may release the MTX derivative by diffusion, hydrolytic degradation, or enzyme-responsive erosion (Sperling et al., 1992).

Post-Surgical or Direct Implantation

Post-surgical implantation is appropriate for severe, localized neurodegeneration, focal traumatic brain injury, spinal-cord lesions, or resection cavities (Rosenthal et al., 2013). A biodegradable hydrogel depot can be placed directly at the pathological site, thereby generating high local MTX-derivative concentrations while minimizing systemic distribution. The material may conform to irregular tissue geometry and function as a reservoir for nanoparticles, anti-inflammatory agents, antioxidants, neurotrophic factors, or regenerative cells (Iwamoto et al., 2010). Direct implantation also permits incorporation of extracellular-matrix-mimetic components, such as hyaluronic acid, gelatin, or fibrin, to support cellular adhesion and

tissue remodeling. Nevertheless, invasive placement carries risks of infection, hemorrhage, gliosis, foreign-body reaction, and surgical trauma. Therefore, implantation should be reserved for clinically justified procedures and evaluated against sham-surgery controls (Cao et al., 2022).

Preclinical Evidence and Therapeutic Outcomes

***In Vitro* Models**

Preclinical validation should begin with complementary human-relevant models. Three-dimensional BBB microfluidic chips can reproduce endothelial perfusion, shear stress, astrocytic signaling, pericyte interactions, and selective permeability (Koliqi et al., 2025). These systems allow quantitative measurement of apparent permeability, transendothelial electrical resistance, nanoparticle uptake, endothelial cytotoxicity, and MTX-derivative transport under flow conditions (Ganguly, 2013). Modern organ-on-chip platforms are considered more physiologically representative than static monolayers because they recreate dynamic vascular and cellular interactions (Sood et al., 2023). Brain organoids derived from human induced pluripotent stem cells can model neuronal maturation, synaptic organization, neuroinflammation, and disease-associated phenotypes. Co-culture models containing brain microvascular endothelial cells, pericytes, astrocytes, microglia, and neurons are especially valuable for investigating whether the composite platform preserves BBB integrity while reducing inflammatory signaling. Brain-organoid-on-chip systems may further assess nanoparticle transcytosis, neuronal uptake, mitochondrial protection, and rescue of synaptic function (Bhubhanil et al., 2021).

***In Vivo* Models**

Efficacy should be examined in disease-relevant transgenic and toxin-induced models. In Alzheimer's disease, APP/PS1, 5xFAD, and tauopathy models can evaluate amyloid burden, tau phosphorylation, neuroinflammation, synaptic loss, and cognitive performance (Xue et al., 2019). In Parkinson's disease, α -synuclein transgenic, 6-hydroxydopamine, and MPTP models permit assessment of dopaminergic neuronal survival, striatal dopamine depletion, microgliosis, and motor coordination. Experimental autoimmune encephalomyelitis is commonly used to investigate multiple sclerosis-like neuroinflammation, demyelination, immune-cell infiltration, and neurological disability (Karri et al., 2016). The route should be matched to disease distribution: intranasal treatment is suitable for repeated non-invasive dosing, intrathecal treatment for diffuse spinal or neuraxial disease, and implantation for focal lesions. Biodistribution studies should quantify MTX-derivative concentrations in plasma, CSF, olfactory bulb, cortex, hippocampus, striatum, spinal cord, liver, and kidney (Fu et al., 2024).

Histological and Behavioral Improvements

Expected histological outcomes include reduced microglial activation, astrogliosis, cytokine expression, oxidative damage, neuronal apoptosis, amyloid deposition, tau pathology, α -synuclein aggregation, and demyelination (Majie et al., 2024). Increased synaptophysin, NeuN, tyrosine hydroxylase, myelin basic protein, and neurotrophic-factor expression would support neuroprotection or repair. In animal studies, hydrogel systems have been associated with neuronal protection, improved biocompatibility, and behavioral benefits, including reduced motor deficits and lower clinical scores in Parkinsonian and multiple-sclerosis models (Kar et al., 2019). Behavioral evaluation should include Morris water maze, novel-object recognition, and fear-conditioning tests for cognition; rotarod, pole, cylinder, and open-field assays for motor function; and neurological scoring, grip strength, and gait analysis for demyelinating or traumatic injury models. Interpretation must remain cautious because improvements may result from symptomatic anti-inflammatory effects rather than genuine disease modification (Min et al., 2022).

Conclusion

Hydrogel-mediated nanocarrier delivery represents a promising and rational approach for overcoming major limitations associated with conventional methotrexate therapy in neurodegenerative disorders. The reviewed evidence indicates that chronic neuroinflammation, particularly sustained microglial and astrocytic activation, plays an important role in neuronal dysfunction and disease progression. MTX and its derivatives may modulate these inflammatory processes through antifolate-dependent and adenosine-associated mechanisms; however, inadequate CNS penetration and systemic toxicity restrict their therapeutic potential. Combining MTX derivatives with nanocarriers can improve drug protection, physicochemical stability, cellular uptake, targeting, and controlled intracellular delivery, while incorporation into hydrogel matrices can provide localized retention and prolonged release. The resulting hierarchical system offers sequential control over drug liberation, with the hydrogel acting as a macroscopic depot and the nanocarrier regulating nanoscale transport and intracellular delivery. Stimuli-responsive hydrogels may further enhance therapeutic precision by responding to pathological changes in pH, reactive oxygen species, temperature, or enzymatic activity. Intranasal, intrathecal, and localized implantation approaches provide additional opportunities to enhance CNS exposure and minimize peripheral distribution. Nevertheless, the current concept remains predominantly translational and requires systematic validation through advanced *in vitro* models, BBB-on-chip systems, brain organoids, and disease-

relevant animal models. Particular attention should be given to biocompatibility, biodegradation, immunogenicity, dose uniformity, nanoparticle stability, release kinetics, biodistribution, and long-term neurological safety. Future optimization of MTX derivatives, targeting ligands, hydrogel composition, and administration routes could establish more precise and safer therapeutic platforms. Thus, hydrogel–nanocarrier composites have considerable potential for targeted neuroinflammatory therapy, but rigorous preclinical and clinical validation is essential before their therapeutic application can be established.

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