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Review

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Injectable nanogel systems for brain drug delivery

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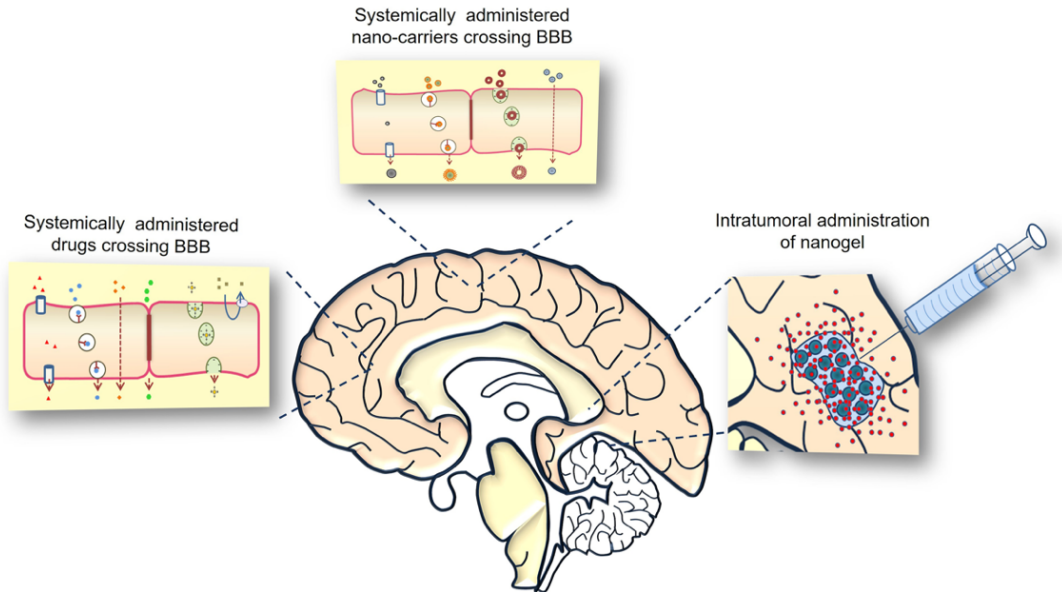
Graphical Abstract



Abstract

The central nervous system (CNS) controls many critical functions, such as intelligence, emotion, sense, and memory, and modulates many other functions of the entire human body. Diseases affecting the CNS are challenging to treat because of the CNS's sensitive functional and structural features and limited accessibility. The brain is secured from the systemic circulation by a unique biological fence, the blood-brain barrier (BBB), that limits molecular exchange between the peripheral and CNS systems. Most drugs used for treating CNS diseases fail to reach the brain in effective therapeutic concentrations due to the presence of BBB. During the last decade, specifically engineered drug delivery systems (DDS) have offered the opportunity to overcome systemic barriers in brain drug delivery. However, even with the best-engineered nano-carriers, delivering sufficient drug doses into the brain through systemic circulation remains challenging. Here, we review various systems for 'direct' drug delivery to the brain using implantable or injectable intracranial nano-drug delivery systems (nDDS), which can ensure 100% release of drug to the brain in a sustained or controlled fashion for prolonged periods, bypassing the BBB, thereby radically improving the therapeutic efficacy while minimizing systemic toxicity. Brain injectable nanogels or nanoparticle systems can be considered superior compared to other local drug-delivery systems because they possess better diffusivity of drugs. The nanogels can be injected through minimally invasive procedures, precisely delivering drugs to specific target sites. This review covers a detailed discussion about locally injectable DDS and various nanogel-based drugs, oligonucleotide, and theragnostic delivery systems used for treating brain malignancies, infections, and neurodegenerative diseases

Keywords: Brain drug delivery, Injectable systems, blood-brain barrier, CNS, Neurodegenerative Diseases

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1. PURPOSE AND RATIONALE

Diseases affecting the central nervous system (CNS) constitute almost ~ 6.3% of all diseases[1] and 12% of deaths globally.[2] These diseases can not only cause morbidity or mortality of the affected individuals but can influence the emotional, financial, and social well-being of the patient and caregivers. CNS diseases include neurodegenerative diseases such as Alzheimer's, Parkinson's, Huntington's disease, multiple sclerosis, brain malignancies, infections, epilepsy, and other genetic diseases such as lysosomal storage disease. Substantial research is happening to cure or at least control the disease. In most scenarios, a potential drug that is proven effective against the pathophysiology of disease exists but is not efficiently made available to the target site due to the body's own biological fence, the blood-brain barrier (BBB).

The BBB stands as a checkpoint restricting the free movement of substances (internal and external agents) between peripheral systemic circulation and CNS, thereby maintaining the homeostasis of the CNS microenvironment. Components of BBB include a complex system of endothelial cells, astroglia, pericytes, and perivascular mast cells.[1–6] Adjacent endothelial cells are connected by complex tight and adherent junctions (Figure 1A). These junctions are formed with a significant trans-endothelial electrical resistance (TEER) >1500Ωcm^2 (which, in contrast, for peripheral circulation is less), thereby creating a barrier limiting the transport of molecules between the two compartments.[2] Proteins like occludin and claudin form the backbone of the tight junctions, restricting the passive movement of most small molecules across the BBB. Junction adhesion molecules are the prime proteins that regulate the trafficking of immune cells, such as T-lymphocytes, neutrophils, and dendritic cells, during an immune response. Cadherins and catenins, the integral proteins that form adherence junctions, strengthen the mechanical attachment between the endothelial cells. All these factors contribute to limited paracellular transport of molecules between the two compartments (Figure 1B).[3]

Due to the BBB, systemic routes of drug delivery reach the brain at levels below the therapeutic dose; therefore, the development of local DDS would help circumvent the difficulties of overcoming the BBB. In this review, we have made an attempt to discuss locally administered nanogel/nanogel hybrid systems that carry chemotherapeutic drugs, immunotherapeutics, and contrast agents to treat various CNS disorders.

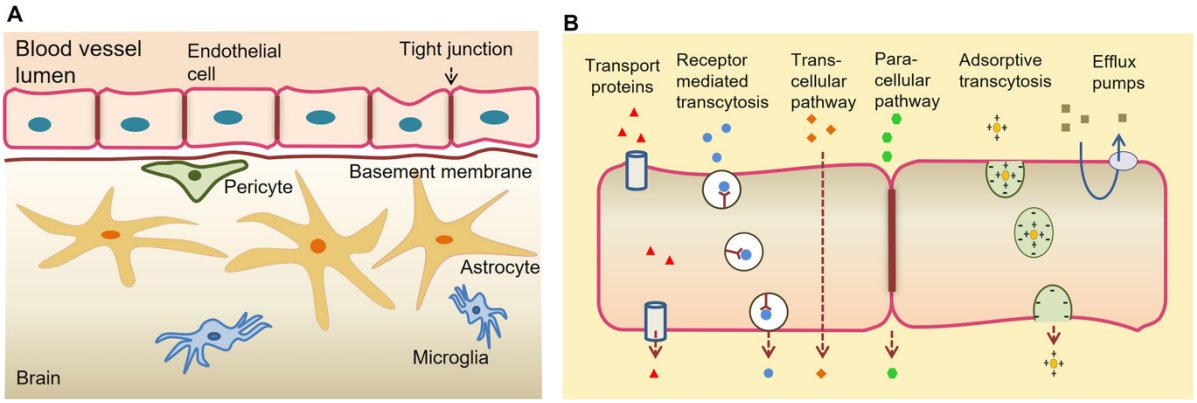

Figure 1. (A) longitudinal section of blood-brain barrier. (B) Transport pathways across the blood-brain barrier (1) substrate-specific transport proteins for transport of glucose amino acids, nucleosides, etc., (2) receptor-mediated transcytosis for transport of insulin, transferrin, LDL, etc., (3) transcellular pathway for lipophilic drugs (4) paracellular pathway for hydrophilic drugs (5) adsorptive mediated transcytosis for albumin and other plasma protein exchange.

2. SUMMARY OF RELEVANT LITERATURE

2.1 PARAMETERS FOR DESIGNING A LOCAL DRUG DELIVERY SYSTEM

Local delivery has many advantages over systemic delivery; the most important ones are the concentration reaching the target and minimizing systemic toxicity by avoiding non-specific accumulation. Many approaches have been proposed for the direct delivery of the drug into the brain, which include intracerebral/intra-parenchymal delivery (delivery of drugs into parenchymal space),[4] intraventricular delivery / transcranial delivery (delivery into cerebral ventricle)[5] and intrathecal/intra-CSF delivery (drug administered into cistern magna of the brain).[6] Most polymeric implants are intracerebral implants such as GliadelTM, which releases the anti-glioma drug carmustine for 5–7 days directly into the brain.[7] Further, long-term (>30 days) releasing polymer wafers were reported.[8] Intracerebral delivery needs higher doses to achieve appropriate concentration in parenchyma due to limited diffusion coefficient. In contrast, intraventricular delivery helps to achieve higher concentrations due to the absence of interaction with interstitial fluid. The intrathecal route is the least invasive method, but the drug fails to reach deep brain structures.[9]

The following are the important factors to consider while designing a local drug delivery system.

Controlled release of the drug: The local drug delivery system intended to be administered to provide a pharmacological action for a prolonged period should be carefully designed to release the drug in a controlled fashion, avoiding local exposure to higher doses and the potential damage created by overdosing. Having a thorough understanding of the release pattern is a critical factor. The anticipated burst release should be less than the maximum tolerable dose.[10]

Sustained / prolonged drug release: The duration of drug release is as important as the amount of drug released. Sustained release refers to the slow release of a drug over a time period, thereby maintaining constant drug levels at the target tissue. Local drug delivery is an invasive procedure that makes repeated dosing or implantation impossible, especially with susceptible organs such as the CNS. So, the developed system must provide a sustained drug release with a single injection.

Adequate penetration of the drug from the site of implantation: The drug should penetrate into the brain tissue to obtain the desired therapeutic effect. In the case of brain malignancies such as glioma, local infiltration of tumor cells results in recurrence within a depth of 2 cm from the initial lesion.[7] Drugs should penetrate adequately from the implantation site, and the penetration depth depends on the disease condition and diffusion from the nanogel (NG). Usually, the concentration of the drug decreases drastically as the distance increases. So far, clinically proven drug penetration is up to a distance < 8 mm from the implant site.[8] Also, the anatomical complexity of the brain with extremely tortuous extracellular space should be taken into account. Considering all these factors, the nanogel must be loaded with a suitable concentration of drug that will avoid local toxicity but at the same time adequate to penetrate deeper tissues.

Biocompatibility of nanomaterial: Biocompatibility is a prime requisite of implant irrespective of the site, disease condition, or purpose. Invasive procedures are accompanied by a local inflammatory response. The aim should be to develop a local drug delivery system that will not aggravate this response. Many studies are aimed at creating a biocompatible interface between the brain implant and neuronal tissue.[11–13] Low undesired cytotoxicity, non-immunogenic, and absence of mutagenicity are key factors of a biocompatible material. Also, the implant should not be separated from host tissue by glial scar formed due to the inflammatory reactions. A list of widely used biocompatible materials for the brain is summarized in Table 1.

Biodegradability: Most of the DDS are supposed to degrade, and the end product of degradation should be non-irritating to the surrounding tissues. Even though local drug delivery and nanocarrier-mediated drug delivery are highly explored for many disease conditions, the fate of these carriers is rarely addressed.

Table 1. List of Biocompatible Materials for Brain Drug Delivery and Neuro Regeneration

Material	Description	Ref
Hyaluronic acid	Linear polysaccharide, component of ECM. HA can form perineural nets (PNNs) and is widely studied for neuro regeneration	81
Collagen	Most abundant protein in human body, excellent candidate for drug and cell delivery	82
Alginate	Natural polysaccharide. Non-biodegradable in brain due to absence of alginase hemocompatible	83
Chitosan	Natural polysaccharide widely used as drug delivery systems. Chitosan may increase the inflammatory response	84
Methyl cellulose	Methylcellulose + hyaluronic acid systems are widely studied for cell and drug delivery	85
Fibrin / fibrin glue	Natural and degradable (enzymatically) polymer involved in blood clotting. Fibrin may increase neuro inflammation by increasing plasmin activity.	86
Polyethylene Glycol (PEG)	Extensively studied synthetic polymer, least inflammatory reaction. PEG alone has poor physical properties	85
Poly (lactic-co-glycolic acid) (PLGA)	Excellent biocompatibility, Degradation products (lactic and glycolic acid) are naturally occurring metabolites. Can be functionaly modified.	87
Polylactic acid (PLA)	PLA-PLGA PEGylated systems are widely studied for drug delivery. Mild inflammatory reaction can occur	88
Poly Urethane (PU)	PU integrated composites can form excellent biocompatible interface between electrodes and neural tissue and also can be used in neuro regeneration	89
Methyl methacrylate (MMA)	Widely used for the fabrication of PEG based drug delivery systems	90

Possible routes of clearance are (i) metabolic enzyme-mediated (aminopeptidase, hyaluronidase etc.) extracellular degradation[14] (ii) intracellular degradation after being taken up by neurons and glial cells[15,16] (iii) via CSF circulation (glymphatic pathway) and then drained into bloodstream or cervical lymph nodes[16] (iv) brain to blood efflux mediated by P-glycoproteins, multidrug-resistance proteins and breast cancer resistance proteins (BCRP)[17]. Most of the polymeric systems are degraded by hydrolytic, oxidative, or enzymatic reactions; the degradation rate depends on the type of polymer, the ratio of monomers, and even local changes in pH, whereas inorganic/metallic particle-based systems are cleared via CSF or brain-to-blood efflux.[18]

Extensive research is happening into direct drug delivery to the brain, and many such systems are reported. Many drug release depots are in the market for CNS disorders but are not intended for direct delivery to the brain. SABER® and Risperidol Consta® are examples of implantable drug-releasing depots that release risperidone for schizophrenia.[19,20] Gliadel™ is a successful, FDA-approved, and clinically used local delivery system against glioblastoma where 7–8 drug-loaded polymer wafers are implanted in the tumor resection cavity for controlled release of drug intending to improve patient survival. However, median survival was improved by only ~ 2. 3 months. This could be attributed to the limited penetration of the drug in the brain tissue (~ 5mm) and almost complete

drug release within 7 days, limiting the overall efficiency, with disease recurrence seen in most patients.[7,21] To overcome the limitations of Gliadel™, many studies were carried out using different polymers and drugs. A study reported using an electrospun polymeric wafer capable of releasing temozolomide for up to one month[8]. *In vivo* studies on tumor-bearing rat models had shown significant antitumor effects and prolonged survival of treated animals. Combinatorial drug therapy using wafers has also drawn wide attention, considering that tumor recurrence is mainly attributed to drug resistance.[22] Although implantable wafers help in improving median survival, they are associated with several complications. Gliadel™ wafers are associated with complications, including brain edema, CSF leakage, intracranial infection, delayed healing, and cyst formation.[23]

CLASSIFICATION OF NANOGELS BASED ON SYNTHESIS

Nanogels (NGs) are polymer networks composed of hydrophilic, hydrophobic, or amphiphilic materials that have the capability to entrap and release various drugs and biological agents in a controlled and sustained manner. They are called NGs when the cross-linked units or pores formed are in nanoscopic dimensions. They are extensively studied for the incorporation of organic molecules such as bioactive molecules (drugs, peptides, and proteins), antigens, oligonucleotides, genes, carbohydrates, DNA, and inorganic molecules such as quantum dots, magnetic nanoparticles, and metallic nanoparticles. The general properties of NGs that make them promising carriers for drug delivery are nanoscopic dimensions. They can reach areas inaccessible to the hydrogel and provide better permeability. They can be safely delivered to the cytoplasm; hence, they can be used for intracellular drug delivery.

Similar to polymer synthesis, NGs can be prepared broadly through chemical and physical means.[24,25] Preparation through a chemical approach typically includes the addition of a crosslinker to form covalent bonds during the process of polymerization. In contrast, physical cross-linking is the type of polymerization through the self-assembly of polymers involving weaker interactions among them. Free radical polymerization is one of the chemical cross-linking methods in which most stimuli-responsive NGs can be prepared.

Subtypes of free radical polymerization include nanoemulsion, microemulsion, precipitation, and atom transfer radical polymerization (ATRP), and the appropriate method is selected based on the respective application.

Polymerization by precipitation: In this method, all the materials required for polymer formation (monomers, crosslinkers) are brought to the same phase, producing a homogenous reaction. Within the homogenous phase, the polymerization occurs and continues till the desired extent of polymerization is achieved, following which the homogenous phase is separated to form polymer particles and eventually converted to NGs using a suitable medium. This technique is most employed to prepare temperature-sensitive NGs like poly(N-isopropylacrylamide) (PNIPAM). Gong et al. [26] developed a PNIPAM hydrogel to mimic the intracerebral hemorrhage (ICH) in the brain due to difficulty in developing the ICH model *in vivo*. Following injection into the brain, PNIPAM turned into a hydrogel, exhibiting a pore structure and morphology like a hematoma.

ATRP: This method employs catalysts that transfer atoms to the dormant species to activate free radicals and initiate the process of polymerization reversibly. NGs prepared through this method are usually hydrophilic in nature with properties of good colloidal stability, controlled structure, uniform size distribution, and biodegradability. Lignin-g-p (NIPAM-co-DMAEMA) cross-linked with gold nanoparticles was prepared using the ATRP method, following which they self-assembled into NGs.[27] These NGs co-loaded with curcumin and piperine showed enhanced cytotoxicity and uptake by U-251 MG glioma cells, with an increased drug release observed at pH 4. However, these methods suffer the limitation of producing polydisperse particles, the introduction of an ultrasonic device to induce shear

stress (in the case of microemulsion), and the addition of excessive concentration of surfactant and co-surfactants, which could result in non-specific toxicity.[25]

Physical cross-linking relies on hydrogen bonding, hydrophobic interactions, and Van der

Waals forces to perform self-assembly or aggregation of the polymers to form NGs. They are relatively simple and easily reproducible; the drug entrapment is mainly through physical encapsulation. Micellar and hybrid NGs explore the potential of using hydrophilic and hydrophobic moieties to incorporate different types of drugs. The hydrogen bonding binds them together, resulting in a large surface area for the drugs to be entrapped.[28] However, non-uniform size distribution and uncontrolled drug release are critical drawbacks for NGs prepared using physical methods.

2.2 DRUG RELEASE MECHANISM FROM STIMULI-RESPONSIVE NANOGELS

i) pH-responsive nanogels: In pH-responsive NGs, alteration in pH causes swelling of polymers or vice-versa due to the presence of acidic or basic groups in their structure (Figure 2). By regulating microenvironment pH, we can control drug release. The pH-responsive system comprises cross-linked polyelectrolytes with weakly acidic or weakly basic groups, which can be used as either proton donors, acceptors,, or both. These gels have volume phase transition pH, below which NG particles are swollen and beyond which they collapse. Most commonly used polymers are methacrylic acid (MA), poly-(acrylic) acids (PAA), poly ethylenimine (PEI), poly[2-N-N-(diethylamino)ethyl methacrylate] (PDEAEMA) derivatives and so on.[29]

ii) Temperature-responsive nanogels: Among NGs that respond to external stimuli, thermoresponsive NGs have been researched extensively since temperature changes are a common pathological state feature and can be externally applied. In these types of NGs, changes in temperature result in the expansion of polymer chains, allowing drug diffusion.[30] They swell at low temperatures and collapse at a higher temperature in aqueous solutions, exhibiting volume phase transition temperature (VPTT). A polymer NG system designed with VPTT close to physiological temperature will be an ideal candidate for drug delivery. Poly (alkylacrylamides) are widely used for the synthesis of thermoresponsive nanogels using poly(N-isopropylacrylamide) (PNIPAM), being the most common one. Another widely used polymer for various biomedical applications is poly(vinyl caprolactam), a highly biocompatible polymer with VPTT close to the physiological range (32–38°C).[31] A study by Lee et al. utilized temperature-induced volume transition for necrotic damage of cells.[32] Once these NGs entered the necrotic cells and when exposed to cold shock, the gel volume increased, leading to the bursting of cells.

iii) Photo-responsive nanogels: In these NGs, photo-controllable cross-linking in polymers controls their swelling and deswelling. When exposed to light, the cross-linking density in NGs changes their volume, leading to drug release.[33] Parameters like wavelength, intensity, and duration of exposure are used to control the release of molecules. They can be classified as NGs prepared from light-responsive polymers containing azobenzene, spiro benzopyran, and triphenyl methane, which are photoactive in nature, or NG systems composed of nanoparticles containing noble metals like silver or gold. When irradiated, NGs with thermoresponsive polymers can change their shape, size, and ionic nature. Irradiation by ultraviolet or visible short-wavelength light is a limitation because superficial layers absorb these lights and thus cannot be used for deeper tissues. Metallic nanoparticle-loaded systems absorb near-infrared (NIR) light and generate heat that is negligibly damaging to skin or tissues.

iv) Magnetic nanogels: Superparamagnetic iron oxide nanoparticle (SPION) is an example of magnetic nanoparticles that can be incorporated into NGs, making them magnetically responsive. They can be stimulated when a magnetic field is applied, and once the field is removed, the response disappears, thus preventing further magnetization. Agglomeration of these particles can be prevented by coating them with compounds like citric acid, dextran, or polymers.[34]

v) Multi-sensitive nanogels: Copolymers that can respond to different stimuli lead to the formation of multi-sensitive NGs. Dual pH and temperature-sensitive PDEAMA/PVCL NG prepared by seeded batch emulsion polymerization was reported.[35] More examples for each type of NGs, particularly for brain drug delivery, are discussed in the coming sections.

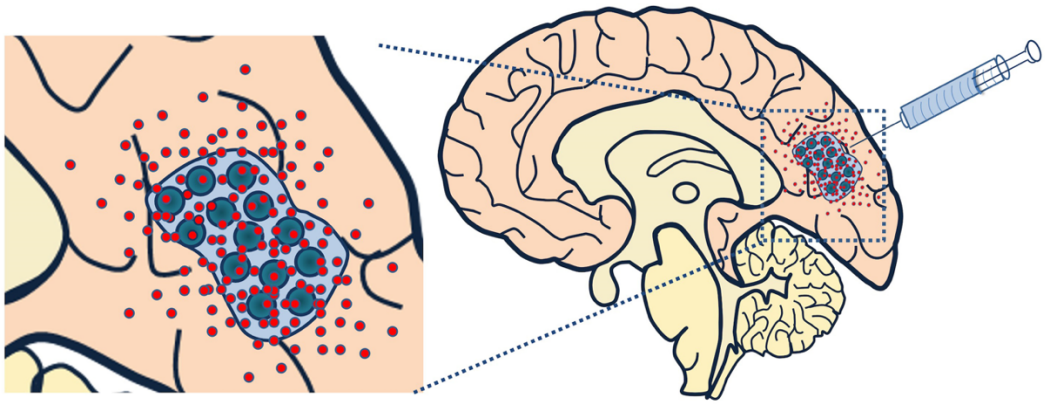

Figure 2. Drug release from nanogels after intracranial injection. Release happens in response to external factors such as light (photo-responsive nanogels), heat (thermoresponsive nanogels), magnetic field (magnetic nanogels), or internal factors such as a change in temperature (thermoresponsive) or pH (pH-responsive nanogels) in the tumor microenvironment or via diffusion from the polymer gel matrix.

2.3 NANOGELS FOR BRAIN MALIGNANCIES

Brain/nervous system malignancy is the 10th leading cause of death worldwide. Five-year survival of patients with CNS malignancy is approximately 35%. Glioblastoma Multiforme (GBM) is the most lethal type, comprising almost 15% of the total malignancies that affect the brain.[36] It has a highly diffusive and infiltrative behavior along with many drug resistance mechanisms leading to higher recurrence rates after surgical resection, chemotherapy, and radiotherapy, which is the standard of care treatment. Even with intense triple therapy, mean survival is only 12–15 months.[22] After surgical resection, some microscopic locally infiltrated cells remain, leading to higher chances of recurrence. As we discussed earlier, BBB complicates systemic chemotherapy using conventional chemotherapy drugs. Also, most of these drugs are hydrophobic, further challenging CNS bioavailability when administered systemically. NGs can be introduced into CNS by intratumoral injection after surgical resection (Figure 3). Various hydrogel systems with nanoparticles as the drug delivery unit (hybrid nanogel systems) are also widely investigated (Table 2)

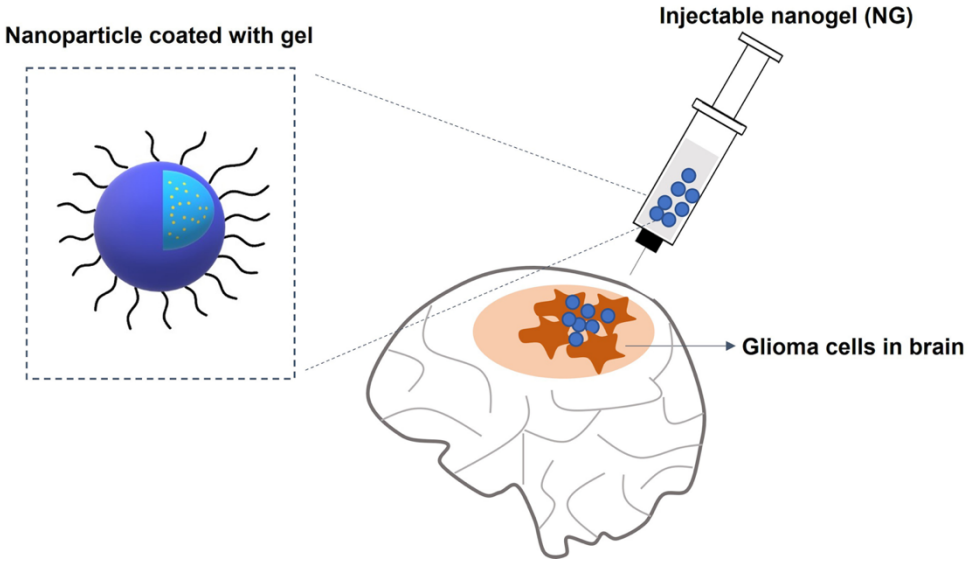

Figure 3. Schematic overview of hybrid nanogel systems for localized treatment of glioma.

Temozolomide (TMZ), a DNA alkylating agent, is used clinically as the first line of treatment for glioma (oral dosage of 150 mg/m^2).[37] TMZ induces cell cycle arrest at the G2/M phase, gradually leading to cell apoptosis. To bypass the systemic side effects of TMZ, local TMZ-loaded NGs were investigated. Poly(ethylene glycol) dimethyl acrylate PEG-DMA-based photopolymerizable gel system loaded with TMZ was designed.[38] TMZ was loaded on PEG_{750} [Poly(ε-caprolactone-*co*-trimethylene carbonate)] polymeric micelles, which aimed at promoting the solubility of TMZ, otherwise hydrophobic in nature. TMZ-micelle containing PEG-PDMA NG was injected into the glioma resection cavity and then photopolymerized using UV light. *In vivo* antitumor studies on U87 MG tumor-bearing mice exhibited good efficiency with high apoptosis and tumor inhibition.

Even though extensive surgical resection is the most efficient way to prevent recurrence from locally infiltrated tumor cells, it is not always possible, especially when highly specialized brain areas are involved. Tao J et al. conducted a study for combatting residual cells' issues wherein they used hydrogel nanocomposite loaded with paclitaxel (PTX) in the glioma resected cavity.[39] The NGs released PTX in a controlled manner and efficiently inhibited the proliferation of tumor cells. NGs containing lipid nanocapsules loaded with prodrug lauryl gemcitabine showed sustained drug release for up to one month and improved cytotoxicity compared to free drugs.[40] OncoGel™ is another controlled release system comprising thermosensitive triblock copolymer releasing chemotherapy drug paclitaxel.[41] Studies conducted in rodent glioma models have proven the safety and efficacy of this system for intracranial injections. A pre-gel solution composed of PEG-DMA polymer, a photoinitiator, and PTX-loaded PLGA nanoparticles was injected into the tumor-resected cavity and polymerized using 400 nm light.[42] *In vivo* studies confirmed delaying recurrence, thus prolonging tumor-bearing animals' survival. TMZ-loaded dextran phosphate gel (Temodex), developed by Karlsson et al., was efficacious compared to the intravenous administration of TMZ and improved median patient survival by 33 weeks.[43] TMZ efficacy is usually affected by the MGMT promoter's methylation status. However, the effectiveness of post-temodex administration was not affected by MGMT. Other than TMZ, 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU)-PLGA-based NG (hydrogel prepared from the extracellular matrix of pigskin) was developed, which showed continuous release of drug and effective inhibition of residual tumor cells for 30 days.[44] Another injectable system of BCNU-loaded PLGA-PEG gel addressed the issues of deep brain penetration along with sustained drug release.[45] This was achieved by the *in situ* formation of < 50 nm PEG-drug nano-complexes for deep brain penetration up to 2–3 cm, while the nanoparticles stayed back at the injection site for sustained drug release for > 15 days. This system exhibited ~ 95% antitumor efficacy in the orthotopic GBM rat model.

Immunotherapy for brain cancer has drawn wide attention recently. Bevacizumab (Avastin®) and dinutuximab (Unituxin®), which targets VEGF/VEGFR and GD2 pathways, are two FDA-approved antibodies for brain cancer immunotherapy. There are many other immunotherapeutics in the clinical trial, including durvalumab (anti-PDL1 antibody) and bispecific T cell engaging antibody (BiTE, against EGFRvIII tumor-specific antigen). An *in situ* sprayed bio-responsive Fibrin-Calcium carbonate gel that delivers anti-CD47 antibody to control tumor recurrence post-surgery was prepared by Chen et al.[46] These NGs scavenged H^+ ions in the resection cavity and helped in the polarization of tumor-associated macrophages to M1-like phenotype. Anti-CD47 blocked the antiphagocytic signal, increasing the phagocytosis of cancer cells by macrophages.

Avoiding immune destruction by immunosuppressive factors secreted in abundance by tumor microenvironment is a hallmark of cancer. Transforming growth factor-beta (TGF-β) and vascular endothelial growth factor (VEGF) are examples of such factors that suppress cytotoxic T-cell activity. NGs can be loaded with antibodies against specific receptors overexpressed by tumor cells. One example is cisplatin-loaded NGs, conjugated with monoclonal antibodies against Connexin 43 (Cx43) and brain-specific anion transporter (BSAT1), which are overexpressed by the tumor cells and also in peritumoral areas.[47] NG was synthesized with 20% cross-linked carboxylic groups of PMMA chains. *In vivo* analysis of rat glioma

models showed enhanced tumor inhibition and life span. Systemic toxicity was reduced in comparison to systemically administered free cisplatin.

Stimuli-responsive nanogel systems are also widely used, and they have leverage over other systems because of their ability to control drug release in response to external stimuli. A light-sensitive NG was synthesized from pentaerythritol poly(caprolactone)-b-poly(acrylic acid) with ferric ions as a crosslinker for delivering doxorubicin (Dox). On exposure to light, in the presence of lactic acid, de-crosslinking was initiated, followed by the release of the drug.[48] Thermoresponsive polymeric PLGA-PEG-PLGA gel loaded with micronized TMZ (Micro-TMZ@PLGA-PEG-PLGA) showed effective *in vivo* suppression of GBM recurrence, with an increased survival rate of 40%.[49] Magnetic nanogel-based hyperthermia therapy for cancer is based on the ability of magnetic nanoparticles to get heated upon the application of an external alternating magnetic field, resulting in hyperthermia. Hyperthermia brings the tissue to a temperature range of 41–46°C and induces physical changes such as protein misfolding and aggregation, eventually leading to apoptosis-mediated cell death. The efficiency of hyperthermia depends on the temperature rise at the target site and the duration of exposure. Conventional systems used for hyperthermia, such as infrared radiation, ultrasound, etc., lack specificity and can create damage to healthy tissues as well. Studies have shown that NGs help to overcome these drawbacks to some extent. An example of such a system is a polymer hydrogel nanocomposite made from poly(ethylene glycol) methyl ether methacrylate and dimethacrylate loaded with iron oxide nanoparticles. This system can be co-loaded with a chemotherapy drug, such as paclitaxel, to amplify the antitumor activity of the system.[50–52] Interestingly, machine learning techniques and algorithms are being employed along with magnetic NGs to understand the drug encapsulation within the nanoparticles (NPs) and to observe the extent of magnetic NPs accumulation within the desired brain area.[53]

Developing resistance to drugs is a very common scenario in cancer chemotherapy. Resistance can arise from abnormal functioning of DNA repair mechanisms due to overexpression of certain genes. Methyl guanine methyltransferase (MGMT) is an enzyme involved in DNA repair, overexpression of which will lead to resistance to alkylating agents such as TMZ. Combination therapy is one way to overcome resistance. A study conducted by Xu Y et al. [54] reported a dual drug-loaded PEG-PLGA nanocomposite thermosensitive gel system for glioblastoma. Chemotherapeutic agents temozolomide (TMZ) and paclitaxel (PTX) were loaded on monomethoxy (polyethylene glycol)–poly(D,L-lactide-co-glycolide) (mPEG-PLGA) thermosensitive gel delivery system. *In vitro* studies had shown the sustained release of drugs from the gel system, and elimination was also delayed, leading to prolonged growth inhibition of tumor cells. Clindamycin (CLD) was observed to reduce the ribosomal protein p70S6K (functions by suppressing the T cell proliferation) in a dose dependant manner, thus found to be useful in combinatorial therapy with TMZ, wherein CLD inhibited MGMT expression in cultured cells.[55] Also, co-administration of CLD and TMZ significantly reduced tumor growth in a mouse xenograft model.

Chemicals that sensitize cells towards radiation, thereby increasing the lethal effect of radiation therapy, are called radiosensitizers. Radiosensitizer administered during radiation therapy will help to minimize radiation dose and alleviate the side effects, including damage to healthy cells. A study used Alginate NG co-loaded with potential radiosensitizers gold nanoparticles (AuNP) and cisplatin against a U87-MG human glioblastoma cell line.[56] The outcome of the study has shown enhancement in the therapeutic ratio of radiation therapy. The addition of AuNP can also be utilized in photothermal therapy (PTT) assisted cell death. PTT uses light to induce hyperthermia-mediated cell death with the help of a photosensitizer or photoabsorbing agent. On irradiating with a 532 nm laser, AuNP and cisplatin NG-complex-treated tumor cells had a faster temperature rise due to the peculiar absorption properties of AuNPs, thereby increasing antitumor efficacy.

2.4 NANOGELS AS VEHICLES FOR OLIGONUCLEOTIDE DELIVERY IN THE BRAIN

Oligonucleotides (ODN) are widely explored as potential therapeutics and diagnostic agents for cancer, viral infections affecting the brain,

and neurodegenerative diseases (Figure 4). They can engineer RNA processing by altering target gene levels, inducing RNA degradation, blocking or mimicking miRNA, inhibiting mRNA translation, or modulating pre-mRNA slicing. Antisense ODN, siRNA, miRNA, and aptamer CpG ODN are examples of oligonucleotides used for therapy. Even though it has proven effective, the use of ODN is restricted due to its lack of stability. They are susceptible to enzymatic degradation inside the body and have rapid renal clearance and limited penetration within the brain.

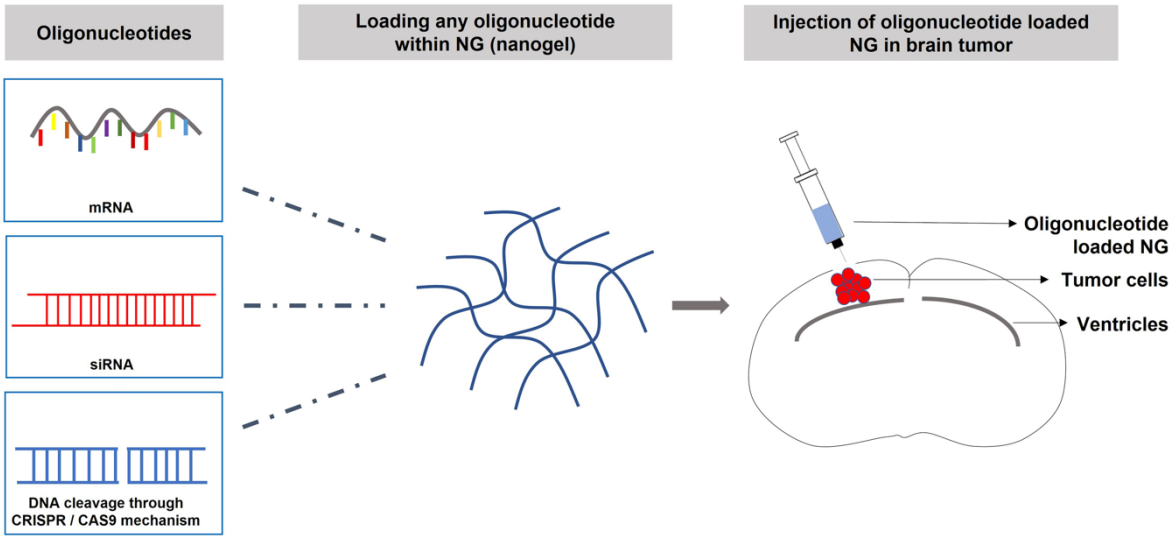

Figure 4. Schematic diagram representing oligonucleotide (mRNA, siRNA, or CRISPR) loaded NG local delivery for brain tumor therapy.

Cationic NGs consisting of PEO and PEI [Poly (ethylenimine)] can bind to and encapsulate oligonucleotides of any charge through ionic interactions, which include antisense ODN, ribozymes, siRNA, etc. A drug delivery system, NanoGel™ was synthesized by cross-linking of PEI and carbonyl diimidazole activated PEG using emulsification or solvent evaporation technique.[57] Antisense phosphorothioate oligonucleotide specific to the human mdr1 gene was incorporated into this system. This system inhibited the expression of the P-glycoprotein efflux pump. CA Manju et al. prepared NGs loaded with siRNA by self-assembly with protamine and hyaluronic acid.[58] This system exhibited simultaneous gene silencing of FAK, NOTCH-1, and SOX-2, inhibiting the neurosphere formation by glioma stem cells observed in the C6 cell line and patient samples. *In vivo,* patient-derived xenograft models showed no tumor growth by day-30 following injection of n-siRNA$_{FNS}$.

Micro RNA's ability to target many genes involved in cellular pathways is exploited immensely to treat the heterogeneous cancer cell population. Many miRNAs identified in glioma are either upregulated or downregulated and function as an oncogene or tumor suppressor gene. GBM progression is often caused due to dysfunctional miRNA pathways. J Marcos et al. proposed the development of cationic lipids containing miRNA and TMZ, with a coating of R8 peptides, in which the arginine content helps interact with the glioma surface. R8 binding to VEGFR and LanCL-1 also initiates clathrin-mediated endocytosis.[59] Once the nanoparticles are taken in, MiRNA and TMZ are released to inhibit tumor proliferation by intervention in molecular pathways and producing DNA damage, respectively.

CRISPR/cas9 system depends on endonuclease Cas9 to identify and cut the target DNA directed by single-guide RNA. Its application in treating inherited diseases is wide because of its ability to target and edit the genome. It is widely used for genomic silencing and knock-in and knock-out for targeted gene mutation. A study by Chen Z et al. used core-shell liposome–templated NGs to deliver CRISPR/cas9 system to tumors.[60] The core was formed from PEI-based

hydrogel cross-linked with cyclodextrin and adamantine encapsulating Cas9 protein. Shell consisted of cationic 1,2-dioleolyl 3-trimethyl ammonium-propane chloride salt (DOTAP) lipids. *In vitro* studies on U87 MG cells demonstrated that this NG could deliver CRISPR/cas9 more efficiently than commercial agent Lipofectamine 2000 and confirmed cytotoxic effects. NG administration in orthotopic tumor-bearing mice models resulted in the effective reduction of tumor size and improved survival. Pandey et al. developed VPX (vitamin B6-coupled polydixylitol vector) complexed with CD133 targets both CSCs (cancer stem cells) and non-CSCs, by the delivery of SMO (smoothened) CRISPR/Cas9 to the CSCs and SHMT1 (serine hydroxymethyl transferase 1) siRNA (siSHMT1) to non-CSC cells.[61] This delivery strategy reduced the xenograft tumor volume in mice by 81%.

2.5 NANOGELS IN BRAIN THERAGNOSIS

NGs are widely studied in diagnosis and imaging with more focus on developing new theragnostic systems (Table 3). NGs-based imaging systems have better specificity and respond to different stimuli based on which they either swell or de-swell to release the entrapped agents (Figure 5). They are highly tunable due to their ability to conjugate with antibodies, proteins, drugs, and fluorescent agents.[62] A study reported the use of a hydrogel system to prevent tumor recurrence from residual cells and locally infiltrated cells, co-loaded with bovine serum albumin nanoparticles containing the hydrophilic drug epirubicin and hydrophobic drug paclitaxel (PTX).[63]

These NPs were incorporated into an MRI traceable thermosensitive gel system made from carboxymethylcellulose (CMC) grafted poly(N-isopropylacrylamide-co-methacrylic acid) (CMC-g-PNIPAAmMA) and gadopentetic acid / branched polyethylenimine ($DTPA_{Gd}$/bPEI). This system exhibited excellent T2 contrast on MRI, allowing real-time hydrogel distribution and degradation monitoring.

Another theragnostic NG system was developed from poly(organophosphazene) hydrogel loaded with PEGylated cobalt ferrite and PTX. PEGylation reduced the toxicity of cobalt ferrite particles.[64] Poly(or)-PEGylateCoFerrite-paclitaxel system showed MRI contrast up to 3 weeks *in vivo* in tumor-bearing mice. As discussed earlier, along with AuNP-cisplatin-loaded alginate NGs used as a chemotherapeutic and radio-sensitizing agent, it can also be used as a theragnostic system due to the unique properties of AuNPs. Computed tomography (CT) images of NG-loaded tumors showed increased brightness, CT number value, and contrast-to-noise ratio (CNR).[65] Thus, biodistribution or accumulation of the NG could be accessed via CT imaging along with induction of chemotherapeutic effect.

Knowing the exact surgical margin is a key factor determining the success of clinical resection and preventing tumor recurrence, which is usually limited due to poor contrast between neoplastic and normal tissues. MRI is the gold standard in the preoperative diagnosis of tumors and also for identifying tumor margins. Optical imaging during the procedure is an emerging field that can provide real-time imaging of the tumor tissue and can precisely guide physicians to delineate the boundary between normal and malignant tissue. So, a combination of MRI and optical imaging can predict the tumor margin and guide physicians during the planning of surgery as well as during the procedure. Recently, Jiang L and team [66] developed a pH-temperature-sensitive magnetic NG conjugated with Cy5.5–labeled lactoferrin. This gel was capable of changing its hydrophilic/hydrophobic properties and size in response to pH and temperature. Thus, it was easily internalized by the malignant cells. *In vivo* studies on tumor-bearing rats showed MR/fluorescent images with high sensitivity and specificity.

Core-shell structured hybrid NG system was reported by Wu et al.,[67] which could be used for optical temperature sensing, targeted tumor cell imaging, and chemo-photo thermal therapy. Ag-Au bimetallic nanoparticle core was coated with a thermoresponsive nonlinear poly (ethylene glycol) based hydrogel as the shell. Drug release from this system was controlled by heat generated by external NIR irradiation and temperature rise in the tumor microenvironment.

Hasegawa and colleagues reported using NG-quantum dot hybrid nanoparticles for live cell imaging.[68] Cationic liposomes are used to deliver quantum dots to cells.

Table 2: Summary of Nanogel/nanogel hybrid systems used for Brain Malignancies

Drug delivery System	Therapeutic agent	Purpose	Description	Ref
PEG-DMA gel system	TMZ	Chemotherapy	Directly injectable gel system which could be photo polymerized post insertion.	38
Lipid nanocapsule gel system	Lauroyl gemcitabine	Chemotherapy	Injectable nanogel like system	40
PLGA nanoparticle loaded photopolymerisable gel	PTX	Chemotherapy	Intra operative injectable photopolymerizable gel	42
Nanogel hybrid system prepared from fibrin and CaCO3 nanoparticles	Anti-CD47 antibody	Immunotherapy & H+ scavenger	Insitu sprayed gel that could deliver antibody to prevent tumour recurrence and polarisation of macrophages to M1 phenotype	46
PMMA nanogel	Antibody against Cx43 & BSAT1	Immunotherapy	Cisplatin loaded nanogel conjugated with monoclonal antibodies against connexin43 & brain specific anion transporter	47
Polymer hydrogel nanocomposite loaded with iron oxide nanoparticles	Paclitaxel	Hyperthermia and chemotherapy	Magnetic nanogel based hyperthermia therapy along with chemotherapeutic effect	50,51
PEG PLGA nanocomposite thermosensitive gel	TMZ & PTX	Chemotherapy	Thermosensitive gel system capable of releasing two drugs resulting in prolonged tumour inhibition	91
Alginate nanogel	AuNP & Cisplatin	Radiosensitizer , Phototherapy & Theragnosis	Radiosenstiser enhancing the therapeutic ratio and phototherapy. Enhances the anti-tumour efficiency.	78

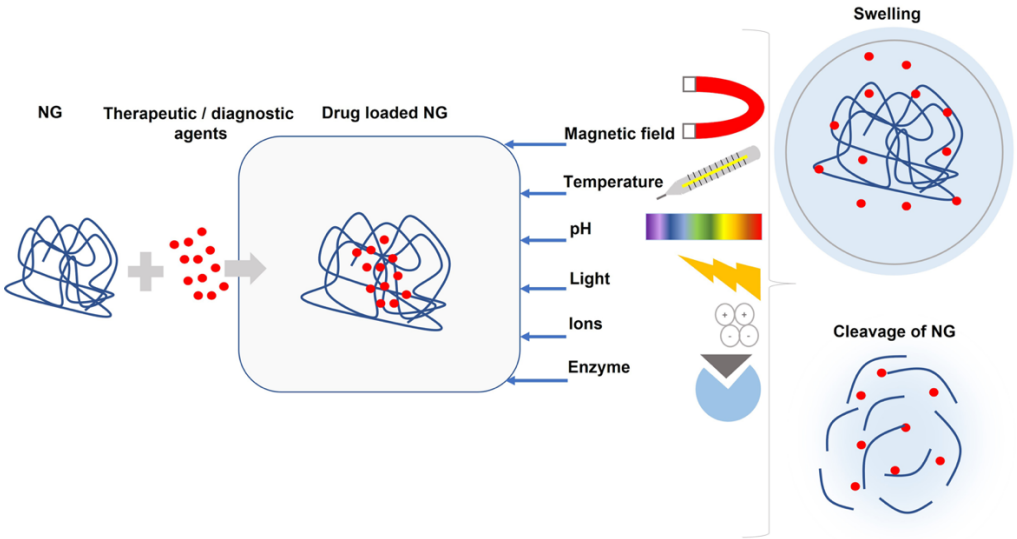

Figure 5. Schematic representation of stimuli-responsive nanogels for theragnostic applications in the brain.

Cholesterol-bearing pullulan (CHP) can form complexes with various drugs and proteins by hydrophobic interaction and released upon exposure to specific proteins or cyclodextrin. They can be used as a drug carrier system as well as artificial chaperones.

2.6 NANOGELS FOR NEURODEGENERATIVE DISEASES

Neurodegenerative diseases refer to a heterogeneous type of disorders that can cause brain or peripheral nerve malfunctioning. Neurons, the primary building block of the nervous system, cannot regenerate or repair. As age advances, susceptibility to neurodegenerative diseases increases. Parkinson's disease (PD), Alzheimer's disease (AD), Huntington's disease, and motor neuron diseases are a few examples of neurodegenerative diseases (Figure 6). Many of them are genetically inherited, while some medical conditions can also cause them. Alzheimer's and Parkinson's disease are the most prevalent (Figure 6A-B). Even though there is no cure, oligonucleotides have shown potential in controlling the disease. As discussed earlier, the use of ODN is limited by its instability in biological conditions and inability to penetrate the BBB. PD is characterized by selective degeneration of dopaminergic neurons in the substantia nigra that finally leads to decreased dopamine in the striatum. Clinically, the disease is manifested by rigidity, resting tremors, bradykinesia, and postural instability. Lewy bodies are seen, which are α synuclein and protein inclusions present in neurons. Other features are downregulation of P-gp expression, reduction in cerebral blood flow, and vascular aberrations. Current therapy for PD is levodopa (L-DOPA) along with carbidopa, a peripheral decarboxylase inhibitor. A study conducted by Rashed et al. used dopamine-loaded polyvinyl pyrrolidone-poly(acrylic acid) (PVP/PAAc) NG to deliver dopamine in PD-induced rat brain models. Administration of nano-dopamine improved the catalepsy state, along with an enhanced increase in dopamine in the rat striatum.[69] They have also shown improved mitochondrial function after multiple dosing, pointing to a disease-modifying effect. AD is an irreversible, progressive brain disorder that slowly destroys memory and thinking skills, and gradually, the person loses his ability to think and carry out simple tasks. The main features of this disease are brain atrophy, amyloid beta (Aβ) peptide aggregation (senile plaque), hyperphosphorylated tau filaments, and modification in the cerebrovascular area that results in cerebral amyloid angiopathy.

In healthy conditions, P-glycoprotein and low-density lipoprotein receptor (LRP) related protein clears Aβ peptide while the receptor for advanced glycation end products (RAGE) controls Aβ influx to the brain. A study aimed to inhibit Aβ protein formation, which is the key step in disease progression.

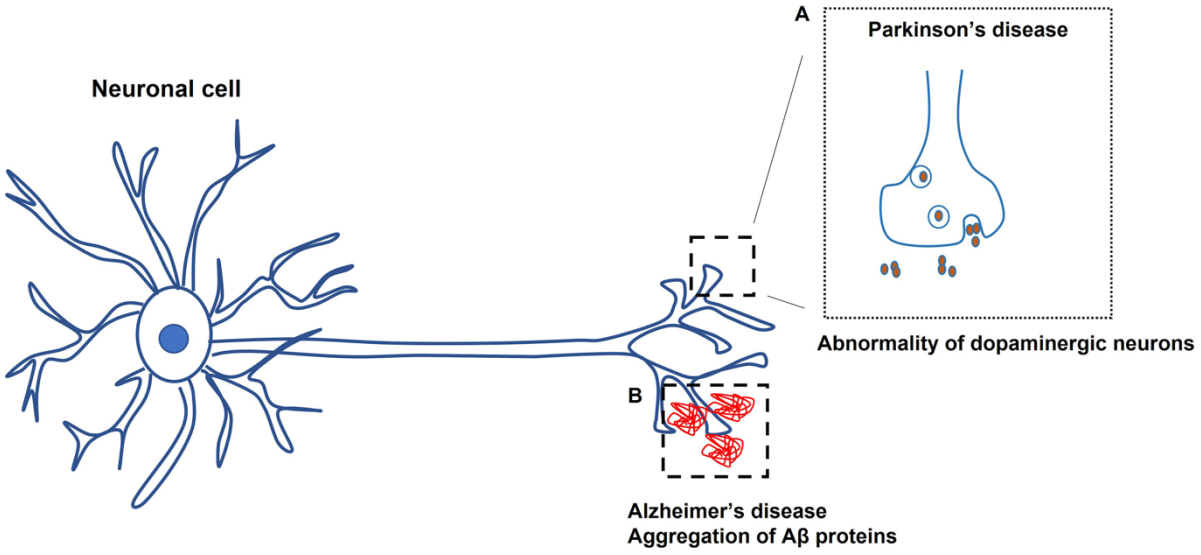

Figure 6. Schematic overview of neuronal degeneration in (A) Parkinson's disease and (B) Alzheimer's disease.

Table 3: Summary of Nanogel/nanogel hybrid systems used for other CNS diseases

Drug delivery System	Therapeutic agent	Purpose	Description	Ref
NanoGel™ Cationic nanogel with PEI & activated PEG	Antisense phosphorothioate	Gene therapy	Enhanced cytotoxicity by preventing oligonucleotide degradation andinihibit P-gp expression	57
Nanogel hybrid system- liposome templated hydrogel nanoparticle	CRISPR/cas9	Gene editing	Controlled DNA and protein release	60
CMC-g-PNIPAAmMA&DTPAGd/bPEI gel with albumin nanoparticles	Epirubicin, PTX	Theragnosis	Pinhole injections introduce the MRI traceable thermosensitive gel capable of releasing chemotherapeutic agents	63
Poly(organophoshazene) gel with PEGylated cobalt ferrite nanoparticles	PTX	Theragnosis	System shows MRI contrast and anti tumour effect	92
Fe3O4 encapsulated poly(N-isopropylacrilamide-co-acrylic acid) nanogel	Cy5.5 labelled Lactoferrin conjugated	Diagnosis	pH temperature sensitive magnetic nanogel provides excellent MR/flourescent images	66
Hybrid nanogel made from Ag-Au bimetallic nanoparticle core coated with PEG based hydrogel	TMZ69	Theragnosis	Bimetallic core emits visible flourescence and allow thermotriggered release of TMZ	67
PVP/PAAc nanogel	Dopamine	Parkinson's disease	Nano-dopamine improved treatment outcome and mitochondrial function in rat models	69
CHP nanogel	Cholesterol bearing pullulan	Alzheimers's disease	CHP act as artificial chaperone inhibiting Aβ fibril formation	70
Carboxy functionalisedpoly(N-vinyl pyrrolidone) nanogel	Insulin	Alzheimers's disease	Insulin triggers AKT pathway providing neuroprotection	71
Hyaluronic acid nanogel	EGCG & curcumin	Alzheimers's disease	Nanogel delivers inhibitors of Aβ aggregation	72
Chitosan modified PMSDT nanogel	Mn2+	Deep brain electrical stimulation	High resolution real time MEMRI.	73
PEG PEI nanogel with apolipoprotein E	Triphosphorylated NRTI	HIV infection of brain	Increased concentration of drug inside brain with effective suppression of retro viral activity	74
Nano- lipogel	CaMK4 inhibitor	Autoimmune disease	CaMK4 inhibitor suppresses IL2 & IL7 more effectively than systemically administered drugs	77

An amphiphilic NG was formed from cholesterol-bearing pullulan (CHP) composed of a polysaccharide backbone and hydrophobic cholesteryl moieties.[70] It formed complexes with denatured protein and thereby prevented its non-specific aggregation and formation of Aβ (1-42) fibrils. The amino group-modified CHP NG displayed a greater inhibitory effect. A carboxy functionalized poly(N-vinyl pyrrolidone) NG system was developed to deliver insulin to the brain.[71] Insulin-conjugated NG could bind to the insulin receptor, thereby triggering insulin signaling via AKT activation, providing neuroprotection against dysfunction induced by the Aβ protein. Jiang et al. used hyaluronic-acid-based NG to deliver inhibitors of Aβ aggregation, epigallocatechin-3-gallate (EGCG), and curcumin.[72] This NG could deliver drugs with different hydrophobicity, and synergistic inhibition was inferred.

Chronic brain stimulation is a physical therapy method used to treat neurodegenerative diseases. Deep-brain electrical stimulation and manganese-enhanced MRI (MEMRI) provide information about communication between brain areas. A challenge faced by the current system is poor resolution real-time images since the $MnCl_2$ solution introduced through microinjection results in image blurring and toxicity due to uncontrolled diffusion. A study conducted on NG composed of chitosan-modified poly(3,4-ethylenedioxythiopene) (PMSDT) exhibited properties like structure, mechanical properties, and conductivity similar to that of Au.[73] Electrical stimulation could form a metal-ligand bonding with Mn2+, thus releasing Mn2+ in a precisely controlled manner, leading to high-resolution real-time MEMRI.

2.7 NANOGELS FOR BRAIN INFECTION AND AUTOIMMUNE DISEASE

Nanotherapy for human immunodeficiency virus (HIV) brain infection needs highly efficient antiviral drugs and targeted drug delivery systems. Nucleoside reverse transcriptase inhibitors (NRTI) are still the drugs of choice for AIDS treatment, but decreased concentration reaching the brain due to the efflux mechanism of MRP4, MRP5, and BCRP transporters in BBB hinders the effectiveness of this therapy. Also, neurotoxicity is a significant concern with NRTI therapy. A study by Gerson et al. used triphosphorylated NRTI loaded onto PEG-PEI-based cationic NG, decorated with apo-lipoprotein-E specific peptide to treat HIV infection of the brain.[74] Nano NRTI decreased cytotoxicity and increased drug concentration inside the brain, leading to a 10-fold suppression of retroviral activity. Preparation of Poly(N-vinyl caprolactam) (PVCL) based NGs cross-linked with 4% N, N'-methylene bisacrylamide (BIS) cross-linking agent showed > 70% inhibitory effect against R5-HIV-1 *in vitro* was reported.[75]

Autoimmune diseases are generally treated using systemic immune suppressants. Systemic therapy usually brings more side effects than desired pharmacological action. Look et al. developed NGs loaded with mycophenolic acid (MPA)for lupus treatment and demonstrated decreased production of IFN-γ and IL-12 (inflammatory cytokines) post-dendritic cell internalization.[76] MPA-nanogel improved the median survival of lupus-prone mice by 3 months. Otomo et al. used nanolipogel to deliver a CaMK4 (calcium/calmodulin-dependent protein kinase IV) inhibitor to $CD4^+$ T cells to suppress pro-inflammatory markers IL2 and IL17 in encephalomyelitis, lupus, and multiple sclerosis.[77] This nanogel-based delivery was 10 times more potent than systemic therapy.

3. SAFETY CONCERNS, TRANSLATION DIFFICULTIES AND REGULATORY CHALLENGES WITH INJECTABLE NANOGELS

Most of the studies discussed in the above sections were carried out with the primary goal of delivering a drug at the target site. Directly introducing a delivery system to the brain raises major challenges, such as a risk of infection and all other complications of any invasive procedure, such as hemorrhage or thrombosis. The drug should not only reach the target site but also be maintained in therapeutic concentrations for an adequate time period[78]. Increased surface area is a specific property of nanosized material that has been exploited for better drug delivery. However, the same property can increase their reactivity in the brain, leading to neurotoxicity.[79] Nanoparticles can cause excessive reactive oxygen species (ROS) production, leading to oxidative stress, increased cytokine release, and neuro-inflammation and cell death. Rapid washout by CSF leading to a sink effect is another major drawback of local NG delivery. Also, there is minimal restriction on the

boundary of distribution. Most studies unaddressed the uptake/efflux and metabolic fate of NGs in brain cells. For implantable medical devices, existing regulations mainly point to the choice of material used with more importance for toxicity issues.[80] Hence, adequate research must critically evaluate the therapeutic vs. side effects of nanogels used for brain drug delivery.

4. CONCLUSION

This paper reviewed recent advances in injectable nanogel systems for delivering drugs locally to the brain. Although we may design very effective drugs against central nervous system (CNS) diseases, their efficient delivery to the disease site is a major challenge. Hence, together with discovering newer molecules, we should optimize new delivery systems that can overcome the challenges of BBB. Nanoparticles and nanogels are being investigated for sustained and targeted drug delivery, and some of the systems are already used in the clinics for systemic use. These injectable systems can be optimized for direct drug delivery to the brain to avoid systemic toxicity and BBB to deliver 100% of the drug to the disease site. Administration using minimally invasive procedures or intra-surgical injections can be done with minimal adverse effects, practically in any location in the brain. Most studies reviewed in this paper were successful in animal models; however, human clinical translation remains a major challenge. Future work in this area will focus on human clinical trials of brain-injectable nanogels. We strongly believe that our ability to locally deliver 100% of drugs directly to the brain in a controlled manner will provide dramatic opportunities for managing CNS diseases.

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Conflict of Interest

The author is an associate editor of the journal and declares no other conflicts of interest. For a signed statement, please contact the journal office at editor@precisionnanomedicine.com.

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