Local delivery of antineoplastic agents using biodegradable polymers for the treatment of malignant brain tumors

Federico G Legnani, Gustavo Pradilla, Paul P Wang, Henry Brem, Alessandro Olivi and Francesco DiMeco[†]

The prognosis for patients diagnosed with malignant brain tumors has remained dismal despite advances in both neuroimaging and conventional treatment modalities. The use of biodegradable polymers for controlled local delivery of antineoplastic agents represents a major advance in the treatment of brain tumors. By implanting polymers loaded with chemotherapy agents directly onto the brain tumor resection bed, therapeutic doses of a drug can be administered intracranially for prolonged periods of time meaning high systemic doses associated with debilitating toxicities can be avoided. This technological advance has expanded the spectrum of available treatments for neoplasms of the CNS and has facilitated new approaches for the treatment of malignant brain tumors.

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CONTENTS

[†]*Author for correspondence*
Department of Neurological Surgery Istituto Nazionale Neurologico 'C. Besta',
Via Celoria 11,
Milano, Italy
Tel.: +39 348 913 7180
Fax: +39 348 7063 5017
fdimeco@bs.jhmi.edu

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Malignant brain tumors are the most commonly diagnosed primary tumors of the CNS. In the USA, approximately 16,800 people are diagnosed with primary brain tumors each year and 13,100 Americans die from these lesions [1]. Conventional treatment includes stereotactic biopsy and subsequent neuropathological diagnosis or surgical debulking of the accessible tumor followed by chemotherapy and radiation therapy [2–7]. Despite significant advances in neuroimaging, microneurosurgery and radiation therapy, the prognosis is dismal. The median survival after surgical resection alone is 6 months with only 7.5% of patients surviving for 2 years. Additional radiation therapy extends the median survival to 9 months, and systemic chemotherapy does not provide a significant impact on survival of patients [8,9].

There are significant obstacles to improving treatment because a large surgical resection or increments in radiation doses increase the risk of damaging functional brain areas that cause immediate morbidity and possible neurological deficits. Thus, improvement in chemotherapy and novel approaches are urgently needed to treat this devastating disease.

In this review, the authors describe the necessary steps to develop a novel approach in brain tumor therapy. The authors first focus on the unique issues of drug delivery into the brain. Biodegradable polymer technology is described and the safety and efficacy of this new brain tumor treatment strategy demonstrated in preclinical and clinical trials is summarized. Finally, future technologies to deliver antineoplastic agents are discussed.

Drug delivery rationale

The regulatory interface that mediates the restricted movement of substances between the bloodstream and the cerebral parenchyma is called the blood–brain barrier (BBB). The structural basis of the BBB resides in the continuous-type brain capillaries whose endothelial cells (ECs) display well-developed intercellular junctions. There are two properties of ECs responsible for the functional characteristics of the BBB: a very low rate of transcy-

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totic vesicular transport and highly electrically resistant tight junctions [10]. The low permeability of the BBB usually allows entry into the brain of only small hydrophobic molecules, a limited set of specifically transported nutrients, such as glucose and certain amino acids, and a few macromolecules [11]. In addition, small and large hydrophilic molecules can penetrate the brain by active transport [12]. Conventional chemotherapeutic agents are large, ionically charged or hydrophilic and do not fall into this category, making them difficult to transport into the CNS.

To overcome the intrinsic characteristics of the BBB and improve drug delivery to the brain, three different approaches have been followed. First, adapting to the natural permeability properties of the BBB, using more lipophilic variants of conventional chemotherapeutic agents, for instance, is theoretically possible and increases drug delivery. Clinical trials with systemic administration of lomustine and semustine, both lipophilic variants of carmustine (Gliadel®, Aventis, NJ, USA) 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU), which has shown an increase in survival of patients with malignant brain tumors, have not achieved better results than BCNU. Another variation of this approach is binding antineoplastic drugs to modified or unmodified carriers capable to overcome the BBB [13].

The second alternative to increase drug delivery to the brain is fenestrating the BBB. For example, William and colleagues examined the efficacy of the administration of carboplatin and etoposide after intra-arterial infusion of hyperosmolar mannitol in 34 patients with intracranial tumor. Unfortunately, despite a modest improvement in four patients with primitive neuroectodermical tumors (PNETs) and two patients with lymphoma, no benefits were observed in patients with glioblastoma multiforme (GBM), oligodendrogliomas and metastatic carcinomas [14].

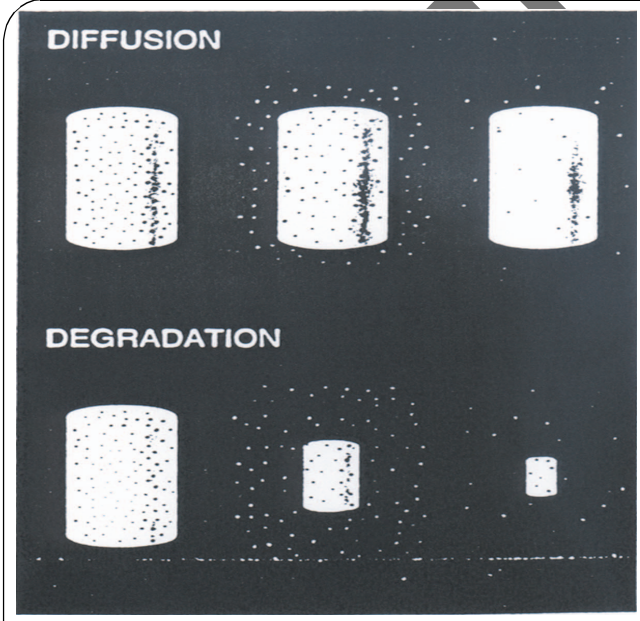

Figure 1. Desired properties of polymer implants include drug release by degradation instead of diffusion (Reprinted with permission [36]).

The third approach to overcome the limitations imposed by the BBB and improve drug delivery achieving very high local concentrations of antineoplastic agents and no systemic toxicity, is to develop a local delivery system capable of releasing drugs surgically placed in the tumor site in a sustained fashion. Polymer-mediated delivery of drugs provides a continuous release of active drugs which systemically would have a very short life. This technology has opened the door to the evaluation of new therapeutic agents for the treatment of brain tumors. Moreover, the fact that 80% of malignant gliomas recur within 2 cm of the original tumor site and that extra CNS metastases are exceedingly rare strengthen the rationale for strategies aiming at locally controlling this disease [15].

Development of a polymer-based local delivery treatment for brain tumors

Selection of a controlled-release polymer

Although many types of polymers are available for local delivery of drugs, only a few meet the criteria required for their use in clinical settings, such as absence of toxicity and sustained release of drugs with both high and low molecular weight. In 1976, Langer and Folkman described a diffusion-regulated polymer system capable of highly predictable and reproducible drug-release profiles [16]. Macromolecules incorporated in this nonbiodegradable ethylene-vinyl-copolymer (EVAc) diffuse through the micropores of its matrix with a diffusion rate depending on the pharmacological characteristic of the binded drug. This system is used in several clinical applications, such as glaucoma treatment, dental care prevention, contraception and chemotherapy but it has not been approved for clinical applications in the CNS. Its major limitation is that the empty polymer matrix remains at the site of implantation indefinitely, thus, theoretically requiring a follow-up surgery to remove the foreign body.

Conversely, a new generation of biodegradable polymer systems provides controlled drug delivery through a combination of drug diffusion and the gradual surface erosion of the matrix itself (FIGURE 1) [17]. Since these polymers degrade as they release the drug, surgical removal is not necessary. In 1985, Leong and colleagues formulated the development of the polyanhydride poly (1,3)-bis (carboxyphenoxy (propane-co-sebacic-acid) (PCPP:SA) matrix [18]. By spontaneous reaction with water-forming dicarboxylic acids, polyanhidride copolymer leads to the sustained release of the incorporated drug. In addition, it exhibits several important properties. First, an extremely hydrophobic nature protects chemotherapeutics agents incorporated into the matrix from hydrolysis and enzymatic degradation. Second, the steady rate degradable matrix allows drugs that would last only few minutes when systematically administrated, to be released in a biologically active form for long periods at a relatively steady concentration. The rate of PCPP:SA breakdown is regulated by varying the rate of PCPP and SA. For instance, a 1 mm disk of pure PCPP degrades in about 3 years and a PCPP:SA matrix obtained mixing 20% of CPP and 80% of SA of equal thickness has a 3-week biological life (FIGURE 2A)

[19]. Moreover, the polymer synthesis is performed at very low temperatures (-37°C) and under high pressure. These physical conditions allow it to be shaped into many configurations, such as, wafers, rods, sheets and microspheres facilitating surgical delivery (FIGURE 2B). The brain biocompatibility of this polymer has been confirmed through many studies in rats and rabbits [20–22]. The histological evaluation of the implantation sites revealed transient and minimal inflammatory signs similar to the reactions elicited by common hemostatic implants, such as oxidized cellulose (Surgicel®) and gelatin sponges (Gelfoam®, Pharmacia & Upjohn, USA). Finally, PCPP:SA polymers were implanted into the frontal lobe of cynomolgus monkeys (*Macaca fascicularis)*. No signs of behavioral or neurological deterioration nor hematological changes were detected in these animals after the polymer implantation [23]. Postmortem analysis of the brains of these animals showed only localized reaction at the site of implantation.

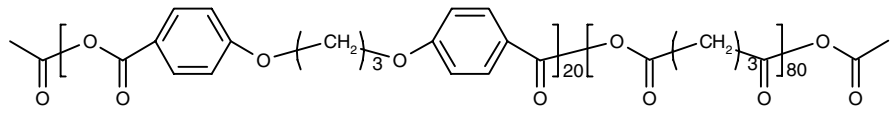

Figure 2A. Chemical structures of PCPP:SA.
PCPP: 1,3-bis [carboxyphenoxy] propane; SA: Sebacic acid

Another biodegradable polymer has been proven to improve the spectrum of deliverable drugs. To release hydrophilic compounds that can not be delivered by PCPP:SA, a fatty acid dimer:sebacic acid (FAD:SA) copolymer system has been developed [24]. Similarly to PCPP:SA, this system can be shaped into any configuration and its release kinetics can be manipulated by varying the ratio of FAD to SA. Its ability to release both proteins and antineoplastic agents has been assessed in rats and was found to be comparable with that of PCPP:SA [25,26].

A widely used type of biodegradable polymer capable of releasing drug by combining degradation and diffusion is polylactic-co-glycolic-acid (PLGA). Its biocompatibility was fully demonstrated initially with polymer fashioned into sutures and confirmed in the rat brain [27–29]. These polymers, often shaped into injectable microspheres have been successfully used to deliver anti-inflammatory agents, narcotics, antibiotics, anesthetic and antineoplastic agents [30–34]. In addition, a microsphere variant has been introduced that can be stereotactically implanted into the brain [35]. Although PLGA polymers remain an interesting alternative, their drug release by bulk erosion (like a sugar cube) can result in a sporadic release of drug leading to a suboptimal tissue exposure [36].

Preclinical studies

The first clinical trials for the treatment of brain tumors using a local delivery system were conducted during the 1970s. There were 14 patients with both malignant gliomas and metastatic diseases who were treated intracranially with 5-fluorouracil (5-FU) and urochinase loaded into silastic tubes [37]. Out of 14 patients, 13 survived for more than 8 months after implantation. Additional clinical trials using silastic tubes loaded with a chemotherapy cocktail containing 250 mg 5-FU, 6000 IU urokinase, 1.5 mg mitomycin C and 250 mg bromodeoxyuridine demonstrated a medain survival of 18 months for patients with malignant gliomas, with a 3-year survival rate of 16% [38,39]. Tumoricidal levels of 5-FU were measured as long as 2 years after implantation. In 1986, Kubo and colleagues incorporated 5-FU, adriamycin and mitomycin into a matrix consisting in glassified monomers with 10% polymetacrylic methyl and tested it on 55 patients. A 47% 1-year survival rate in patients with malignant gliomas was noted [40].

The first chemotherapeutical agent that was used to test the real efficacy of drug delivery to the brain released by polyanhydride polymers was BCNU. The well-known alkylating activity against the nitrogen bases of DNA, its existing use as a systemic agent to treat brain tumors and its low molecular weight and liposolubility theoretically allow it to cross the BBB at potentially tumoricidal concentrations. Unfortunately systemic administration of BCNU is limited by toxicity, especially bone marrow suppression and pulmonary fibrosis and by a very short serum half-life (only 15 min). Clinical trials with systemic administration of BCNU have shown only a very modest increase in survival. To increase its effectiveness and to limit the side effects, BCNU was incorporated in polymers and tested for treatment of malignant brain tumors.

The first set of preclinical experiments analyzed the *in vivo* release kinetics. Grosmann and colleagues compared the biodistribution of BCNU delivered by PCPP:SA and direct stereotactic injection [41]. Radioactively labeled BCNU was delivered and its distribution was assessed by quantitative autoradiography in brain sections. This study demonstrated that a drug released from the polymer had more extensive distribution which lasted for longer periods of time. Tissue concentrations of BCNU in the ipsilateral hemisphere, 21 days after implanting the polymers, were increased by two standard deviations when compared with controls while no detectable levels were observed after 72 h post direct injection. High performance liquid chromatography (HPLC) was used to confirm that the radioactivity was associated with active BCNU. Other kinetic studies were performed in cynomologus monkeys showing that tumoricidal concentrations of BCNU released intracranially by 20% loaded PCPP:SA polymer were found at 4 cm from the implantation site at 24-h, at 2 cm on day 7 and at 1.3 cm on day 30 [42]. PCPP:SA polymers were implanted on the left frontal lobe where a small cavity was developed by gentle suction of the cortex.

The second set of experiments investigated the efficacy of BCNU-loaded polymers. Tamargo and colleagues implanted a 9L gliosarcoma tumor subcutaneously and orthotopically into the brain of Fischer 344 rats demonstrating a statistically significant improvement in survival in animals treated with BCNU polymers compared with animals treated with empty polymers or intraperitoneal injections of BCNU [43]. In addition, local

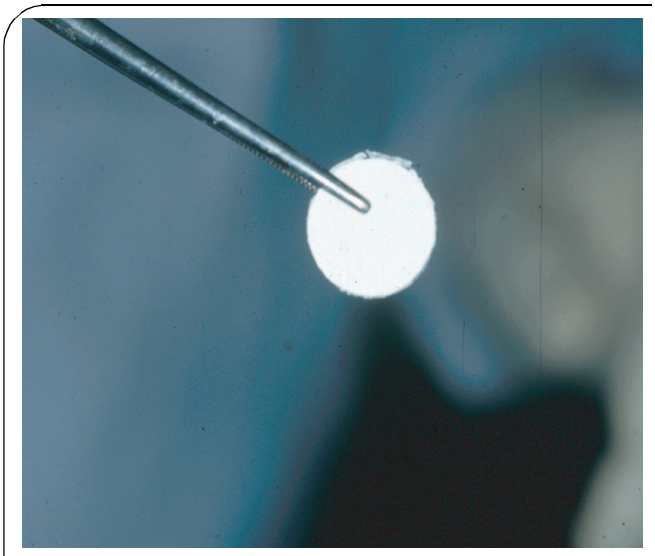

Figure 2B. Macroscopic aspect of a PCPP:SA polymer (Gliadel).
PCPP: 1,3-bis [carboxyphenoxy] propane ; SA: Sebacic acid.

delivery of BCNU resulted in 17% long-term survivors, while no long-term survivors were observed among systemically treated animals.

The third set of preclinical experiments investigated the efficacy of local delivery of various antineoplastic agents, including BCNU, for the treatment of brain metastases in several mouse metastatic models [44]. Tumor cell lines included B16 melanoma, RENCA renal cell carcinoma, CT 26 colon cancer and Lewis lung carcinoma all of which were tested with encouraging results. These findings led to clinical trials of BCNU-loaded PCPP:SA for the treatment of metastatic brain tumors.

Clinical trials

Biocompatibility, biodistribution and efficacy studies mentioned demonstrated that the BCNU–PCPP:SA polymers are biocompatible, nontoxic, capable of releasing tumoricidal quantities of a chemotherapeutic agent for a long time and effective in increasing the survival rate in animals with long-term survivors. This evidence constituted the basis for the subsequent translation of this work into the clinical arena.

Treatment of recurrent malignant brain tumors

The safety of implanting 20:80 PCPP:SA BCNU-loaded polymers wafers into the human brain has been assessed with a Phase I–II clinical trial. In 1987, 21 patients affected by recurrent malignant glioma that had previously undergone surgical debulking and in whom conventional therapy had failed, were enrolled at five institutions in the USA [45]. The admission criteria were: indication for reoperation, a unilateral single focus of tumor in the cerebral cortex with an enhancing volume of at least 1.5 cm^3 on CT scan, a Karnofsky Performance Scale (KPF) equal or greater than 60, previous radiation therapy, no chemotherapy within 1-month and no nitrosureas within 6 weeks of enrollment. In these patients, after maximum debulking, the resection cavity was lined with up to eight BCNU-loaded wafers (FIGURES 3A & B). There were three different formulations of loaded polymers studied: 1.93, 3.85 and 6.35% yielding 3.85, 7.7 and 12.7mg of BCNU, respectively; each polymer weight 200 mg. Out of 21 patients, 11 received the highest BCNU-wafer dose (102 mg), five the intermediate concentration (62 mg), and five the lowest concentration (31 mg). There was no evidence of systemic toxicity nor signs of neurological deterioration. Blood chemistry and urinalysis tests did not show signs of bone marrow, hepatic or renal injury. Postoperative T-1 weighted MRIs displayed the implanted polymer as areas of decreased signal and CT scans were able to detect in 13 out of the 21 patients, a thin layer of contrast-enhancing ring surrounding the wafer. These changes were detectable only up to 7 weeks from the time of surgery and there was no correlation between this signs and neurological decline. The overall median survival time was 46 weeks after polymer implantation and 87 weeks after initial diagnosis. Only eight patients (38%) survived more than 1-year.

These encouraging results assessed safety and efficacy on survival improvement of local delivery of 20:80 BCNU-loaded polymers and layed the bases for a Phase III clinical trial. A multicenter, prospective, randomized, double-blinded, placebo-controlled trial was carried out to evaluate if 3.85% PCPP:SA-BCNU wafers could reduce the 6-month mortality period in patients with recurrent malignant glioma [46]. In 27 medical centers in the USA and Canada, a total of 222 patients were enrolled and randomly divided into two equal groups for all known prognostic factors (e.g., age, KPF score, prior treatment, time from first surgery and histological grade). The admission criteria were similar to those used for Phase I–II studies except that no chemotherapy was allowed for 4 weeks before surgical intervention and systemic chemotherapy was allowed as early as 2 weeks after surgery. After 6 months, the data from the last patients were collected and the code was broken: 110 patients had received the BCNU wafers and the other 112 patients had received placebo. The overall postoperative median survival was 31 weeks for the BCNU group and 23 weeks for the placebo group (hazard ratio = 0.67; p = 0.006) (FIGURE 4). The 6-month survival rate was 60% in the active group and 47% in the placebo group. Moreover, in patients (n = 145) who underwent surgery for glioblastoma, the 6-month survival rate was 50% greater in the BCNU-loaded polymer group compared with placebo (p = 0.02). Again, neither remarkable bone marrow suppression signs nor systemic or neurological toxicity were shown. There was not a statistically significant difference regarding the incidence of either seizures or intracranial infections after wafer implantation.

In 1996, this evidence, together with previous findings, led to the approval by the Food and Drug Administration (FDA) of 3.85% BCNU-loaded PCPP:SA wafer Gliadel®, (Aventis Pharmaceuticals Inc., NJ, USA) for the treatment of recurrent glioblastoma. This represents the first new treatment approved by the FDA in 23 years and has received regulatory approval in 21 countries.

Treatment for newly diagnosed malignant gliomas

Based on the success of the Phase III clinical trial for the palliative treatment of recurrent glioblastoma multiforme, 3.85% BCNU-loaded PCPP:SA polymer has been hypothesized to be even of greater efficacy and safety for initial treatment of malignant brain tumors. To prove this hypothesis, a Phase I-II study was carried out and 22 patients underwent surgical debulking and placement of up to eight wafers [47]. Inclusion criteria were: unilateral tumors focus greater than 1 cm^3, age over 18 years, KPS 0.60 or greater. All the patients received external beam radiation therapy (5000 rads). MS was 44 weeks for the treatment group with four patients surviving more than 18 months. The mentioned study demonstrates Gliadel safety and efficacy in conjunction with radiation beam therapy for treatment of newly diagnosed malignant brain tumors.

The encouraging results obtained with the Phase I–II studies prompted a Phase III randomized placebo-controlled clinical trial [48]. Originally planned for 100 patients, the study was interrupted for temporary unavailability of the drug with 32 patients. Admission criteria were similar to the Phase I–II study except that a histopathological diagnosis of grade III astrocytoma or GBM was performed on intraoperative frozen sections. Out of 32 patients, 27 affected by glioblastoma and five by anaplastic astrocytoma were randomized to receive either placebo or BCNU wafers (61.6 mg of BCNU) and after maximum surgical debulking underwent radiation therapy. The median age was 55.5 years for the BCNU group and 53 years for placebo. The median KPS score was 75 for the active group and 90 in the control group. The median survival was 58.1 weeks for the active arm of the study and 39.9 weeks for the placebo arm ($p = 0.012$). The subgroup of GBM patients had a median survival of 53.1 weeks when treated with BCNU wafers compared with 39.9 weeks when treated with placebo ($p = 0.0083$). The 1-, 2- and 3-year survival rates were 63, 31 and 25% for the BCNU group compared with 19, 6 and 6% for the placebo group, respectively. Again no systemic or local signs of deterioration attributable to the polymer were detected.

The definitive role of Gliadel in initial therapy has been assessed with a large Phase IV, multicenter, randomized, double-blinded, placebo-controlled clinical trial. At 38 centers in 14 countries, a total of 240 adult patients underwent initial surgical resection for a high-grade malignant glioma. In the surgical cavity either BCNU wafers or placebo wafers were placed followed by radiation beam therapy at approximately 2 weeks. The primary goal of this trial was survival. A follow-up of up to 48 months was obtained in 239 patients. The median survival was 59.6 weeks for the treatment group compared with 49.7 weeks for the placebo group ($p < 0.05$). In total, 11 patients survived longer than 48 months, nine of which received Gliadel wafers. The overall risk of dying during 3–4 years after treatment was reduced in the Gliadel wafer treatment group, as reflected by a hazard ratio of 0.73 (95% confidence interval [CI] 0.56–0.95; $p < 0.05$) [49]. Based on these results, on February 26, 2003, the FDA approved Gliadel for use in newly diagnosed patients with high-grade malignant glioma as an adjunct to surgery and radiation.

Recently, laboratory experiments in rats and monkeys demonstrated that the efficacy of Gliadel wafers can be improved simply by increasing the BCNU loading dose [50]. Based upon these encouraging preclinical results, the author's group led a National Institute of Health (NIH)-funded dose escalation trial at 11 medical centers in the USA to evaluate the safety of Gliadel wafers containing between 6.5 and 20% BCNU in patients with recurrent malignant brain tumors. This Phase I–II dose-escalation study established that the maximal nontoxic PCPP:SA-BCNU loading dose is equal to 20% [51]. A Phase III clinical trial will follow to evaluate the efficacy of the highest-tolerated BCNU loading dose.

Other chemotherapeutic agents

Local drug delivery has renewed interest in chemotherapeutic agents shown to be safe and efficacious for the treatment of other tumors but that have never been used for malignant gliomas because of brain-barrier impermeability or systemic toxicity. The following agents have been incorporated into polymers and used to treat experimental brain tumors.

O6-Benzylguanine (O^6-BG)

AGT, a DNA-repair protein, is responsible for most of the resistance seen in malignant brain tumor cells against BCNU

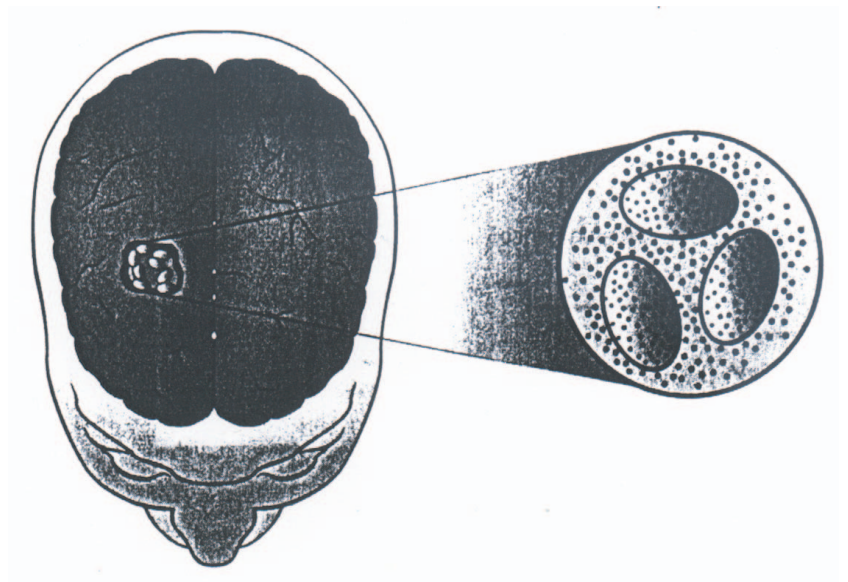

Figure 3A. Up to eight polymer implants line the tumor resection cavity, where the loaded drug is gradually released as they dissolve.
The inset shows conceptually how drug molecules diffuse away from these implants. (Reprinted with permission) [36].

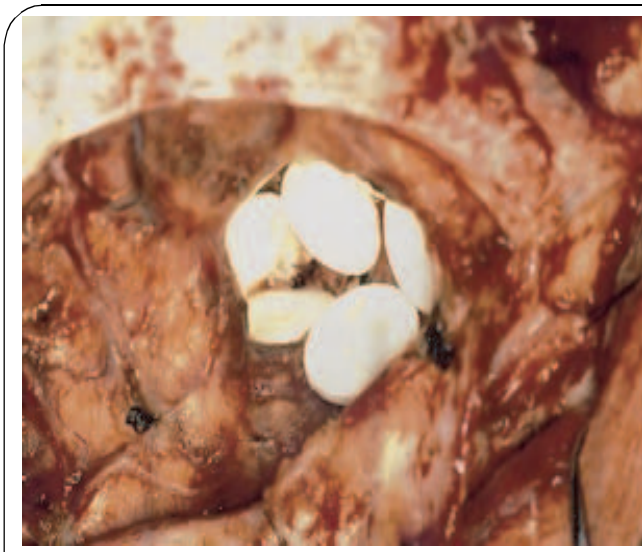

Figure 3B. Intraoperative picture showing the resection cavity lined with Gliadel.

[52]. O^6-BG is capable of irreversibly inactivate AGT [53]. By combining locally delivered BCNU with O^6-BG, tumor resistance to BCNU could be avoided in theory. Rhines and colleagues combined systemic O^6-BG treatment with intracranial locally delivered BCNU polymers [54]. Using the established intracranial F98 rat glioma model, an improvement in median survival was observed (34 days) in animals receiving combination therapy as compared with animals receiving O^6-BG alone (22 days; $p = 0.0002$) or BCNU polymer alone (25 days; $p = 0.0001$).

These results suggested that concomitant use of O^6-BG and BCNU polymers may be an important addition to the treatment of malignant brain tumors. Therefore, a Phase I clinical trial was carried out and a dose-escalation study is currently under way.

Taxol®

Taxol (paclitaxel, Bristol-Myers Squib Co., NY, USA) is a microtubule stabilizer with potent antineoplastic activity against glioblastoma cell cultures and several human tumors [55]. Its utility is limited because it does not penetrate the BBB [56]. This makes it an ideal drug for local delivery to brain tumors via sustained-release polymers. Walter and colleagues incorporated Taxol into 40 and 20% loaded PCPP:SA polymers and showed sustained release up to 1000 h [57]. *In vivo* biodistribution studies revealed tumoricidal concentrations of Taxol for more than 30 days up to 5 cm from the site of implantation. In the 9l gliosarcoma model local delivery of Taxol increased rat survival two- to three-folds (38 days MS with 40% Taxol and 61.5 days with 20% Taxol vs. 19.5 days with placebo).

4-hydroxyperoxycyclophosphamide (4-HC)

4-HC the *in vivo* active derivative of cyclophosphamide, has been incorporated into a FAD:SA polymer and used for local drug delivery to the brain [26,58]. *In vitro* and *in vivo* pharmacokinetic experiments demonstrated both favorable release and biodistribution [59]. In the rat brain, after establishing a maximum tolerated dose (MTD) of 20%, efficacy was tested comparing survival in the 9l gliosarcoma model. Animals treated with an empty polymer had a median survival of 14 days with no long-term survivors, while animals treated with 4-HC-loaded polymers had a median survival of 77 days with 40% surviving beyond 80 days ($p = 0.004$).

5-Fluorouracil (5-FU)

5-FU is a tymidine analog that both normal and tumor cells metabolize to 5-fluoro-2′-deoxyuridine monophosphate (FdUMP) and 5-fluorouridine triphosphate (FUTP). These metabolites cause cell injury by two different mechanisms. First, FdUMP and the folate cofactor, N 5-10-methylenetetrahydrofolate, bind to thymidylate synthase (TS) to form a covalently bound ternary complex. This binding inhibits the formation of thymidylate from 2′-deoxyuridylate. Thymidylate is the necessary precursor of thymidine triphosphate, which is essential for the synthesis of DNA, so that a deficiency of this compound can inhibit cell division. Second, nuclear transcriptional enzymes can mistakenly incorporate FUTP in place of uridine triphosphate (UTP) during the synthesis of RNA. This metabolic error can interfere with RNA processing and protein synthesis. While this drug has been used for the treatment of several malignancies, it has not been considered for the adjuvant treatment of malignant brain tumors due to its inability to cross the BBB and its systemic toxicity which includes myelosuppression and gastrointestinal mucosal injury [60]. Nevertheless, 5-FU exhibits little neurotoxicity making it an appealing candidate for local treatment of malignant brain tumors.

In 1995, 5-FU was successfully incorporated into PLGA polymers revealing good pharmacokinetic profiles [61]. When used against the intracranial C6 rat glioma model, treatment with 5-FU-loaded microspheres demonstrated a significant decrease in mortality without toxicity [62].

Subsequently, in 1999, Menei and colleagues treated eight patients with newly diagnosed malignant brain tumor with 5-FU-loaded PLGA microspheres following surgical debulking and external beam therapy [63,64]. This pilot clinical trial demonstrated clinically relevant concentrations of 5-FU in the CSF up to 1-month after surgical implantation and a significant median survival (98 weeks). At the time of the publication two patients exhibited disease remission at 139 and 153 weeks.

Adriamycin®

Adriamycin (doxorubicin hydrochloride, Pharmacia Italia SPA, Milan, Italy) is a cytotoxic anthracycline antibiotic that seems to act by nucleotide base intercalation and cell membrane lipid binding. Intercalation inhibits nucleotide replication and activity of DNA and RNA polymerases. Its tumoricidal activity has been documented in lymphoma, leukemia breast cancer and other malignancies [60]. It has been successfully incorporated into EVAc polymers demonstrating significant brain tumor

growth inhibition in nude mice [65]. Adriamycin has also been incorporated into PCPP:SA polymers demonstrating an improvement in median survival (33 vs. 13 days in control; $p < 0.0006$) in the intracranial 9l gliosarcoma model [66].

Platinum drugs

Platinum drugs, which exert antitumor effects by binding to DNA and producing interstrand crosslinks, are promising alternatives in the treatment of CNS tumors including malignant glioma, medulloblastoma, optic pathway glioma, brainstem glioma and ependymoma [67]. Unfortunately, the systemic use of platinum-based drugs is limited by marked toxicity, particularly hematologic [68]. Furthermore, being water soluble, these drugs hardly cross the BBB. Hence, controlled release polymers constitute a good tool to overcome those limitations and exploit the platinum drugs antitumor potential. Among all platinum derivatives, carboplatin (Paraplatin®, Bristol-Myers Squibb Co., NY USA) is the most suitable for local treatment of brain malignancies because it is effective *in vitro* against CNS tumors and when delivered directly to the CNS, exhibits less neurotoxicity than other platinum-based compounds [69,70]. Thus, the authors have optimized the delivery of carboplatin by incorporating it into both the FAD:SA and PCPP:SA biodegradable polymers, obtaining sustained release [71]. The authors have also developed a method to encapsulate carboplatin into ethylcellulose microcapsules that offers the possibility of stereotactical injections when surgical resection is not indicated [72]. The authors found that local delivery of carboplatin via either polymers or microcapsules is safe and highly effective against F98 gliomas in rats [71]. Based on these promising findings, current studies are underway in order to assess safety and toxicity of carboplatin based polymers in primates.

Camptothecin

Camptothecins are a chemotherapeutic family interacting specifically with the enzyme topoisomerase I which relieves torsional strain in DNA by inducing reversible single-strand breaks [73]. Clinical trials found severe systemic toxicity preventing its use as a systemic agent for the treatment of malignant gliomas [74]. Due to the encouraging *in vitro* results against brain tumor cell lines, camptothecin was incorporated into polymers for local delivery to the brain. Weingart and colleagues loaded 50% EVAc polymers with sodium camptothecin and tested its efficacy in the intracranial 9l model showing a very significant extension in median survival [75]. Rats treated with local delivery of sodium camptothecin survived for more

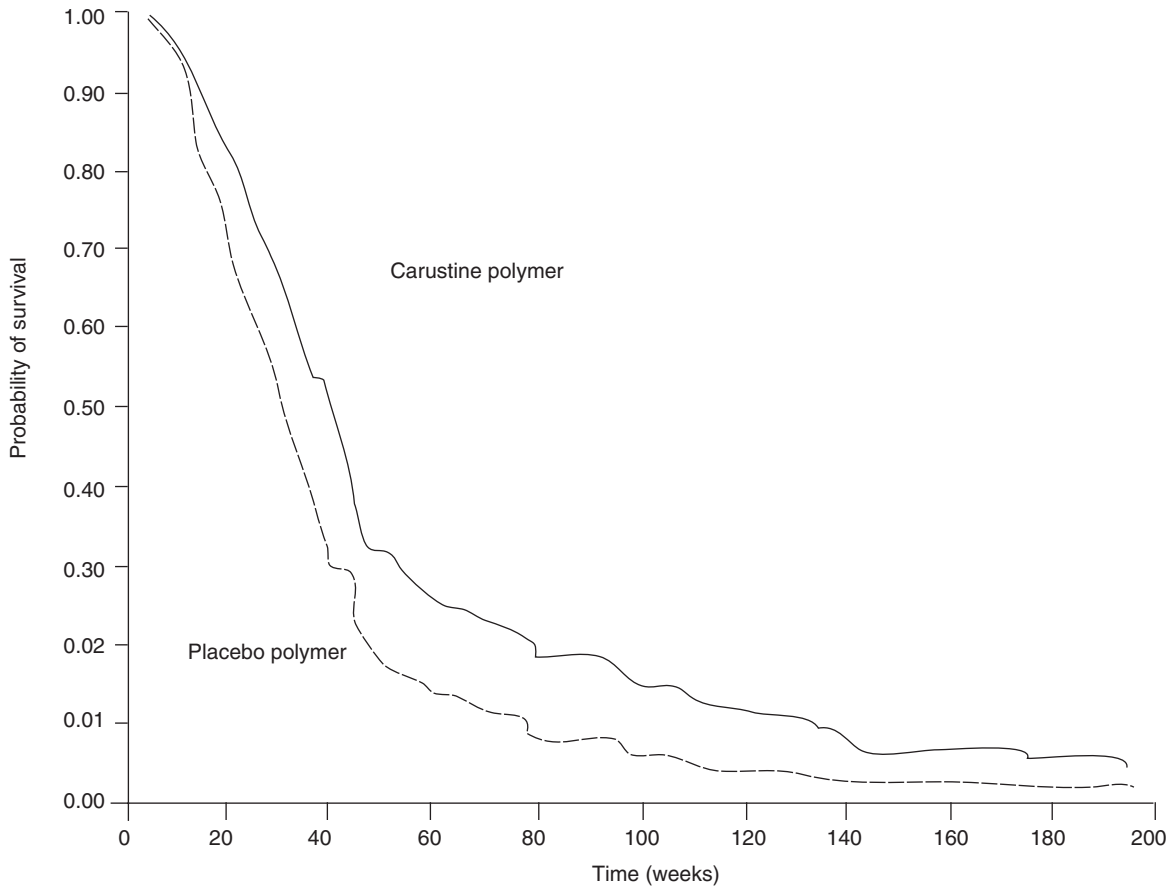

Figure 4. Overall survival for patients receiving BCNU-loaded polymers versus controls at the time of the operation for recurren t malignant gliomas after adjustment for prognostic factors. (Reprinted with permission) [46].
BCNU: 1,3-bis(2-chloroethyl)-1-nitrosourea.

than 120 days compared with a mean 19 day survival in controls ($p < 0.001$). Furthermore, none of the controls survived beyond 32 days but, 59% of treated animals survived for more than 120 days. Recently, sodium camptothecin has been loaded into 50% PCPP:SA polymer and evaluated against established intracranial 9l gliosarcoma in rats. In this study, median survival was 69 days in the treatment arm versus 17 days in the controls. No local or systemic toxicity was noted in any of the animals. Currently a variety of potent camptothecin derivates are being prepared for future clinical trials.

Mitoxantrone

Mitoxantrone is a DNA-reactive agent that intercalates into DNA through hydrogen binding and causes crosslinks and strand breaks. Mitoxantrone also interferes with RNA and is a potent inhibitor of topoisomerase II, an enzyme responsible for uncoiling and repairing damaged DNA. This drug is used in the treatment of advanced breast cancer, nonHodgkin lymphoma, acute nonlymphoblastic leukemia and chronic myelogenous leukemia in blast crisis and has been approved for clinical use in hepatic and ovarian cancer. It has also been identified as one of the most potent drugs against malignant glioma cell lines *in vitro*. Systemic administration of mitoxantrone for the treatment of malignant brain tumors, however, has been hampered by poor CNS penetration, bone marrow suppression and myocardial toxicity. Therefore, we incorporated mitoxantrone into PCPP:SA polymers and determined its release kinetics, toxicity, biodistribution and efficacy for the treatment of experimental brain tumors using the 9l gliosarcoma model in rats [76]. We demonstrated tumoricidal drug concentrations in the brain for more than 35 days and a significant improvement in survival. The combined median survival for each treatment group was: controls, median survival = 19 days; 1%-wafers, median survival = 30 days ($p < 0.0001$); 5%-wafers, median survival = 34 days ($p < 0.0001$); 10%-wafers, median survival = 50 days ($p < 0.0001$). This study has demonstrated that MIT delivered in biodegradable wafers is successful in the treatment of malignant gliomas in rodents and has strong potential for future clinical applications.

Others agents

Angiogenesis inhibitors

Angiogenesis is a complex process involving endothelial cell proliferation and migration, with a parallel remodeling of the extracellular matrix (ECM) that results in the development of new blood vessels [77]. The development of new blood vessels is fundamentally required for tumors to expand and it also facilitates metastatic spreading. Avascular tumors grow slowly until they reach an equilibrium at which the rate of cell proliferation at the periphery is equal to the rate of cell death in the center of the tumor [78]. Once new vessels form, nutrients can reach the central cells and the tumor growth becomes exponential. GBM is one of the most angiogenic of all tumors and its treatment with antiangiogenic factors generated wide interest.

Early experimental studies with local delivery of angiogenesis inhibitors have been carried out with a combination of heparin and cortisone coreleased by nonbiodegradable EVAc polymers [79,80]. This combination reduced VX-2 carcinoma-induced neovascularization in the rabbit cornea assay. In the same study, local sustained release of cortisone and heparin by PCPP:SA polymers was effective against tumor growth in flank-implanted 9l gliosarcoma.

A second antiangiogenic factor, minocycline, is a semi-syntetic tetracycline with anticollagenase properties. Tamargo and colleagues demonstrated that local delivery of minocycline by controlled-release EVAc polymers has been effective in inhibiting the tumor-induced neovascularization in the rabbit cornea VX-2 carcinoma model with angiogenesis indexes of 4.5 4.4 and 2.9 at days 7, 14 and 21, respectively [81]. Minocycline was also capable of extending survival of rats challenged intracranially with 9l gliosarcoma cells. Local treatment with minocyline also demonstrated synergistic properties with the systemic administration of BCNU.

A novel aminosterol, squalamine, was shown to be effective in angiogenesis inhibition by blocking mitogen-induced proliferation and migration of endothelial cells in the rabbit cornea VX-2 tumor model [82]. Currently, squalamine is involved in a Phase I clinical trial to evaluate its efficacy in patients with advanced cancers [83].

Endostatin is a 20 kDa proteolytic fragment of collagen XVIII with potent antiangiogenic activity [84]. It was first isolated from conditioned media of nonmetastatic murine hemangioendothelioma and appears to inhibit angiogenesis and tumor growth by inhibiting proliferation and migration of endothelial cells through blocking the activity of proteolytic enzymes, particularly matrix metalloproteinase (MMP)-2 produced by endothelial cells [85,86]. Antiangiogenic activity of endostatin has been demonstrated in animal models of malignant gliomas but production of human recombinant endostatin is an expensive and difficult process [87]. A synthetic fragment of endostatin is currently being tested for antiangiogenic activity by the author's group and others and might constitute the best alternative for endostatin therapy.

Immunotherapy

Another approach to malignant brain tumor therapy is the development of a treatment strategy that specifically targets tumor cells using the immune response of the host. To participate and maintain this immune response, white blood cells (WBC) produce cytokines, such as interleukins (IL), interferons (IFN) and colony-stimulating factors. Several cytokines have demonstrated an antineoplastic effect *in vivo*. For example, IL-2 is FDA approved for systemic treatment of patients with metastatic renal cancer. Given the rationale for using local chemotherapy to treat brain tumors, as well as considering the notion that cytokines exert their immunomodulatory activity in a paracrine fashion, the authors hypothesized that local cytokine-based immunotherapy could be used to control tumor growth in the brain.

The first strategy pursued was to deliver cytokines to the CNS bypassing the BBB and providing high concentrations of immunoregulatory factors at the site of the tumor, a process called *ex vivo* gene transfer. It consists in tumor cells transfected to secrete cytokines in a paracrine fashion. In Legnani and colleagues' laboratory this strategy was proven to be effective in treating established brain tumors [88,89]. Although efficacious in animal models, genetically modifying cells may constitute and obstacle in the treatment of human brain tumors. In consequence, the authors used controlled-release technology to achieve paracrine cytokine production at the tumor site. The authors tested the ability of IL-2 to induce and maintain an immune response against metastatic and primary brain tumor models. By employing a coacervation procedure a bead matrix (microspheres [MS]) consisting of gelatin and chondroitin sulfate was produced. The MS produced have been precisely characterized and optimized for reproducibility with respect to size (15 µm mass-average diameter), encapsulation efficiency (85–90%) and cytokine release (over 2 weeks *in vitro*). The efficacy of local IL-2 therapy from gelatin chondroitin sulfate MS has been tested extensively in the treatment of brain tumors in rodents. IL-2 MS were highly effective in the treatment of B16-melanoma metastases to the brain in mice and 9l gliosarcoma in rats [90]. Histological analysis of the tumor site in animals receiving IL-2 MS showed massive necrosis of tumor with associated lymphocyte infiltrate surrounding distinct IL-2 polymer spheres. Immunohistochemistry showed that the lymphocyte infiltrate included $CD4^+$ and $CD8^+$ T-cells, naturalkiller cells and polymorphonuclear cells. On the contrary, placebo MS were inert in the brain. Further laboratory studies identified strong antiangiogenic and immunomodulatory properties of IL-12 making it a suitable candidate for local paracrine delivery. The authors implanted IL-12-trasduced rat 9l gliosarcoma cells intracranially. *In vivo* expression of IL-12 was confirmed by reverse transcriptase polymerase chain reaction (RT-PCR). In this study, local paracrine delivery of IL-12 in addition to prolonging survival also induced immunological memory in the animals [91]. In this study the authors proved that a second injection of wild type 9l gliosarcoma tumor cells elicited an immune response.

Since intracranial chemotherapy cannot reach all residual tumor cells after surgical debulking in patients with malignant gliomas, the authors hypothesized that the combination of paracrine immunotherapy and local delivery of chemotherapy by biodegradable polymers may provide a synergistic antitumor response. Sampath and colleagues demonstrated that engineered tumor cells to produce IL-2 and local delivery of 10% BCNU-PCPP:SA polymer produced a synergistic increment in survival in mice challenged intracranially with a lethal dose of B16-F10 tumor cells [92]. Histological examination of animals treated with combined therapy at day 14 revealed rare degenerating tumor cells with marked mixed chronic inflammation, no tumor cells and resolution of the inflammatory reaction by day 72.

Finally, the authors displayed a synergistic effect by local delivery of BCNU wafers and IL-2 microspheres in the treatment of 9L gliosarcoma in rats [93]. In this study, the median survival was 28.5 days in the group receiving IL-2 median survival and 3.8% BCNU polymer and 45.5 days with IL-2 MS combined with 10% BCNU polymer. Median survival of animals receiving monotherapy with IL-2 microspheres or 3.8% BCNU polymer or 10% BCNU was 24 days and 32.5 days. Control animals had a median survival of 18 days. The combination of 3.8 or 10% BCNU polymer with IL-2 MS resulted in 7 versus 25% long-term survivors. Continued research on this field is underway.

Expert opinion

Local drug delivery using biodegradable polymers has significantly improved the treatment of malignant brain tumors. Gliadel represents not only the first successful drug developed from this technology but also the first new treatment approved by the FDA for recurrent and newly diagnosed malignant gliomas in 23 years. It provides an effective means of bypassing the BBB delivering tumoricidal amounts of antineoplastic agents in the tumor site minimizing systemic toxicity. Moreover, the success achieved in the preclinical and clinical studies with BCNU-loaded polymers validates the concept that biodegradable polymers represent an effective vehicle for several other drugs otherwise not suitable to treat brain tumors. This can be considered, in our opinion, the major advancement provided by this strategy, which has undoubtedly paved the way for new avenues in the field of glioma therapy. New classes of therapeutic agents, such as antiangiogenic agents, immunotoxins and antisense nucleotides are all amenable to local delivery to the brain with controlled-release polymers. In addition, this new approach has the potential to improve quality of life for

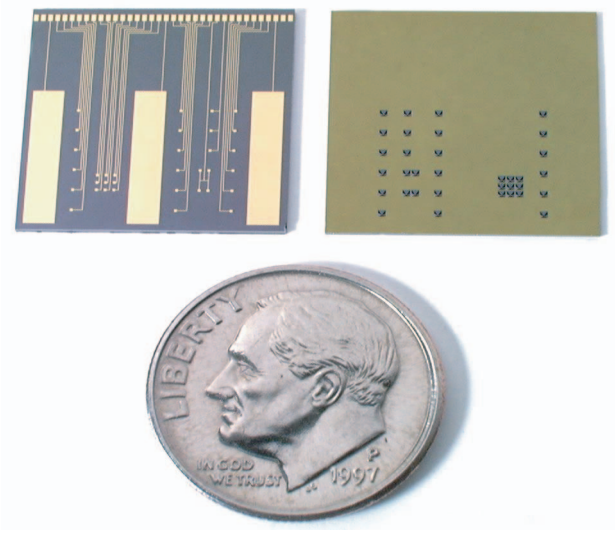

Figure 5A. Microchip with dime caption.
Front (left) and back views of a new microchip for controlled local release of chemicals. The dots between the three large bars (cathodes) on the front are the caps (anodes) covering the reservoirs holding the chemicals. Electrical voltage applied between the cap and cathode causes a reaction that dissolves the cap, thus releasing the reservoir's contents. The back view shows the larger openings through which the contents of the reservoirs are deposited. (These openings are sealed after filling). Photo by Paul Horwitz, Atlantic Photo Service Inc., USA.

patients when combined with steroids that minimize brain edema and anticonvulsants that control seizure activity. Finally, the success achieved by the polymeric strategy has put big emphasis on any sort of therapy aiming at achieving a local control of disease. Consequently, new local delivery technologies, such as convection-enhanced delivery system and silicon microchips are the object of ongoing studies holding considerable promise for the field of neurosurgery in general and for the treatment of patients with malignant gliomas in particular.

Five-year view

Local delivery of antineoplastic agents using biodegradable polymer is an established therapeutic technology capable of increasing survival and quality of life in patients with malignant brain tumors. Active research in the development of other exiting approaches to achieve sustained drug delivery to the brain is currently ongoing.

An area of interest involves convection-enhanced delivery system. Convection results from a simple pressure gradient and is independent of molecular weight. When a drug is infused into the cerebral white matter creates a pressure gradient that increases convection and can be used to deliver high concentrations of drugs to large regions of the brain without structural or functional side effects [94–96]. Primate trials have been conducted using convection-enhanced drug delivery to treat Parkinsonian symptoms [97]. Convection-enhanced delivery may offer another important way of delivering chemotherapeutic agents to surgically inaccessible brain tumors.

A novel and potentially powerful method of drug delivery involves the use of newly developed microchips [98]. This technology depends on a solid state silicon microchip that can provide controlled release of single or multiple chemical agents (FIGURE 5A). The release is based on the electrochemical dissolution of a thin anode membrane covering multiple microreservoirs which can be filled with solids, liquids or gels. The releasing time of each reservoir is scheduled to deliver its content and can be programmed independently. The device is an integrated circuit providing its own battery, memory and multiplexing circuitry. It can be mounted on a tip of a small probe, surgically implanted and swallowed. Active investigation is also ongoing developing a biodegradable passive chip system (FIGURE 5B) in which the release mechanism of each reservoir is based on slow degradation of a thin polymeric membrane covering each drug reservoir. With proper selection of a biocompatible device material, this pharmacy-on-chip may be used to deliver up to 1000 different drugs on demand.

Therefore, in the near future, when a patient with a malignant brain tumor undergoes surgical debulking, a microchip will be programmed and loaded with a combination of chemotherapy tailored to the intraoperative frozen pathology diagnosis. Other wells of the microchip could be loaded with steroids to treat brain edema or anticonvulsants to treat seizures. Portions of the tumor could be irradiated and either directly placed in the tumor site with cytokine-loaded microspheres, or loaded into other wells of the microchip, along with cytokines. Local and controlled drug delivery to the

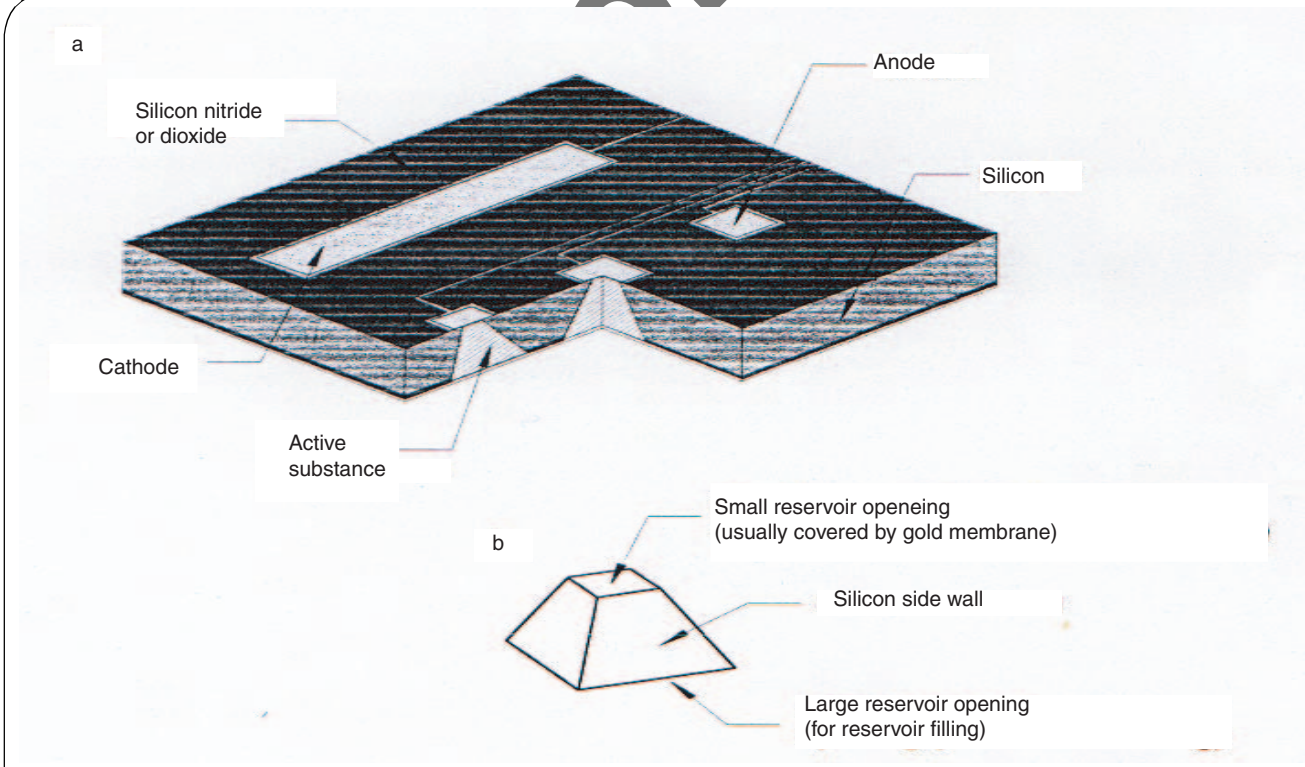

Figure 5B. Schematic of the passive microchip.
Initial models will use PLGA and other existing polymer matrices for the substrate. The entire chip will be biodegradable.

CNS represents a major and exiting discovery in the field of neuro-oncology and holds great promise for both patients and physicians.

Information resources

The following internet websites were useful in providing accurate information about local delivery of antineoplastic agents:

- www.ncbi.nlm.nih.gov/entrez/queryfcgi (Accessed May 2003). This Pubmed website, maintained by the National Library of Medicine, provides a database search for over 12 million MEDLINE citations from all the major medical journals dating back to the 1960s.
- www.clinicaltrials.gov (Accessed May 2003). A website provided by the National Institutes of Health and maintained by the National Library of Medicine listing all active clinical research studies.
- www.nih.gov (Accessed May 2003). The official website of the National Institute of Health, which provides general information about various cancers and existing clinical trials.
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- www.gliadel.com (Accesssed May 2003). The official website of Gliadel.
- web.mit.edu/cheme/langerlab/index.html (Accessed May 2003). The official website of Robert Langer at Massachusetts Institute of Technology (MIT).

Disclosure

Under a licensing agreement between Guilford Pharmaceuticals and the Johns Hopkins University, Dr Brem is entitled to a share of royalty received by the University on sales of products described in this work. Dr Brem and the University own Guilford Pharmaceuticals stock, which is subject to certain restrictions under University policy. Dr Brem also is a paid consultant to Guilford Pharmaceuticals. The terms of this arrangement are being managed by the Johns Hopkins University in accordance with its conflict of interest polices.

Key issues

This review of local delivery of antineoplastic agents for brain tumors focused on:

- Development of biocompatible polymer technology.
- Preclinical and current clinical experience of using biodegradable polymer in the treatment of brain tumors.
- Future technologies, such as implantable microchip, capable to deliver up to 1000 different drugs on demand.

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Affiliations

- *Federico G Legnani, MD, Department of Neurological Surgery, Johns Hopkins Medical School, Hunterian 817, 725 North Wolfe Street, Baltimore, MD 21205, USA*
- *Gustavo Pradilla, MD, Department of Neurological Surgery, Johns Hopkins Medical School, Hunterian 817, 725 North Wolfe Street, Baltimore, MD 21205, USA*
- *Paul P Wang, MD, Department of Neurological Surgery, Johns Hopkins Medical School, Hunterian 817, 725 North Wolfe Street, Baltimore, MD 21205, USA*
- *Henry Brem, MD, Departments of Neurological Surgery and Oncology, Johns Hopkins Medical School, Hunterian 817, 725 North Wolfe Street, Baltimore, MD 21205, USA*
- *Alessandro Olivi, MD, Departments of Neurological Surgery and Oncology, Johns Hopkins Medical School, Hunterian 817, 725 North Wolfe Street, Baltimore, MD 21205, USA*
- *Francesco DiMeco, MD, Departments of Neurological Surgery, Johns Hopkins Medical School, Hunterian 817, 725 North Wolfe Street, Baltimore, MD 21205, USA*
  Department of Neurological Surgery, Istituto Nazionale Neurologico 'C. Besta', Via Celoria 11, Milano, Italy Tel.: +39 348 913 7180, Fax: +39 348 7063 5017, fdimeco@bs.jhmi.edu