Nanotherapeutic systems for local treatment of brain tumors

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Malignant brain tumor, including the most common type glioblastoma, are histologically heterogeneous and invasive tumors known as the most devastating neoplasms with high morbidity and mortality. Despite multimodal treatment including surgery, radiotherapy, chemotherapy, and immunotherapy, the disease inevitably recurs and is fatal. This lack of curative options has motivated researchers to explore new treatment strategies and to develop new drug delivery systems (DDSs); however, the unique anatomical, physiological, and pathological features of brain tumors greatly limit the effectiveness of conventional chemotherapy. In this context, we review the recent progress in the development of nanoparticle-based DDSs aiming to address the key challenges in transporting sufficient amount of therapeutic agents into the brain tumor areas while minimizing the potential side effects. We first provide an overview of the standard treatments currently used in the clinic for the management of brain cancers, discussing the effectiveness and limitations of each therapy. We then provide an in-depth review of nanotherapeutic systems that are intended to bypass the blood–brain barrier, overcome multidrug resistance, infiltrate larger tumorous tissue areas, and/or release therapeutic agents in a controlled manner. © 2017 Wiley Periodicals, Inc.

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INTRODUCTION

Cancer originating in the brain and other parts of the central nervous system (CNS) is a devastating disease with extremely low survival rates, with 23,770 new cases and 16,050 deaths estimated in 2016.1 Other than primary brain tumors, brain metastasis of other types of cancers during their later stages occurs in around 15% of all cancer patients.2 In particular cases such as lung adenocarcinoma, 54% of patients will develop brain metastases that are refractory to the systemic treatments for lung cancer.3,4 Thus, the treatment of brain tumors has become a significant challenge in cancer therapy and management.

The presence of the blood–brain barrier (BBB), the blood–tumor barrier (BTB), and the invasiveness of brain tumors are the leading causes responsible for the low 5-year survival rate (35%1) of brain tumor patients (Figure 1). The BBB is a physiological barrier composed of tightly bounded cerebral capillary endothelium, pericytes, astrocytes, and basal membranes, with scarce endocytosis and transcytosis but active efflux due to the highly expressed P-glycoproteins on cerebral endothelial cells.5 The BBB prevents the penetration of almost all macromolecules and >95% of small molecules (including anticancer drugs) into the brain,5 and is thus, to a large extent, responsible for the failure of most chemotherapies. Recent studies have shown some evidence that BBB breakdown can occur to some extent as a result of glioma-induced remodeling, or can be induced by some secreted chemicals that could potentially degrade tight junctions and disrupt the BBB.6–9 However, these disruptions

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are spatially and temporally heterogeneous in tumors and vary from case to case,\textsuperscript{10,11} making it difficult to develop generic drug delivery systems (DDSs) for optimal and effective treatments. The BTB consists of three different types of microvessels: the first are continuous, nonfenestrated capillaries (similar to those in a normal brain), the second are continuous, fenestrated capillaries, and the third contain endothelial gaps. This variation in microvasculature forms parts of a controversial argument on whether tumor blood vessels exhibit an increase in permeability to small molecules as the permeability surrounding the tumor has been shown to return to normal within a few millimeters.\textsuperscript{12} Many studies, however, have proven the existence of such a barrier and studied its biological characteristics, while others have worked to disrupt it through focused ultrasound.\textsuperscript{13–17} The invasive and aggressive nature of brain cancer cells is another critical cause for the poor prognosis, as the cancer cells can outgrow into their surroundings and even distant healthy brain parenchyma, making it almost impossible to completely eradicate cancer cells through surgery or chemotherapy. Also, there are many multidrug resistance mechanisms that brain tumors have developed and are associated with the tumor microenvironment, tumor progress, and metastasis, all of which impede the effectiveness of chemotherapy. According to the WHO (World Health Organization) guidelines, more than two-thirds of primary brain tumors are invasive and thus high grade (grade III or IV). For patients with glioblastoma, a grade IV glioma that alone makes up 54.7% of all gliomas and is the most common brain tumor in adults,\textsuperscript{18} the survival time is less than 1 year in 50% of patients, and 5 years in 5% of patients.

Given the aggressive nature of brain tumors and the presence of the BBB, there is a lack of curative treatments for patients with malignant gliomas. Additionally, the existence of cellular and extracellular barriers, and the development of drug resistance by cancer cells over the course of the treatment make the situation more complicated and uniquely challenging. However, continuous progress has been made, resulting in the establishment of the current standards of treatment, which comprise surgical resection, carmustine wafer implantation, radiation therapy, systemic chemotherapy, and antiangiogenesis therapy.\textsuperscript{3}

**CURRENT TREATMENT: STRATEGIES AND LIMITATIONS**

The treatment for brain tumors of a specific type is highly dependent on the stage of the disease. Currently, the four widely adopted clinical treatments are surgery, radiotherapy, chemotherapy, and immunotherapy (Figure 2), with each treatment having its advantages and limitations. A maximized response and safety can be achieved using combined therapies, the regimen of which is carefully chosen based on factors such as the rate of progression, type and stage of the tumor, and the age, condition, and life expectancy of the patient.

**Surgery**

Surgical resection is usually the first and most common treatment method applied in the clinic for patients diagnosed with brain tumors (Figure 2(a)). Tumor removal by surgery helps relieve symptoms and pressure on the brain, providing the opportunity for local chemotherapy and also possibilities for enhancing the efficacy of radiotherapy and chemotherapy.\textsuperscript{21–23} The prognosis of patients who undergo surgery depends heavily on the pathological diagnosis, but other factors such as the extent of resection also play a role, especially in the cases of gliomas.\textsuperscript{22,24,25} Patients with low-grade primary brain tumors such as pilocytic astrocytomas, a type of noninfiltrative astrocytoma mainly common in children, can be effectively treated or cured with surgery only.\textsuperscript{26,27} Preventative treatment such as radiotherapy and chemotherapy may be included after surgical removal of grade II gliomas (e.g., diffusive infiltrative gliomas), which are more invasive and poorly
For high-grade gliomas (grade III and IV), surgery alone cannot eradicate all the cancer cells, and thus its combination with other treatments such as chemotherapy and radiation is routinely adopted.

Other than the tumor grade, the tumor location may also affect the patient survival. Patients with glioblastoma in the frontal lobe (11.4 months median) survived longer than those with tumor in the temporal (9.1 months) and parietal lobe (9.6 months) after surgery. Surprisingly, it appears that the tumor size had no significant effect on survival rate. The prognosis of patients through surgery also relies on the effectiveness of the procedure, particularly the extent of resection. Patients undergoing total resection had longer median survival (90 weeks vs 43 weeks) and higher 2-year survival rates (19% vs 0%) compared with those with subtotal resection.

Figure 2(a) shows the magnetic resonance image (MRI) of a patient before and after surgical resection, indicating the lesion location and size, as well as the resection cavity postsurgery. Despite the popularity of surgery in treating brain tumor patients, its limitations are evident and...
include patient discomfort and the possibility of loss or hindered body functions due to the resection of brain tissue surrounding the tumor. The inability to precisely determine the exact extent and area of the tumor before and during the surgery is the major challenge for surgical resection of brain tumors. Although many techniques are used to help surgeons identify and distinguish tumor cells from normal cells, such as microscopy, MRI, and three-dimensional (3D) probe imaging, it is unlikely that all the tumor cells can be removed via surgery, leaving the potential for recurrence. This recurrence can be limited by the local implantation of carmustine wafers into the resection cavity and through postsurgical radiation therapy or systemic chemotherapy.

**Radiation Therapy**

Radiation therapy penetrates deeper into the tissues, breaks down DNA, and kills infiltrated brain tumor cells that cannot be removed by surgery (Figure 2(b)). High-energy X-ray therapy, proton therapy, and gamma knife radiosurgery are the three main forms of radiotherapy, which can take the form of full brain radiotherapy or localized radiotherapy. Full brain radiotherapy maximizes the extent of brain cancer cells that can be eradicated but has the risk of leading to memory loss. Localized radiotherapy is more specific and likely less severe in side effects, but it is not as efficient in eradicating cancer cells that are far away from the main bulk of the tumor where cancer cells infiltrate the normal brain. Regardless of the form of radiotherapy used, the high-energy beams would affect all the cells in their pathway to some extent, thus causing many potential side effects and patient discomfort. To reduce the side effects, a second form of radiation therapy called alternating electric field therapy has been developed where electric fields are generated to treat high-grade gliomas and stop tumor recurrence. However, the effectiveness of this new technique still requires further validation.

**Chemotherapy**

Chemotherapy eradicates tumorous cells through a number of different action mechanisms, including preferentially killing cancer cells that proliferate more rapidly, blocking a specific signaling pathway, depleting nutrients essential for cell growth, or targeting tumor microenvironment or blood vessels. Owing to the presence of the BBB, targeted drug delivery to brain tumors has been a major challenge. As such, local delivery methods have been developed in parallel to bypass the BBB, such as implanting carmustine wafers into the resection cavity left by the surgery. Systemic DDSs include the orally administered temozolomide that can diffuse across BBB and intravenously administered bevacizumab that targets the glioblastoma neovascularature.

**Localized Treatment**

Currently, the only drug approved for the local treatment of brain tumors in the USA is the Gliadel wafer (carmustine wafer or bis-chloroethylnitrosourea (BCNU) wafer), a therapy typically used after resection and in combination with other treatments (Figure 2(c)). Carmustine (BCNU), an alkylating agent, is the active ingredient that kills cancer cells via binding to the O6 position of guanine in DNA so as to cross-link the two strands and prevent DNA replication. In the wafer design, BCNU is embedded and dispersed in the matrix material polifeprosan 20, comprising poly[bis(p-carboxyphenoxy)propylene and sebacic acid in a 20:80 molar ratio. This polyanhydride copolymer matrix is biodegradable and would gradually break down through hydrolysis to release the encapsulated BCNU. In the in vivo setting, the majority of BCNU is released within the first 5–7 days. The polymer material itself, however, requires ~6–8 weeks to degrade fully. The released BCNU maintains a high concentration within 2–3 mm from the wafer but suffers a significant drop as the distance from the implanted sites increases. A meta-analysis on the survival outcome of patients with newly diagnosed high-grade glioma revealed that the median survival for patients receiving Gliadel wafer is 16.4 months compared with 13.1 months for those without Gliadel wafer. Although proven effective, the broader application of Gliadel wafer is limited by several weaknesses. First, carmustine has a half-life of 15 min, which is too short for the active drug to diffuse across large distances before drug metabolism/degradation occurs. Second, the active compound carmustine could potentially enter the circulatory system by capillary loss or through other pathways. Third, the disc-like structure of the wafers does not allow for intimate contact with the tissue, and the uneven contact may lead to uncontrolled hydrolysis of the matrix. Some studies have shown that the wafers produce an excess amount of fluid in the brain cavity as they degrade, which could lead to postsurgery leakage of cerebrospinal fluid and a higher chance of infection, though other studies suggested that this may not be the case. Lastly, the prolonged patient...
survival by Gliadel wafer is only a few months, and the choice of using Gliadel wafer likely precludes the patients from registering for other therapies in clinical trials. The limited benefits of carmustine implantation have led brain tumor patients to seek other medical solutions.

**Systemic Treatment**

Temodar® (Temozolomide, TMZ), an orally administered chemotherapy, was approved by the United States Food and Drug Administration (FDA) in 2005, as a result of its efficacy and ease of use (Figure 2(d)). Temozolomide is an alkylating agent that kills cancer cells through a similar mechanism to carmustine. In addition, when taken following the prescribed schedule, Temodar can inactivate O⁶-methylguanine-DNA methyltransferase (MGMT) that is primarily involved in DNA repair and cell rescue, enhancing its anticancer efficacy.⁵⁵,⁵⁶ In a randomized prospective study in which TMZ was combined with radiotherapy in treating newly diagnosed glioblastomas, the median survival time of patients was 14.6 months compared with 12.1 months for those receiving radiotherapy only, accompanied by an increase in the 1- and 2-year survival rates.⁵⁶ Because of its proven efficacy and convenience for administration, temozolomide has become an important part of the treatment regimen for patients diagnosed with brain tumors.

Although not yet advanced to clinical use, there has been extensive exploration over the past three decades on the utilization of a great diversity of nanoparticles as effective carriers to treat brain tumors systemically.⁵⁷–⁶¹ The ability to accommodate both therapeutic and diagnostic agents enables the natural use of nanoparticles for theranostic agents.⁶²,⁶³ In most cases, targeting or biologically active agents were used to increase their accumulation at the tumor sites, to cross the BBB, and/or to overcome the multidrug resistance mechanisms.⁶⁴–⁶⁶ Given the scope of this review, we will focus our discussion on local treatment strategies for brain tumors. Some excellent reviews on nanoparticle-based systemic treatment of brain tumors can be found elsewhere.⁵⁸,⁶⁶,⁶⁷

**Immunotherapy**

Avastin (bevacizumab) is a recombinant humanized monoclonal antibody that received FDA approval in 2009 to treat recurrent glioblastomas. The action mechanism of bevacizumab is to inhibit neovasculature growth via blocking of Vascular endothelial growth factor A (VEGF-A) (Figure 2(d)). Consequently, bevacizumab would deprive the tumor of oxygen and other nutrients, causing tumor cells to die of starvation.⁶⁸ Clinical studies showed that patients with recurrent glioblastoma who were treated with bevacizumab had a median overall survival of 9.0 months compared with only 6.1 months for those not receiving the drug. The progression-free survival rate during the first 6 months also increased to 41% from 18% when bevacizumab was included in the treatment regimen.⁶⁹ Similar to other therapies, bevacizumab alone has limited efficacy. Consequently, combination use with other treatments is necessary and has to be optimized. One major concern of including bevacizumab into the regimen is the duration of treatment, as a rebounding effect has been recorded for bevacizumab and other anti-VEGF antibodies.⁷⁰

One major advancement in cancer therapy, which is still under clinical research, is the use of checkpoint inhibitors. Tumors have been found to adopt immune checkpoint pathways, allowing them to overcome immune responses triggered by certain T cells specific to tumor antigens. For that, antibodies or other forms of ligands or receptors could be used to block the receptor–ligand interaction, preventing these tumors from achieving immune resistance. The first immune checkpoint receptor to be targeted was the cytotoxic T-lymphocyte-associated antigen 4 (CTLA4). The blockage of this receptor, expressed exclusively on T cells, by certain antibodies facilitates T-cell activation. This method has been FDA approved for melanoma and is currently under clinical trial for other cancers such as lung and brain tumors.⁷¹ Other immune-checkpoint inhibitors being targeted include programmed cell death protein 1 (PD1) receptors and PD1 ligands (PDL-1), which naturally work to limit the activity of T cells during inflammatory responses and to limit autoimmunity.⁷²–⁷⁴ Overexpression of PDL-1 leads to major immune resistance within the tumor microenvironment, and thus its inhibition can make the cancer cells vulnerable to the immune system.⁷⁵,⁷⁶ Many other checkpoint inhibitors are also under study helping to transform human cancer therapeutics.⁷¹,⁷⁷,⁷⁸

**Local Versus Systemic Delivery**

There are benefits and drawbacks for both local and systemic treatments (Table 1). Systemic therapy allows the regime and dosage of the treatment to be readily adjusted in response to therapeutic outcomes. It is highly desirable to maximize the efficacy and also to minimize the toxicity. Nevertheless, the non-specific accumulation of the drug in major organs other than the brain, coupled with the low drug deposition in the brain tumor, remains a major hurdle
for systemic chemotherapy. To address this, techniques that can temporarily open the BBB have been explored, but apparently this strategy would put the brain at risk because potentially toxic species could also gain access to the brain and may cause unexpected side effects. For example, magnetic resonance-guided focused ultrasound (MRgFUS) has been used to temporarily disrupt the BBB. Preclinical models have shown that this method can effectively open up the BBB without causing any lesions or permanent damage. Furthermore, MRgFUS was proven to increase the ability of chemotherapeutics to cross the BBB and to achieve higher drug concentrations within brain tumors.

Chemical modifications to current therapeutics have also been explored to improve their permeability to the BBB. The development of nanotechnology platforms provides an opportunity to improve the efficacy of chemotherapy, as the drugs could be protected from degradation and rapid clearance during their transportation, and to be specifically delivered to the diseased sites such as the brain. Many brain-targeting nanomedicines have been developed, greatly improving the drug’s accumulation within the brain tumor, especially for those that are inherently BBB impermeable. For instance, nanocarriers have been developed to cross the BBB through carrier-mediated transport, receptor-mediated transport, and adsorption-mediated transport. These examples suggest that through electrostatic interactions, cationic molecules can bind to negatively charged glycoproteins within the plasma membrane or to the transferrin receptor, which can facilitate the traversal of nanocarriers across the BBB. However, the brain accumulation efficiency of these nanomedicines is still far below the expected therapeutic level, and as such further investigation is necessary to improve the transportation efficiency across the BBB.

The main advantage of local drug delivery is that the drug would bypass the BBB, thus allowing a therapeutic concentration of drug to be placed directly in and near the targeted area. Consequently, significant systemic exposure is avoided and any associated side effects such as reduced blood counts and patient discomfort are minimized. However, local therapy also suffers from limited distribution and poor tissue penetration, as the released drug could be potentially deactivated by metabolism or premature degradation, or cleared from the brain through capillary loss. The potential toxicity and biocompatibility of the matrix are another concern for local therapy. Also, local delivery faces the same drug resistance challenges as systemic delivery, and multiple-dose drug administration is complicated and often proves difficult to implement for surgery-assisted local treatment. Numerous strategies have been developed to address these issues and will be discussed in the following sections.

### NANOTHERAPEUTIC SYSTEMS FOR LOCAL TREATMENT

Nanotechnology and nanosciences have served as a major driver over the past three decades for developing new strategies for the localized treatment of brain tumors, as they could potentially address the drawbacks of the Gliadel wafer through rational design of nano-based DDSs. First, nanoparticles of well-defined sizes and shapes allow for physical encapsulation or chemical conjugation of multiple types of therapeutic
agents within synthetic or naturally derived materials, improving the aqueous solubility of hydrophobic molecules, preventing their premature degradation, and providing possible pathways to bypass drug resistance mechanisms. Second, nanoscale carriers can be used to regulate the release rates of the carried drugs, realizing cancer-specific and prolonged drug release. Third, nanoparticles with optimized surface chemistry could improve the penetration within the brain and possess the ability to differentiate cancerous cells from healthy. Many forms of nanostructures are currently being investigated for drug delivery such as liposomes, dendrimers, lipid nanoparticles, polymeric micelles, nanocapsules, nanoemulsions, nanoparticles, and nanofibers. In this section, we examine how nanoparticles and hydrogels have been designed in several research laboratories as new platforms for locally treating brain tumors. We will also discuss new methods being explored for the local administration of drugs into the brain.

Implantable Microdevices for Local Delivery

Microreservoir Devices

Microdevices are attractive candidates that could potentially improve the efficacy of local chemotherapy of brain cancer, as they can deliver large doses of drugs in either solid or liquid form. Cima and coworkers developed a microdevice made of poly(L-lactic acid) (PLLA) and a liquid crystal polymer (LCP) (Figure 3(a)). The microdevice has a single 889-μm hole in the cap and several smaller holes in the wall through which the encapsulated temozolomide could be released over a period of several days. Intracranial implantation of the devices into the brain tumor, either primary glioma brain tumor or secondary brain tumor metastasized from breast cancer, resulted in prolonged survival of the treated animals. Although proven effective, the fast drug release of the microdevice hampers its further application as a long-lasting local treatment for brain cancer. Consequently, a new version of the device was developed, consisting of a reservoir and a cap that were micromachined and made of the biocompatible material, polysulphone, as a delivery system for cediranib or dexamethasone to treat brain tumors and tumor-related edemas. The device can also be extended to deliver anticancer drugs (Figure 3(b)).

The reservoir is a cylinder 2 mm in diameter and 2 mm in height and is designed to be drug impermeable. The cap of the device was machined with 300-μm-diameter holes through which the encapsulated drug could penetrate across. Compared with a systemic delivery strategy, only a small percentage of the systemic dose is required to achieve similar efficacy. More importantly, the release of the encapsulated drug from the microdevice is highly controllable with a linear release profile—the more holes machined in the cap, the faster the release of the drug. For instance, only ~5% of the encapsulated drug was released in 10 days for a microdevice with only a single hole in the cap, and thus it is theoretically possible to develop a drug depot that can release a drug for up to 200 days (Figure 3(c)).

Another form of microreservoir is the polymeric microchip device designed by the Langer lab. These microchips were 1.2 cm in diameter and 480–560 μm thick, possessing 36 reservoirs that could each be loaded with a different substance. PLLA was the matrix polymer used to make these systems and a membrane consisting of poly(D,L-lactic-co-glycolic acid) (PLGA) covered each reservoir. By altering the molecular mass of the PLGA membrane, the time to release the drug within the particular reservoir can be controlled. This delivery system provides the ability to load different drugs within the microchip and release them at various intervals after implantation.
Drug-Eluting Beads
The microdevices developed, although much smaller than the Gliadel wafer, still require surgical implantation. Baltes et al. downsized the device into drug-eluting beads (DEBs) with diameters of 100–300 μm, allowing implantation through intracerebral injection in the treatment of glioma.124 DEBs have been used as drug delivery embolization systems that can both kill cancer cells and block the blood supply to the tumor.125 In their study, DEBs produced from polyvinyl alcohol (PVA) hydrogels were loaded with doxorubicin and irinotecan drugs. The PVA hydrogels were modified with sulfonate groups to allow controlled loading and delivery of chemotherapeutic drugs. Prolonged survival was observed from the rats treated with the DEBs loaded with both drugs (21 days vs 16 days for the nontreated group), while blank DEBs caused no significant toxicity to the animal.124 Although these DEBs have proven to be an innovative way of treating brain tumors, the quality control of DEBs (e.g., the size and size distribution, the bead erosion rates, and the drug release rates) remains a challenge that may limit their clinical translation.126

Microelectromechanical Systems
Microelectromechanical systems (MEMSs) are implantable devices that consist of many microreservoirs covered with a gold membrane, each containing a specific dose of drug.127 When a positive potential is applied, the gold membrane electrochemically corrodes into soluble gold chloride, and the substance within that particular microreservoir is free to dissolve or diffuse into solution.128 This property gives MEMSs the ability to deliver multiple drugs or molecules either in parallel or in series.129 Advantages of using this method include the capability to produce complex drug release profiles, the capacity to use a variety of drugs, and the potential for precise dosing. Disadvantages, however, arise from the required surgical implantation and removal of the devices, and the limited size of the microreservoirs.127 MEMSs have been tested in vitro on brain tumors by loading drugs such as BCNU,127 BCNU co-formulated with poly(ethylene glycol) (PEG),130 and TMZ.131 Results showed that the device proved effective in delivering each molecule with a well-defined time-dependent profile.127 Furthermore, the use of this device prolonged survival rates, reduced tumor growth, and improved the rate of drug delivery.130,131

Discrete Nanoparticles
The ability to produce nanoparticles using a range of materials and to accommodate both therapeutic and diagnostic agents, combined with the potential for further surface modifications, opens up many possibilities to generate new platforms designed to address the challenges in the local treatment of brain tumors.

Nanoengineered Titanium Wires for Local Drug Delivery
Gliadel wafers as polymer-based implants are highly suitable for local delivery of hydrophobic drugs. However, for hydrophilic drugs such as doxorubicin, the drug loading is limited and the release rate is poorly controlled. Given that inorganic nanoparticles usually have a strong affinity for doxorubicin through chelation,81,132 Losic and coworkers prepared titanium wires to act as nanotube holders and drug nanocarriers.82 In this study, a self-ordering electrochemical anodization process was applied on the surface of 0.75-mm-thick and 30–40-mm-long titanium wires, generating titania nanotube arrays 170 nm in diameter and 70 μm in length for doxorubicin loading. These nanoengineered wires were biocompatible and mechanically stable and could load up to 1200 μg of doxorubicin. The drug-loaded wires exhibited a sustained drug release over 8 days, a result attributed to their high surface-to-volume ratio.133,134 The high loading and controllable release profile of these structures make them a suitable drug depot for local therapy applications.

Paclitaxel-Loaded Nanofiber Discs
Paclitaxel (PTX) possesses a high potency against glioma cells in vitro.135 However, the therapeutic outcome was found to be unsatisfactory for locally delivered PTX using polymeric systems.135–138 One possible reason is that the PTX could be removed from the brain by drug efflux pumps that are highly expressed on the BBB, thus limiting the penetration of the drug across a long distance. Wang and coworkers explored how the rate of drug release could affect the penetration of PTX in the brain, engineering four different PTX in PLGA formulations with different size, shape, and composition, including discs made of PLGA nanofibers (about 100 nm in diameter) or microfibers and microparticles embedded in hydrogels.139 Among all the formulations, PLGA nanofiber discs showed the fastest, though still prolonged, drug release due to the relative hydrophilicity of the PLGA and high surface area exposed to biological fluids. They found that PLGA nanofiber discs were also the most efficient in treating glioma cells that are radially distant from the implant site. A high concentration of PTX was achieved even 5 mm from the implants, a distance greater than their previous formulations.139–141 It is thus believed that the
establishment of a high local concentration of PTX in the implanted site is critical for local therapy of brain tumors.

**Magnetic Nanoparticles**

The ability of magnetic nanoparticles to respond to an external magnetic field has had a profound impact on many medical areas including drug delivery and medical imaging. In a recent study by Pillay and coworkers, magnetized nanoparticles loaded with carmustine were developed for brain cancer therapy. Modification of these magnetic nanoparticles with functional polymers such as PVA and polyethyleneimine (Figure 4) enhanced their structural and chemical stability, as well as providing sites for further functionalization. Conjugation of fluorescein isothiocyanate, for example, allowed for the tracking of the nanoparticles upon injection. The elegance of the design is that the mobility and cell internalization of these nanoparticles were enhanced in the presence of an external magnetic field. The BCNU-loaded magnetic nanoparticles inhibited the growth of glioblastoma cells with cell viability percentages ranging from 49.8 to 7.3%. Nanoparticles alone showed no significant cytotoxicity, demonstrating that the carmustine was responsible for the observed cytotoxicity against cancer cells. Furthermore, the release rates of these nanoparticles are adjustable by their composition, ranging from 78.8 to 30.2% after 72 h.

Utilizing the response of these magnetic nanoparticles to an external magnet, Pillay and coworkers demonstrated that they could manipulate the nanoparticle trajectory, enhancing the mobility and leading to greater cell internalization. This study showed that external magnets could serve as a viable method to control the pathway of drug-loaded magnetized nanoparticles and direct them to target sites.

Magnetic nanoparticles can also be triggered by an external magnet field to generate intratumoral heat. Such particles were injected directly into glioblastoma tumors with the purpose of producing an intratumoral thermotherapy. In vivo studies have shown that the median survival time, starting from the initial diagnosis of the glioblastoma tumor, was 23.2 months using this thermo/radiotherapy method, compared with 14.6 months for the reference group. The significant increase in survival time suggested that magnetic nanoparticles can effectively inhibit tumor growth.

The future success of magnetic nanoparticles as a therapy option is not clear, however, as there are many design parameters and physiological factors that could play a role in the application of the system. In the design phase, the magnetic properties and size of the particles can have an important effect on their overall performance. Similarly, the field strength, field geometry, and drug/gene binding capacity can affect the targeting effectiveness, while physiological parameters such as the depth of the target, the rate of blood flow, vascular supply, and body weight could provide undesired barriers and reduce the effectiveness of such systems. Technological barriers may also present a problem, as specialized instruments would be required to precisely manipulate the trajectory of the magnetic nanoparticles in the complex, 3D environment, that is, the human body. Current technology may be able to exert some influence over the particle’s movement, but further advances may be necessary to attain the desired degree of control. The importance of these parameters in the system design should be well understood before clinical trials take place.

**Nonviral Gene Therapy for Glioblastoma Treatment**

Gene therapy is an upcoming exciting method being explored at the preclinical stage for the treatment of cancer. This methodology involves the delivery of therapeutic genes that are capable of promoting apoptosis once expressed in cancer cells, are neuroprotective if expressed by healthy cells, or they could help promote immune response toward tumors. Gene therapy can involve either viral vectors that are incorporated into patients or nonviral vectors through nanoparticle encapsulation. Viral methods have caused hesitation due to safety concerns, while nonviral methods have lacked efficiency and long-term stability. In an initial study,
different poly(β-amino esters) (PBAEs) were used to form the nanoparticle carriers. PBAEs are cationic linear polymers that demonstrate high transfection efficiency and low cell toxicity. These cationic polymers can co-assemble with DNA into well-defined nano-objects, and are capable of encapsulating up to 100 plasmids each. The properties of these particles were analyzed for their effect on brain tumor stem cells (BTSCs), the source of glioblastoma tumors that play a prominent role in tumor recurrence, as well as against glioblastoma astrocytes, neural progenitor cells (NPCs), and noncancerous cells. The results of the study show that incorporating nonviral genes within different PBAE-formulated nanoparticles can help overcome several drawbacks previously experienced with this technique. Some PBAE nanoparticles were shown to be highly efficient in transfecting glioblastoma astrocytes and BTSCs compared with normal cells and NPCs. The PBAE–DNA nanoparticle complex could be prepared, stored, and reconstituted for up to 3 months without experiencing functional losses. After successful results had been achieved, further studies were conducted in which this PBAE–DNA nanoparticle system was tested in vivo. The polymer chemical design has a strong impact on their interactions with cells as a small variation of just one or two monomers could make a significant difference. Different trends were observed among the tested molecules that varied in polymer end caps, drug loading, and molecular design. The results from this study showed that by adding additional sucrose when lyophilizing the nanoparticles, shelf life could be extended to 2 years without loss of efficacy, instead of 3 months without added sucrose. Furthermore, in vivo results suggested that naked DNA possessed poor expression capabilities compared with nanoparticle-encapsulated DNA. Also, nanoparticles transfected significantly better when injected into tumor-bearing mice compared with healthy mice, as expected from the in vitro results.

A later study by the same group used flow cytometry to quantify intracellular and nuclear uptake of these nonviral genes when encapsulated within PBAE nanoparticles. Most recently, these nanoparticles were shown to extend survival in rodents with human-derived brain tumors. Overall, these three studies prove that using PBAE nanoparticles as nonviral gene carriers in both in vitro and in vivo settings can increase stability, enhance the ability to distinguish between tumorous and normal cells, and improve the efficiency of these nonviral genes—three qualities known to be lacking in current nonviral gene therapy.

PEGylated Nanoparticles With Enhanced Brain Tissue Penetration

In most cases, drugs released from the implanted formulation do not penetrate across large distances due to the premature degradation of the drugs, entrapment in tissues and cells they first encounter, and their clearance from the capillaries. The use of nanoparticle carriers is expected to address these issues, such as the difficulty in penetrating the brain parenchyma. Hanes and coworkers demonstrated that this problem could be potentially addressed by adding a dense PEG coating onto the large polymeric nanoparticles, a strategy that proved successful in the local treatment of brain tumors. The Hanes lab used polystyrene nanoparticles up to 200 nm in diameter as model nanoparticles, some of which were densely coated with PEG. The model nanoparticles were then applied to rodent brain tissue, in vivo and ex vivo, as well as to human tissue, ex vivo. They demonstrated that nanoparticles up to 114 nm in diameter could penetrate the endocannabinoid system (ECS) freely when coated.
with PEG, while bare particles of 40 nm could not.\textsuperscript{116} They suggested that 28% of the brain ECS contains pores of more than 100 nm in size and that PEG, an uncharged hydrophilic polymer, could reduce the nanoparticle interaction with charged or hydrophobic components of the brain perenchyma.\textsuperscript{117} The ability to switch from smaller to larger nanoparticles, capable of brain penetration, enhanced drug loading efficiency, larger drug distribution, and longer drug release periods could produce a more effective treatment method with improved efficacy.\textsuperscript{168–170}

Given the promising results from the model nanoparticles, PTX was encapsulated within 70-nm PEG-coated PLGA nanoparticles to test their effect on brain tumor tissue \textit{in vivo} (Figure 6). The control animals receiving no treatment experienced rapid tumor growth, and those receiving free PTX saw only slight tumor growth inhibition (~15% inhibition). The efficacy was slightly improved when encapsulating the PTX into the PLGA nanoparticle (55% inhibition) and was further increased to 92% growth inhibition if PLGA–PEG nanoparticles were used for PTX delivery. Overall, these studies demonstrated convincing evidence that PEG-coated nanoparticles have the tendency to rapidly diffuse through the brain tumor microenvironment to produce a more uniform distribution, and are capable of suppressing tumor growth when loaded with the PTX drug.\textsuperscript{117}

**Radiosensitizing Nanoparticles**

Other than drug delivery, nanoparticles have also found use in radiation sensitization. Radiotherapy involves using cytotoxic levels of ionizing radiation to kill tumor cells through DNA damage. One drawback of this technique is the inability to deliver sufficient doses while minimizing damage to surrounding healthy cells.\textsuperscript{171} For that, gold nanoparticles have been used as radiation sensitizers due to their strong absorption of ionizing radiation. Upon internalization of such particles into the tumor, low doses of radiotherapy will result in a magnified state, localized to the site of the tumor and minimizing damage to surrounding healthy cells.\textsuperscript{172} Many studies have shown that this combination of intravenously injected gold nanoparticles and radiotherapy results in a significant increase in survival when applied to mice.\textsuperscript{171,173,174} For example, one study by Hainfeld et al. demonstrated that 50% of the mice receiving combination therapy showed long-term tumor-free survival whereas those receiving just radiation all died.\textsuperscript{175} Another study by Miladi et al. uses MRI to track intravenously injected gold nanoparticles to help accurately target and determine the optimal time of radiotherapy; a 473.3% increase in lifespan was observed using this method, compared with a 222.2% increase when radiotherapy was solely used.\textsuperscript{176}

A major issue found with radiotherapy is the ability of some tumors, such as glioblastoma, to acquire radioresistance. One way to overcome this is through the use of higher dose radiotherapy, which provides another application for radiosensitizing nanoparticles. Miladi et al. demonstrated this application method by intratumorally injecting ultra-small gadolinium-based nanoparticles (GBNs: 2.9 ± 0.2 nm) to act as radiosensitizing agents and help overcome radioresistance. As predicted, mice bearing radioresistant tumors showed a significant increase in tumor regression when a combination of GBN and radiotherapy was administered, compared with mice

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**FIGURE 6** Schematic illustration of the tissue penetration of non-PEGylated and PEGylated poly(D,L-lactic-co-glycolic acid) (PLGA) nanoparticles loaded with paclitaxel and labeled with fluorescent dyes after the intratumoral injection.\textsuperscript{117} (Adapted with permission from Ref 117)
who received radiotherapy alone. Furthermore, apoptosis increased by 222% in tumors that received a combined treatment, compared with irradiated-only tumors.\textsuperscript{177} Given that a large concentration of GBNs is required in the tumor site for this method to be effective, local intratumoral delivery has proven to be more effective than systemic. However, this technique is limited to tumors that lie close to the body’s surface.\textsuperscript{178}

**Convection-Enhanced Delivery**

Other than using PEGylated nanoparticles as drug delivery vehicles, the penetration of the drug within the brain could also be enhanced with external devices such as a convection-enhanced delivery (CED) system. CED systems have been an extensively researched field in local brain drug delivery for many years. The key concept of CED is to drive the tissue penetration of the delivered drug by establishing a pressure gradient, one created by placing a catheter in the tumor cavity and using a motor to push the drugs through the catheter and into the tumor. As the drug depot is an externally located compartment, it can be easily filled once levels are low. This strategy takes full advantage of local treatment while eliminating the problem of treatment ceasing due to drug depletion.\textsuperscript{179–181} The effectiveness and efficiency of using CED have been well proven and is attributed to its ability to provide an enhanced drug penetration and distribution within the tumor, as evidenced by MRI.\textsuperscript{182,183} In studies that directly compared the brain tissue penetration of liposomes delivered via either CED injection or conventional injection, liposomes delivered with CED technology showed a much broader distribution in the brain, compared with those delivered using conventional injection methods.\textsuperscript{115} Liposomes delivered with CED technology were also superior to those delivered through a vascular route, judging from their tumor growth suppression ability.\textsuperscript{184} Overall, these studies suggest that CED provides a widespread and more efficient drug distribution in intracranial tumors, hindering tumor growth and increasing survival time.\textsuperscript{115,184}

Given the effectiveness of CED technology, Saltzman and coworkers evaluated the anticancer activity of PLGA nanoparticles loaded with camptothecin (CPT)\textsuperscript{185} (Figure 7). Camptothecin has previously been formulated into ethylene-vinyl acetate or 1,3-bis(p-carboxyphenoxo)propane and sebacic acid (PCPP:SA) wafers\textsuperscript{186,187} for the treatment of brain cancer, but the large dosage required to achieve a therapeutic effect could potentially lead to systemic toxicities.\textsuperscript{188} By encapsulating CPT into PLGA nanoparticles and delivering with CED technology, a lower dosage (one-eighth) was required to realize significant tumor growth inhibition. Rats treated with CED-delivered CPT–PLGA nanoparticles showed a median survival time of up to 22 days, compared to those treated with free CPT and unencapsulated nanoparticles, which showed a median survival of 17 and 15 days, respectively. Furthermore, 30% of the group experienced long-term survival and were free of disease after 60 days. Histological examination demonstrated that tumor eradication could be achieved.\textsuperscript{185}

Multifunctional nanoparticles could also be delivered using CED technology to achieve a synergistic effect. Zhang and coworkers utilized magnetic nanoparticles as carriers for O\textsuperscript{6}-benzylguanine, an inhibitor of MGMT\textsuperscript{189} (Figure 8). These nanoparticles were coated with a redox-responsive, cross-linked, biocompatible chitosan–PEG copolymer to improve the stability of nanomedicine during the transportation and to facilitate drug release upon endocytosis. After delivery with CED technology, the distribution of the nanomedicine could be directly imaged through T2-weighted MRI, and when combined with TMZ a threefold increase in median overall survival was observed. Allard et al. explored the synergistic effect of combined chemotherapy and radiotherapy, constructing lipid nanocapsules loaded with the hydrophobic organometallic drug ferrocenophenol.\textsuperscript{190} After intratumoral injection of the nanocapsules with CED technology, radiotherapy was performed. The radiotherapy, while killing the cancer cells itself, also caused the ferrocene unit to oxidize to ferrocenium and eventually generate the alkylating agent quinone methide.\textsuperscript{191} Generation of an additional chemotherapeutic resulted in an improved median survival time (40 days median survival) compared to both the control group, which received no treatment (25 days median survival) and the group which received blank nanoparticles along with irradiation therapy (33 days median survival).\textsuperscript{190} These studies provided insight into the strong impact that nanotechnology and nanoencapsulation can have in the CED-based local chemotherapy of brain tumors.

Although a large and uniform radius of drug distribution is achieved in animal models, there are many factors in human patients that could complicate CED-based treatments. For instance, tumor mass and the effects of edema could play a role in changing the anatomy of the surrounding environment. Creating a pressure gradient within the modified surrounding tissue could lead to uneven and unpredictable drug distributions, and drug leakage into the subarachnoid space is likely to occur.\textsuperscript{192,193} Additionally, the CED system relies heavily on the
infusion technology with improved visualization during catheter insertion and drug infusion. Innovations in the formulation of the infused drug are also required. Nevertheless, the ongoing clinical trials using CED (NCT00431561, NCT02278510, NCT02869243, and others) could provide valuable information for further optimization of the catheter design and the infusion procedure.

**Injectable Hydrogels**

The delivery systems discussed up to this point are either solid implants or liquid nanoparticle suspensions for local injection, with the former being sometimes difficult to administer and the latter having issues remaining at the injection sites. Injectable hydrogels are highly desirable as the drug can be injected into the brain tumor using a needle/syringe and would remain in close proximity to the site of injection. Over time, the hydrogel would provide sustained release of the encapsulated anticancer drugs. OncoGel®, a hydrogel consisting of the polymeric matrix, ReGel, and the drug PTX, was developed as a new DDS to replace Gliadel wafers in brain tumor treatment. ReGel is made of an A-B-A triblock copolymer comprising PLGA and PEG. Like many amphiphilic block copolymers, this triblock copolymer can assemble into micellar structures possessing a hydrophobic core capable of sequestering hydrophobic drugs such as PTX. Importantly, the solution containing the ReGel micelles can transition from a viscous solution at 2–15°C to a hydrogel at

**FIGURE 7** | The anticancer efficacy of poly(ε-caprolactone-co-glycolic acid) (PLGA) nanoparticles loaded with camptothecin (CPT). The PLGA nanoparticles were around 100 nm in diameter (a), and when delivered into the brain tumor through convection-enhanced delivery technology, could significantly prolong the survival of model animals with an intracranial brain tumor (b). Crystal violet staining revealed that CPT–PLGA nanoparticles could eradicate the cancer cells when injected into the center of the tumor (c), or partially inhibit the growth of tumor if injected in the margin of the tumor (d). Free CPT (e) and blank nanoparticles (f) showed no antitumor efficacy. (Reprinted with permission from Ref 185. Copyright 2011 Springer)
This property of the hydrogel allows its usage as injectable hydrogels to fill the brain tumor cavity after surgical resection and act as a PTX drug depot. Compared to the Gliadel wafer, the ReGel was supposed to be more convenient for drug delivery and dosage adjustment. Indeed, OncoGel has been shown to deliver PTX, to surrounding tumor cells, over a period of 6 weeks, with reduced toxicity relative to that of the systemically delivered drug. The median survival time for rats receiving OncoGel and radiotherapy in combination was 83 days in sharp contrast with 31 days for OncoGel alone, 26 days for radiotherapy, 14 days for ReGel, and 13 days for the placebo. Furthermore, there were no signs of toxicity in any of the rats when the OncoGel was administered intracranially.

The in vivo results of OncoGel in animal studies provide valuable insight into the use of hydrogels to locally deliver therapeutic agents into brain
tumors. However, similar significant effects were not observed in the four tested patients, leading to the termination of its clinical trials. Although the exact reasons remain elusive, it is clear that the PTX hydrogel formulation requires further optimization for drug loading, controlled release, tissue penetration, and specific cancer recognition, so as to achieve improved treatment effectiveness in human patients. Also, one of the polymer segments, PEG, is not biodegradable and its clearance from the body remains a concern.

Cancer treatment is most effective in humans when drug administration is homogeneous and long-lasting. On this basis, a possible reason for the failure of OncoGel in humans could be the burst release profile of the delivery system, a consequence of which could be rapid clearance of the drug from the brain that prevents it penetrating across a large distance.

Hydrogels formed from entities that can gelate through self-assembly (hydrogelators) can potentially help address this issue as the encapsulated drugs move into the surrounding tumor cells through simple diffusion.\textsuperscript{206,207} The diffusion rate, in this case, is affected by the strength of interaction between the hydrogelators and the drug itself and can be controlled by the addition of ions or other reagents that modulate the strength of these intermolecular interactions.\textsuperscript{208,209} Materials that are biocompatible, biodegradable, and can form hydrogels would be the most suitable candidates for the local treatment of brain tumors. For example, peptide-based molecular building units can self-assemble into one-dimensional (1D) nanostructures that can subsequently entangle into a 3D supramolecular network through noncovalent interactions such as hydrogen bonds, electrostatic interactions, π–π interactions, or hydrophobic interactions.

**FIGURE 9** (a) Schematic illustrations of the design and assembly of drug-based hydrogelators. A typical drug amphiphile (DA) contains an anticancer drug, a biodegradable linker, and a short peptide. DAs are designed to self-assemble into filamentous nanostructures in water that could further form a hydrogel under physiological condition. (b) Transmission electron microscopy (TEM) characterization of long filaments formed by self-assembly of a camptothecin (CPT) DA. (c) Cryo-TEM image of these ultra-long filamentous nanostructures. (d) A photograph to illustrate the hydrogel form of CPT DAs.
interactions. Furthermore, different components can sometimes be added to the hydrogel to make it susceptible to various stimuli such as magnetic fields, light, ultrasound, nucleic acids, and other small molecules. This added capability could allow external control over the drug release rate. Conjugating drugs to an auxiliary segment could also greatly modify the diffusion rate of the drug, serving as an alternative strategy.

An emerging class of hydrogels that are made entirely of anticancer drugs have been developed over the past 5 years by directly assembling anticancer drugs into a supramolecular network. These drug-based hydrogelators are self-assembling drug amphiphiles (DAs) that incorporate a short peptide segment onto an anticancer drug via a biodegradable linker to promote their assembly. Under physiologically or pathologically relevant conditions, these monodisperse DAs can be designed to spontaneously associate into discrete, stable supramolecular nanostructures that possess the potential for self-delivery and local treatment of malignant brain tumors (Figure 9). Anticancer drugs such as CPT and PTX have been shown to be able to assemble into their respective hydrogels. In vitro studies revealed effective cytotoxicity of these DA hydrogelators against multiple cancer types including primary glioma cells.

Using anticancer drugs as molecular building blocks and hydrogelators for local treatment of brain tumor could have several unique advantages. First, these drug-bearing filaments possess a high and fixed drug loading that is tunable and precisely defined by their molecular design, for example, the drug content was precisely controlled using the two amine functionalities of the amino acid lysine, creating branching points that allow the attachment of one, two, or four CPT molecules as required. Second, functional peptide segments can be incorporated to fulfill different biological roles, such as specific targeting of cancer cells, enhanced cellular uptake, overcoming multidrug resistance mechanisms, and promoting tissue penetration for diffusion across large areas. Third, the drug release rates of hydrogels can be tuned through either the design of the chemical linker that bridges the drug with the peptides or the design of the overall amphiphilicity of the building units to control the disassembly kinetics. Lastly, the creation of hydrogels with anticancer drugs represents a carrier-free drug delivery strategy. There are no additional carriers used to deliver the drugs of interest. Once the incorporated drugs finish their duties, the hydrogels would concurrently disappear. Although there is much to prove, the self-assembling drug-bearing supramolecular hydrogels could represent a new platform technology for the local treatment of brain tumors.

CONCLUSIONS AND FUTURE PERSPECTIVES

As a result of brain tumors, thousands of lives are lost in the USA and much more worldwide. Brain tumors cost billions of dollars to the USA alone and, despite multimodal treatment, they still have a poor prognosis of only months of life expectancy. These tumors are difficult to reach via systemic delivery due to the presence of the BBB. Localized treatment can bypass the BBB and deliver a higher drug amount into the tumor, at the same time diminishing systemic side effects. Gliadel wafer is used for the local treatment of brain tumors, and its clinical success has inspired researchers to develop new implants such as nanoparticles, nanomedicines, and injectable hydrogels. These modern delivery techniques can achieve better drug release and deeper tissue penetration. Despite the early preclinical success, however, there are still many issues that need to be addressed before their translation into the clinic. The first is the limited penetration of the drug into the brain parenchyma. Although a therapeutic concentration of drug could...
be delivered to several millimeters away from the injection site—enough to cover most of the brain in mice or rats—it is still a short distance when compared with the size of the human brain. The second issue comes from the poor selectivity of the drugs. Chemotherapeutics used in the local chemotherapy of brain tumors are often released from their transport vehicles before they encounter cancer cells, leaving healthy noncancerous cells in the brain vulnerable to the effect of the free drug (Figure 10).

Current clinical studies can shed some light on where the field of brain tumor treatment is headed in the near future. One interesting study involves loading doxorubicin into bacterially derived 400-nm EDVTM nanocells, which target cancer cells through cell-surface-receptor binding, they are broken down to release doxorubicin within the cell. This technology has recently entered clinical trials and will be tested on glioblastoma patients.229 Another study focuses on treating metastasized breast cancer tumors in the brain. ANG1005 is a PTX-based peptide compound created from Angiochem’s Engineered Peptide Compound (EPiC), which targets the low-density lipoprotein receptor-related protein (LRP-1). LRP-1 is highly expressed on the BBB and tumor cells, making them ideal targets for chemotherapy drugs. In this study, systemically administered ANG1005 targets the brain cancer cells through LRP-1 binding and acts to kill the cells once inside.210 The field of brain tumor treatment still lacks an effective localized therapy. With time, it is expected that many innovative nanoscale systems and technologies will be developed and clinically tested. Improvement and optimization of the nanoparticle-based DDSs are needed to overcome the specific challenges for localized treatment, including sustainable release over a long period of time, deeper tumor penetration, overcoming drug resistance, targeting cancer stem cells, and so on. As the collective knowledge of brain cancer biology continues to evolve, more and more molecular targets will be identified to enable the specific killing of brain cancer cells with state-of-the-art nanotherapeutic systems.

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