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Local drug delivery to the brain

Paul P. Wang[a], James Frazier[a], Henry Brem[a,b,*]

[a]*Department of Neurological Surgery, The Johns Hopkins Hospital, Hunterian 817, 725 North Wolfe Street, Baltimore, MD 21205, USA*

[b]*Department of Oncology, The Johns Hopkins Hospital, Hunterian 817, 725 North Wolfe Street, Baltimore, MD 21205, USA*

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Abstract

The controlled local delivery of antineoplastic agents by biodegradable polymers is a technique that allows for exposure of tumor cells to therapeutic doses of an active agent for prolonged periods of time while avoiding high systemic doses associated with debilitating toxicities. The use of polymers for chemotherapy delivery expands the spectrum of available treatment of neoplasms in the central nervous system, and facilitates new approaches for the treatment of malignant gliomas. In this article, we discuss the rationale and history of the development and use of these polymers, and review the various agents that have used this technology to treat malignant brain tumors.

Keywords: Brain tumor; Glioma; Local drug delivery; Implantable polymer; BCNU; Gliadel®; Chemotherapy; Astrocytoma; Glioblastoma multiforme

Contents

*Corresponding author. Tel.: +1-410-955-6904; fax: +1-410-614-0478.
E-mail address: hbrem@jhmi.edu (H. Brem).

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1. Introduction

Tumors of the central nervous system (CNS) represent one of the most devastating forms of human illness. In the United States alone, approximately 16 800 people are diagnosed with primary brain tumors each year, and 13 100 Americans die from these lesions [1]. Of these, half originate from glial cells and are thus classified as gliomas; more than three quarters of all gliomas are astrocytomas. Astrocytomas are a heterogeneous group of tumors that range from low-grade to the most aggressive—glioblastoma multiforme (GBM)—based on histopathological classification.

For the malignant gliomas, surgical biopsy for pathological diagnosis with surgical debulking of accessible tumor [2] followed by radiation therapy and chemotherapy [3–7] represents conventional therapy. Unfortunately, the prognosis is dismal; the median survival after surgical resection alone is 6 months with only 7.5% of patients surviving 2 years. Additional radiation therapy extends the survival to 9 months, while systemic chemotherapy has been minimally effective [8,9]. Despite these efforts and advances in neuroradiology and neurosurgical technique, long-term patient survival has not been extended, and new therapies and novel approaches are urgently needed to treat the disease.

There are significant obstacles in improving treatment. Broader surgical resection results in increased risk of removing functional brain tissue, and thus causing immediate morbidity and neurological deficit. Similarly, raising the radiation dose or size of the irradiated field introduces unacceptable acute and chronic side effects. Improvements in chemotherapy require the development of more effective agents or advances in delivery methods.

This chapter chronicles the effort to develop biodegradable polyanhydride polymers for the sustained local administration of chemotherapeutic agents in brain tumor patients. The authors first explore the unique issues of drug delivery into the CNS and how they influence the rationale for local delivery. Next, they discuss the development of biocompatible polymer technology. Third, the authors summarize the preclinical and current clinical experience of using polymers in the treatment of tumors. Finally, future technologies that may advance the ability to deliver antineoplastic agents locally to brain tumors will be explored.

2. Unique issues of drug delivery into the CNS

The unique environment of the CNS presents significant challenges in treating malignant brain tumors. With few exceptions, the neurons and supportive glial tissues within the CNS are physiologically isolated by the blood–brain barrier (BBB). Mechanically consisting of tight junctions between endothelial cells of capillaries that supply the CNS [10], the BBB forms a physiological and pharmacological barrier that prevents the influx of molecules from the bloodstream into the brain. While small, electrically neutral, lipid-soluble molecules can readily penetrate the BBB, many chemotherapeutic agents that are large, ionically charged, or hydro-

philic do not fall into this category [11], and thus are difficult to transport into the CNS (Fig. 1) [12].

The practical consequences of the BBB to chemotherapy are substantial. Intolerably high systemic drug levels are often required to achieve therapeutic doses within the CNS. To circumvent this, efforts to improve drug delivery in brain tumor patients have proceeded along three lines. The first approach makes use of the natural permeability properties of the BBB and tailors chemotherapeutic agents within these properties to maximize delivery. By pharmacological manipulation of existing drugs to create more lipophilic (and thus more BBB-traversable) agents, it is theoretically possible to increase the delivery of chemotherapeutic agents to the tumor site. For example, both lomustine (CCNU) and semustine (methyl-CCNU) are two lipophilic variants of a known chemotherapeutic agent carmustine (BCNU) [13], which has been shown to modestly improve survival in patients with malignant brain tumors. Unfortunately, clinical trials investigating the systemic administration of lomustine or semustine have not shown significant efficacy of either of these agents over BCNU in treating glial tumors [5]. Another variation to this approach is to link an existing chemotherapeutic agent to a carrier capable of traversing the BBB. For example, lipophilic dihydropyridine carrier readily crosses the barrier, and has been shown to increase intracranial concentrations of a variety of drugs, including antineoplastic agents, antibiotics, and neurotransmitters [14]. Alternatively, new transport vectors, such as a modified protein or receptor-specific monoclonal antibody, have also led to the successful delivery of a variety of drugs across the BBB [15].

The second approach to overcoming the BBB

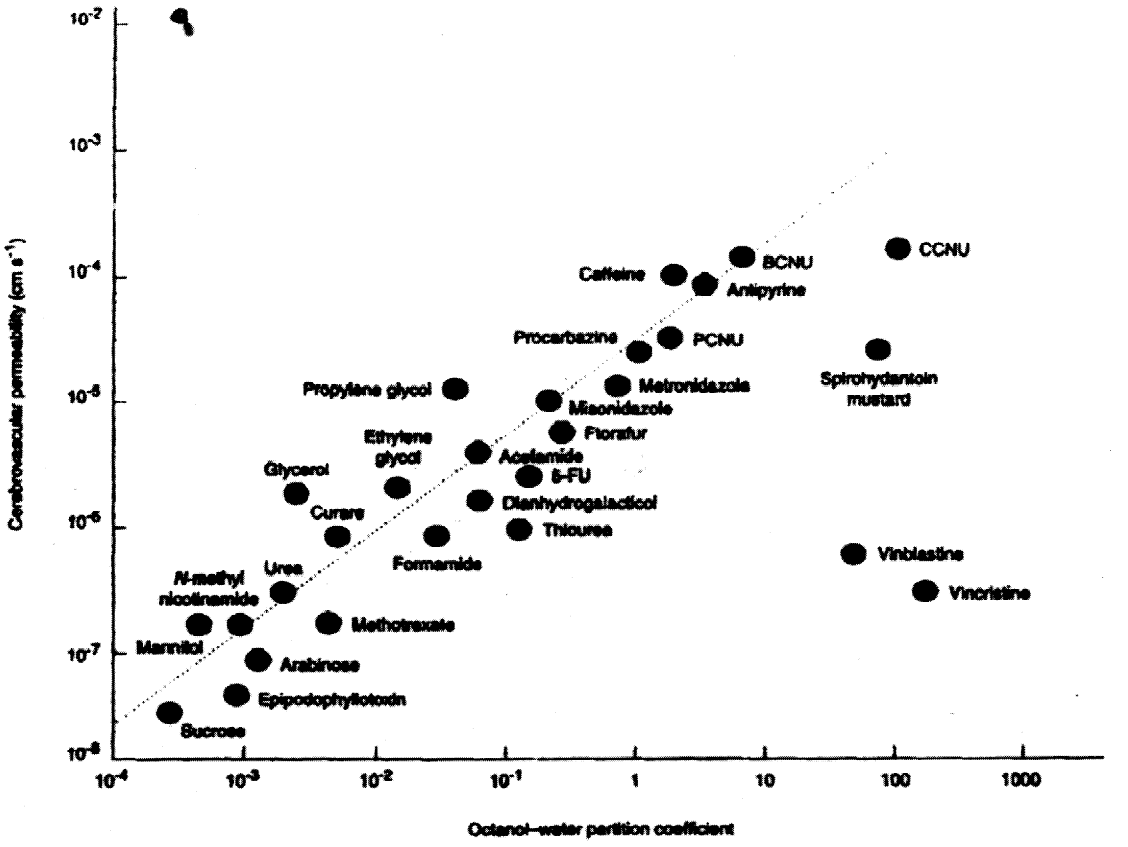

Fig. 1. Cerebrovascular permeability versus octanol–water partition coefficient of selected chemicals and drugs. (Reprinted with permission [12].).

involves first disrupting the BBB, and then delivering the chemotherapeutic agent. For example, infusion of intra-arterial hyperosmolar mannitol can cause an acute dehydration of endothelial cells resulting in cell shrinkage and widening of the tight junctions connecting adjacent membranes. Williams et al. [16] examined the efficacy of the administration of the agents carboplatin and etoposide in conjunction with mannitol in 34 patients with intracranial tumors. Unfortunately, while four out of four patients with primitive neuroectodermal tumors (PNETs) and two out of four patients with CNS lymphomas had some degree of response with the addition of mannitol to the treatment, no benefit was seen in patients with GBM, oligodendrogliomas, and metastatic carcinomas. These results may be due to the finding that while mannitol may increase the passage of hydrophilic substances across the BBB, it does not necessarily improve delivery of drug to the actual tumor site [17]. An alternative to mannitol is the bradykinin agonist RMP-7, which directly disrupts the BBB [18]. RMP-7 has been shown to selectively increase the uptake of carboplatin in experimental brain tumors [19], suggesting that it may be a potentially useful adjuvant for selective delivery of chemotherapeutic agents into the brain.

The third approach to overcoming the BBB is to circumvent it with local delivery into the tumor site. This method offers the advantage of sustained local exposure to concentrated amounts of drug while avoiding significant systemic exposure. In addition, the approach seems particularly appropriate for malignant gliomas, since approximately 80–90% recur within 2 cm of the original site of resection. Cerebrospinal fluid (CSF) infusion is one such strategy, but results have been disappointing secondary to poor penetration of infused compound into the brain parenchyma [20]. Other efforts of this approach can be divided into two categories: (1) administration via catheters; and (2) administration via sustained-release polymers.

Catheter systems have been in clinical use for many years. One such system, the Ommaya reservoir [21], can deliver intermittent bolus injections of antineoplastic agents to the tumor site. Recently, the development of implantable pumps has permitted the constant infusion of drugs over an extended period of time instead of bolus delivery. The prototype model is the Infusaid pump (Infusaid, Norwood, MA, USA) which uses compressed vapor pressure to deliver a solution at a constant rate [22]. Other systems include the MiniMed PIMS system (MiniMed, Sylmar, CA, USA) [23] and the Medtronic SynchroMed system (Medtronic, Minneapolis, MN, USA) [24], which use a solenoid pump and a peristaltic mechanism, respectively, to deliver the infused agent. These devices are all limited by mechanical failure, obstruction by tissue debris or clot, and infection. None has proven superior over the others in the treatment of malignant gliomas.

Biocompatible sustained-release polymers offer an alternative approach to local intratumoral delivery of sustained chemotherapy. Such polymers fall into two categories: (1) biodegradable; and (2) non-biodegradable. Biodegradable polymers release their loaded agents as they break down, while the matrix of non-biodegradable polymers remain intact even after all of the therapeutic agent has been released [25].

3. Development of biocompatible polymers

In 1976, Langer and Folkman [26] reported the sustained and predictable release of macromolecules from the ethylene vinyl acetate (EVAc) copolymer, a non-biodegradable polymer. A drug incorporated into an EVAc polymer diffuses through the micropores of its matrix. The rate of diffusion is dependent upon the chemical properties of the drug, including molecular weight, charge, and water solubility. Biocompatibility was initially established in the rabbit cornea [27], and inertness was confirmed in the rat brain [28–32]. While its numerous clinical applications include glaucoma treatment, dental care prevention, contraception, insulin therapy, asthma treatment, and chemotherapy, the EVAc polymer has never been approved for use in the brain [33]. Its primary limitation has been its inertness; the intact matrix remains permanently as a foreign body. In addition, the release rate of the EVAc copolymer decreases in time, indicating first-order (as opposed to the desired zero-order) release kinetics [34].

In contrast to EVAc, a new generation of biodegradable polymer systems release drugs by a combination of polymer degradation and drug diffusion (Figs. 2 and 3). Initially, the most widely

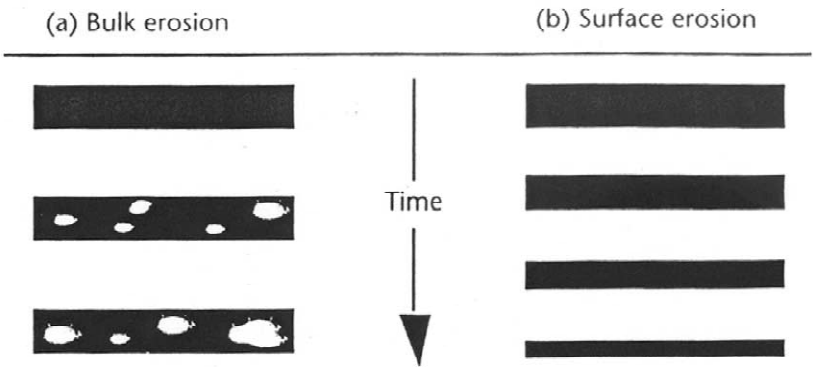

Fig. 2. Biodegradable controlled-release polymer implants are intended to release content at nearly constant rate (zero-order kinetics) as they dissolve in body water. Therefore, desired properties include surface erosion instead of bulk erosion.

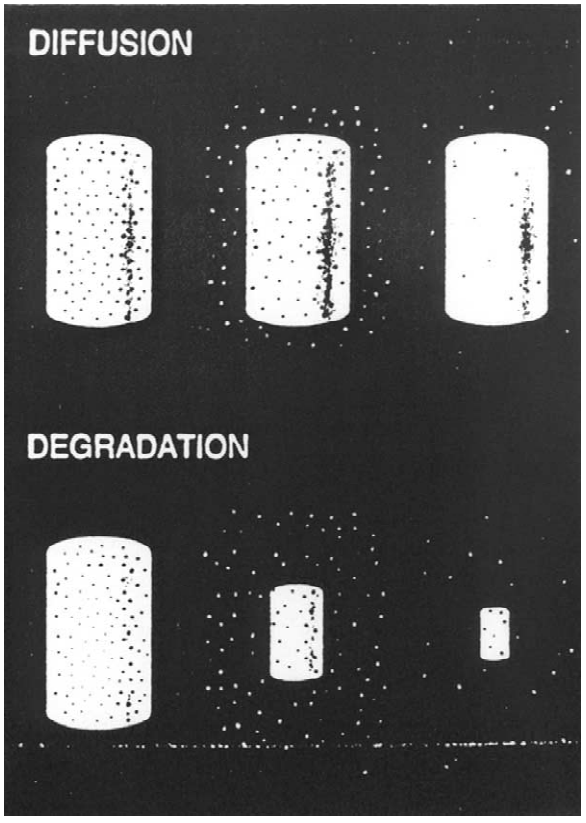

Fig. 3. Desired properties of polymer implants include drug release by degradation instead of diffusion.

investigated family of such polymers was the lactide/glycolide polyesthers, where the monomers (lactic acid and glycolic acid) are polymerized with ester bonds. Varying the ratio of lactic acid to glycolic acid monomers results in polylactic-co-glycolic acid (PLGA) polymers with varying polymer degradation and thus varying drug delivery rates [35]. Biocompatibility was initially shown with polymers fashioned into sutures (which are now in wide clinical use) [36,37], and confirmed in the rat brain [38–40]. These polymers, often shaped into injectable microspheres [41], have been successfully used to deliver a variety of drugs, including steroids, anti-inflammatory agents, narcotic antagonists, antibiotics, anesthetics, and antineoplastic agents [35,42–51]. In addition, a microsphere variant has been introduced that can be stereotactically implanted into the brain [52]. Furthermore, covalent linking of the polymer matrix to a polyethylene glycol coating has been shown to reduce opsonization and elimination by the immune system [53]. While PLGA polymers continue to show great promise, their drug release by *bulk erosion* (like a sugar cube) (Fig. 2b) can result in sporadic dumping of drug, leading to a suboptimal tissue exposure profile, as well as potential for unexpected toxicity [54].

In 1985, Leong et al. [55], formulated the development of the polyanhydride poly[bis(*p*-carboxyphenoxy)] propane–sebacic acid (PCPP:SA) matrix. This biodegradable compound, which breaks down to dicarboxylic acids by spontaneous reaction with water, exhibits several important properties:

- Its extreme hydrophobicity shields the incorporated drug from the surrounding aqueous media,

which is particularly important for drugs with short biological half-lives.

- The macroscopic breakdown of the polymer is limited to its surface, also known as *surface erosion* (like peeling an onion). In contrast to PLGA polymers or other polymers with bulk erosion, surface erosion offers the theoretical advantage of constant-rate or zero-order drug delivery (Fig. 2).
- The rate of breakdown of PCPP:SA polymers can be varied by altering the ratio of the two monomers CPP and SA. For example, a 1-mm-thick polymer composed of pure CPP would require 3 years to degrade. A PCPP:SA matrix with 20% CPP and 80% SA (20:80) of equal thickness would only require 3 weeks [56].
- The polymer can be manufactured in an almost endless variety of shapes, including microspheres, sheets, rods, or wafers, thereby facilitating its clinical applications and surgical delivery [56–62].
- The matrix is completely degradable; there is no residual foreign body after drug delivery.

Biocompatibility of PCPP:SA has been confirmed through extensive investigation in multiple models. The dicarboxylic acid breakdown products were found in standard assays to be neither mutagenic, cytotoxic, nor teratogenic [63]. Both endothelial cell growth and smooth muscle cell growth were not inhibited when plated on a layer of polymer [63]. In the rabbit cornea inflammation assay [27], polymers implanted 6 weeks caused no observable inflammation [61]. Testing using the brains of rats [64], rabbits [65], and monkeys [66] demonstrated its biocompatibility with neural tissue. The combination of its clinical potential and biocompatibility has made PCPP:SA polymers a very attractive system for local drug delivery, and its current applications will be discussed in the next section.

In the effort to improve local polymer delivery technology, a second generation of polyanhydride biodegradable polymers has been developed to broaden the spectrum of deliverable drugs. Because of its hydrophobic properties, PCPP:SA cannot provide constant or sustained release of many hydrophilic compounds when they are densely packed within the polymer matrix. Nor can it prevent the hydrolytic degradation of hydrophilic agents. This has led to the development of the fatty acid dimer–sebacic acid (FAD:SA) polymers [67–69]. As with PCPP:SA, FAD:SA provides drug shielding, zero-order release kinetics, and biodegradability; it can be fabricated into any shape, including microspheres, and its degradation rate and release kinetics can be manipulated by varying the ratio of its two monomers FAD and SA [70,71]. Biocompatibility has been assessed in the rat brain and found to be comparable to that of PCPP:SA [72]. Both proteins [70] and chemotherapeutic agents [73–75] have been successfully loaded and released in FAD:SA. The FAD:SA and PCPP:SA technologies complement each other well; FAD:SA is suited to deliver hydrophilic drugs, the very compounds that give PCPP:SA trouble.

A variety of other local delivery systems have been developed and tested. Polyethyleneglycol-coated liposomes have been introduced to encapsulate anthracyclines [76], and gelatin-chondroitin sulfate coated microspheres have been shown to reproducibly release cytokines in vivo [77]. Many commonly used surgical materials, such as fibrin glue [78], gelatin sponges [79], Surgicel (oxidized regenerated cellulose) [79], polymethyl methacrylate [80], and silastic tubing [81], have all been used to deliver drugs to brain tumors.

4. Clinical applications of polyanhydride polymers for drug delivery

4.1. BCNU (Gliadel®): development and clinical use

The choice of carmustine, or BCNU, as the initial drug in the development of polymer-based intracranial chemotherapy stems from the well-known activity of nitrosoureas against malignant gliomas, and its existing wide use as a systemic agent for treating brain tumors. Like all nitrosoureas, it acts by alkylating the nitrogen bases of DNA. Its low molecular weight and lipid solubility allow it to cross the BBB at potentially tumoricidal concentrations [13]. Unfortunately, its dose-limiting side effects of bone marrow suppression and pulmonary fibrosis, along with its relatively short half-life (< 15 min), limit its

effectiveness as a systemic antineoplastic agent. In fact, clinical trials of the systemic administration of BCNU to treat brain tumors has shown only a modest improvement in survival [4,6]. In an effort to increase its effectiveness and limit the dose-related side effects, BCNU was incorporated into polymers and tested for efficacy against intracranial tumors.

4.1.1. Preclinical trials

The preclinical studies of BCNU-polymer preparations were carried out in several systematic stages. The first set of experiments investigated the in vivo release kinetics of BCNU. The initial study used an EVAc copolymer in the rat brain [32]. After implanting BCNU-loaded polymers intracranially, drug concentrations were measured with the Bratton-Marshall assay in the ipsilateral hemisphere, contralateral hemisphere, and serum at various time points. The ipsilateral BCNU levels peaked 4 h after implantation and remained substantial through day 7. Both contralateral hemisphere and serum levels were at least an order of magnitude less at each time point. A second experiment compared the biodistribution of BCNU via PCPP:SA (80:20) delivery with the distribution after direct stereotactic injection of BCNU [82]. Tritiated BCNU was delivered, and distribution was assessed by quantitative autoradiography in brain sections of animals sacrificed at various time points. With polymer delivery, approximately 50% of the ipsilateral hemisphere was exposed to BCNU at day 3, and 10% was exposed at day 14. A polymer disk containing 600 μg of BCNU produced tissue concentrations of 6 mM at 10 mm from the implantation site on both day 3 and day 7 after implantation. In contrast, direct injection showed an initial spike of broadly distributed BCNU at 1 and 3 h post-injection, which then rapidly disappeared. High-performance liquid chromatography was used to confirm that the radioactivity was in fact associated with active BCNU. A third experiment with 20% (w/w) BCNU loaded PCPP:SA polymers in monkeys found tumoricidal concentrations of BCNU at 4 cm from the implant site at 24 h post-implantation [83]. These efforts confirmed that BCNU can be delivered from polymers to provide local, sustained, clinically significant levels to neural tissue in vivo.

The next set of experiments investigated the efficacy of BCNU-loaded polymers. First, Tamargo et al. [28], compared the efficacy of polymer-delivered BCNU compared to systemic BCNU using both the rat flank and intracranial 9L gliosarcoma models. In the flank model, tumor growth delay was significantly longer in EVAc-delivered BCNU compared to systemic BCNU (16.3 vs. 11.2 days, $P < 0.05$). In the intracranial model, a 10-mg polymer with 20% (w/w) BCNU dramatically improved survival in animals with established 9L gliosarcoma. Compared to controls, the EVAc and PCPP:SA polymers increased survival 7.3-fold and 5.4-fold, respectively. In contrast, the systemic BCNU increased survival only 2.4-fold. A second study using the same established rat intracranial 9L gliosarcoma model compared the delivery of BCNU by 20% (w/w) PCPP:SA polymers and direct stereotactic intratumoral injection with an equivalent dose of BCNU [84]. Compared to controls, median survival was improved 271% in the polymer group in contrast to 36% in the injection group. There were also twice as many long-term survivors in the polymer group.

An additional study was performed to establish the optimal CPP/SA monomer ratio and dosing percentage of BCNU in the polymer [85]. First, in vitro release kinetics were compared between 50:50 and 20:80 PCPP:SA formulations using both 4 and 32% BCNU-loaded polymers. In theory, lowering the proportion of CPP slows polymer degradation, and thus slows drug release. In fact, the release kinetics revealed minimal differences in the profile between the two formulations loaded at 4%, but comparisons between the 32% polymers revealed a 150% increase in release duration (18 vs. 7 days) for the 50:50 formulation. Next, the efficacy of polymer loads of 0, 4, 8, 12, 20, and 32% (w/w) were compared in both 50:50 and 20:80 PCPP:SA polymer formulation. When tested with the established rat intracranial 9L gliosarcoma model, survival was maximized in the 20% loaded PCPP:SA (20:80) formulation, which rendered a 63% survival rate at 200 days compared to a median survival of < 20 days in animals treated with blank polymers. An additional toxicity study was performed using five cynomolgus monkeys [85]. The 20% BCNU-loaded PCPP:SA (20:80) polymers caused no systemic or local morbidities, and MRI images obtained 150 days after

implantation showed no evidence of edema or mass effect.

A final set of preclinical experiments [86] investigated the efficacy of local polymeric delivery of various antineoplastic agents, including BCNU, to combat brain metastases, which represent the majority of newly diagnosed brain tumors [87], and are a major cause of mortality in patients with metastatic cancer [88]. Maximum nontoxic doses of each agent in PCPP:SA polymer were established, and efficacy was tested with and without concurrent radiation therapy in several mouse metastatic models. Tumor lines included the B16 melanoma, RENCA renal cell carcinoma, CT26 colon cancer, and Lewis lung carcinoma. While both BCNU-loaded polymer and radiation treatments were effective alone, they were much more effective in combination against B16 melanoma (median survival 35 vs. 21.5 days for controls; $P = 0.0005$), RENCA renal cell carcinoma (38.5 vs. 12 days for controls; $P < 0.007$), and Lewis lung carcinoma (23 vs. 21 days for controls; $P = 0.001$). While combination was effective against CT26 colon carcinoma (38.5 days; $P < 0.001$ vs. control), irradiation alone was at least equal to the combination (44 days; $P < 0.001$ vs. control). A later study showed intracranial BCNU polymers to be effective with and without irradiation against the EMT-6 breast cancer in mice (median survival 41 and > 200 days, $P = 0.02$ and $P < 0.0001$, respectively, vs. 17 days for controls) [89]. These encouraging findings have led to the planning of clinical trials of BCNU-loaded PCPP:SA treatment for metastatic brain tumors.

4.1.2. Clinical experience for recurrent gliomas

Based on the preclinical findings that the PCPP:SA polymer: (1) is biocompatible and nontoxic with and without BCNU loading and radiation therapy; (2) releases BCNU in vivo and distributes it broadly from the implantation site; and (3) improves survival with its polymer delivery of BCNU compared to systemic treatment in animals, approval was obtained for a multicenter phase I–II clinical trial in humans [90]. Enrollment criteria limited patients to those presenting with recurrent malignant gliomas that had previously undergone a craniotomy for debulking and in whom standard therapy had failed. Other eligibility requirements included an indication for reoperation, a unilateral single tumor focus with ≥ 1 cm^3 of enhancing volume on MRI or CT, completion of external beam radiotherapy, a Karnofsky Performance Scale (KPS) score of ≥ 60, and no exposure to nitrosoureas during the 6 weeks prior to polymer implantation. Twenty-one patients were treated, and three different polymer loads were tested: 1.93, 3.85, and 6.35% (w/w). Each polymer weighed 200 mg, and most patients received a maximum eight wafers implanted within the tumor cavity following debulking (Fig. 4). Tumor volumes were similar in all groups.

There was no evidence of systemic toxicity, as measured by frequent blood chemistry and urinalysis tests. Hematological evaluation also revealed no evidence of bone marrow suppression. While the KPS scores fell slightly in the immediate postoperative period, they returned to baseline and remained stable for at least 49 days, indicating quality of life preservation during the chemotherapeutic period.

Postoperative scans were able to detect the implanted polymers. They appeared as areas of decreased signals on T1-weighted MRIs, and some were visible on CT as long as 49 days after surgery. In 13 of 21 patients, routine protocol scans revealed some areas of marked enhancement around the implant sites. These generally resolved spontaneously, and at no time was there any correlation between

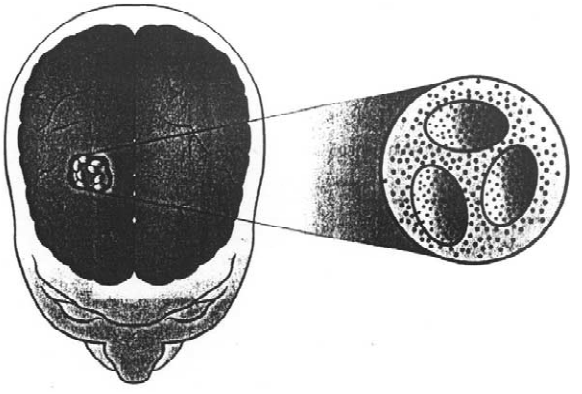

Fig. 4. Up to eight polymer implants line the tumor resection cavity, where the loaded drug is gradually released as they dissolve. The inset shows conceptually how drug molecules diffuse away from these implants. (Reprinted with permission [54].).

the enhancement and neurological decline, or any other toxic sequelae.

Over the course of the study, 10 of the patients required reoperation for declining neurological status with increasing enhancement on MRI or CT. The most notable intraoperative finding was a rim of necrotic tissue up to 1 cm thick, similar to that described in interstitial brachytherapy patients. The KPS scores were generally improved following removal of this tissue.

The overall median survival times were 46 weeks after implant and 87 weeks after initial diagnosis, with 86% of patients alive after more than 1 year of diagnosis. On the basis of this work, the 3.85% BCNU-loaded polymer was eventually chosen for further clinical study. Because the data suggested improved survival, a rigorous phase III clinical trial was designed.

This resulting trial was multicentered, prospective, randomized, double-blinded, and placebo-controlled [91]. It investigated the efficacy of 3.8% BCNU (w/w) PCPP:SA polymers in treating 222 patients with recurrent malignant gliomas at 27 medical centers in North America. Patients were randomized to receive either the BCNU-polymer or a blank placebo. Selection criteria was the same as for the phase I–II study, except that no chemotherapy was allowed for 4 weeks preceding surgery, and systemic chemotherapy was allowed as early as 2 weeks after surgery. Decisions about additional operations for tumor recurrence were made by individual patients and their surgeons independent of participation in the study. Mean age was 47.8 years, and randomization rendered the treatment and placebo groups well matched for important prognostic factors, including age, tumor type, and preoperative KPS scores. All patients previously underwent external beam radiotherapy; 52.7% of the BCNU-polymer group and 48.2% of the control group had previously undergone chemotherapy.

The median postoperative survival of the patients implanted with BCNU-loaded polymer was 34 weeks compared to 23 weeks in the placebo group (hazard ratio 0.67, $P = 0.006$) (Fig. 5). The 6-month survival rate was 60% in the treatment group, and 47% in the placebo group. More remarkable was the finding among glioblastoma patients ($n = 145$), where there was a 50% increase in 6-month survival with BCNU-

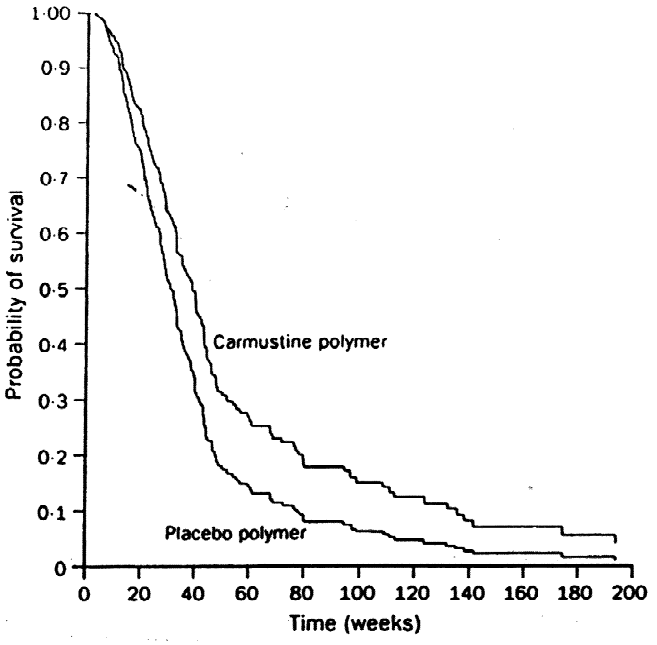

Fig. 5. Overall survival for patients receiving BCNU-loaded polymers versus controls at the time of the operation for recurrent malignant gliomas after adjustment for prognostic factors. (Reprinted with permission [91].).

loaded polymer compared to placebo treatment ($P = 0.02$). The BCNU-loaded polymer was again shown to be safe and well tolerated; there was no evidence of bone marrow suppression or systemic toxicity. Within 6 months of polymer placement, 11.8% of BCNU patients and 11.6% of control patients underwent reoperation. Intracranial infection was more common in the BCNU-treated group than the placebo group (4/110 or 3.6% vs. 1/112 or 0.9%), but this did not reach clinical significance. The brains of 11 patients were examined postmortem, revealing recurrent tumor, as well as mild inflammatory reactions and no marked necrosis.

Consequently, this study established that BCNU-polymers are safe and effective in the treatment of recurrent malignant gliomas. Based on these results, the Food and Drug Administration (FDA) in 1996 approved 3.85% BCNU-loaded PCPP:SA polymer (Gliadel®) for the treatment of recurrent glioblastoma multiforme. This was the first time in 23 years that the FDA approved a new treatment for malignant gliomas.

4.1.3. Clinical experience as initial therapy

In general, any treatment for cancers that has been found effective at recurrence has been subsequently shown to be even more efficacious as initial therapy. After the establishment of Gliadel®'s role in treating recurrent malignant gliomas, attention has naturally turned to examining its possibilities as initial treatment. A phase I–II trial with 22 enrolled patients was designed to determine the safety of Gliadel® polymers at the time of initial surgery [92]. The polymer wafers again weighed 200 mg, and most patients received a maximum of eight wafers. Inclusion criterion required a unilateral enhancing tumor focus ≥ 1 cm^3, age > 18, and a KPS score of ≥ 60. The mean age was 60, and all patients received postoperative external beam radiation therapy averaging 5000 rads. No patients received additional chemotherapy during the first 6 months after surgery.

There was no perioperative mortality, nor was there evidence in any treated patient of systemic or local morbidity attributable to the polymer. Twenty-one of 22 patients received a pathological diagnosis of glioblastoma. Median survival was 44 weeks from the time of implant with four patients surviving > 18 months. This phase I–II study demonstrated that Gliadel® was safe and well tolerated in conjunction with radiation therapy for patients with newly diagnosed malignant gliomas.

Based on the encouraging findings, a phase III, multicenter, randomized, double-blinded, placebo-controlled study of the efficacy of locally implanted Gliadel® against newly diagnosed malignant gliomas was begun [93]. Originally planned for 100 patients, the study was terminated prematurely with only 32 patients enrolled due to temporary unavailability of the drug. However, the results from the abbreviated study were encouraging. Admission criteria included the presence of a single unilateral tumor focus with ≥ 1 cm^3 of enhancement, age between 18–65 years, a KPS score ≥ 60, and a histopathological diagnosis of either anaplastic astrocytoma or glioblastoma multiforme on intraoperative frozen section. Again, 200-mg wafers were used. There were 16 patients in each arm of the study. The median age was 55.5 years for the BCNU group and 53 years for placebo. The median KPS score was 75 for the treatment group and 90 in the control group. There was a discrepancy in tumor pathology; all 16 placebo patients harbored glioblastomas, but only 11 of 16 BCNU patients had glioblastomas.

With all patients included in the analysis, the median survival of the treatment group was 58.1 weeks compared to 39.9 weeks for the control group ($P = 0.012$) (Fig. 6). When limited to the subgroup of glioblastoma multiforme patients, the BCNU-loaded polymer group had a median survival of 53.3 weeks versus 39.9 weeks for the placebo group ($P = 0.008$). Perhaps more striking was that of the four patients with glioblastoma alive 3 years after the termination of the study, three were in the BCNU-loaded polymer group. Age and preoperative KPS score significantly impacted survival, while tumor type impacted survival but not in a statistically significant way. Again, no local or systemic morbidity attributable to the polymers was noted.

Currently, there are several additional clinical trials ongoing involving Gliadel®. A larger phase IV, multicenter, randomized, double-blinded, placebo-controlled study was recently carried out involving 250 patients to definitively assess the role of Gliadel® in initial therapy. A second dose-escalation study was completed to establish the maximal non-

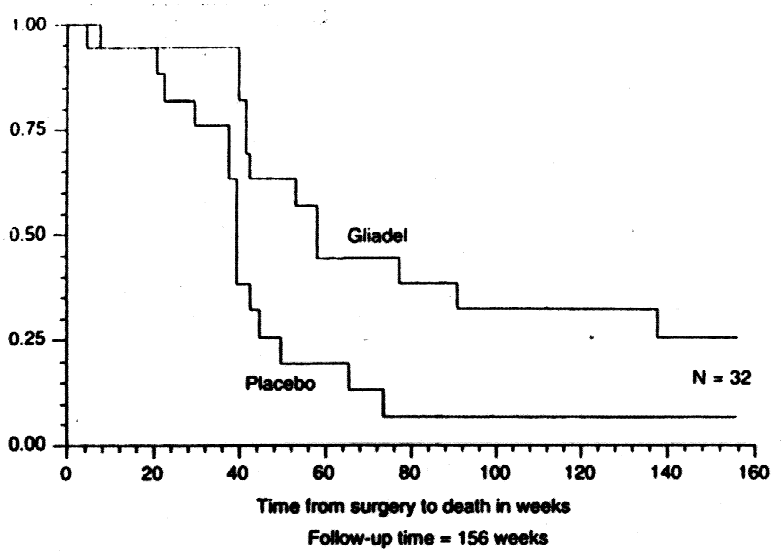

Fig. 6. Kaplan–Meier survival curve for patients with initial therapy for Grade III and Grade IV gliomas treated with BCNU-loaded polymer implants versus placebo. (Reprinted with permission [93].).

toxic BCNU loading in 20:80 PCPP:SA polymers. (In the rat intracranial 9L gliosarcoma model, 20% BCNU (w/w) loaded PCPP:SA (20:80) polymer exhibited maximal efficacy without toxicity in rats or monkeys [85]. In Gliadel®, BCNU is loaded at only 3.8% (w/w).) Future clinical trials are being planned, including a phase III trial to evaluate an improved BCNU-loaded wafer with a safe higher BCNU load than the standard Gliadel® wafer, as well as trials investigating the use of Gliadel® for metastatic tumors and in combination with other drugs.

4.2. Other chemotherapeutic agents

The successful development of BCNU-loaded sustained-release polymers for clinical use in malignant gliomas has served as a model for future investigation. With local delivery, agents that have never been seriously considered for brain tumors due to systemic toxicity can now be examined for clinical potential. The following agents have either been successfully incorporated into polymers or have been successfully used as adjuvants for polymer-based treatment.

4.2.1. O^6-Benzylguanine (O^6-BG)

A limitation of Gliadel® treatment is the resistance of many brain tumors to BCNU and other alkylating agents. AGT, a DNA-repair protein found in the majority of brain tumors [94], is responsible for much of the resistance. BCNU exerts its antineoplastic effects by chloroethylation of DNA at the O^6 position of guanine. By removing adducts at this position before cytotoxic effects can occur, AGT is able to protect tumor cells from BCNU. O^6-Benzylguanine (O^6-BG) is a substrate analog that can irreversibly inactivate AGT by transferring a benzyl group to a cysteine residue at the active site of AGT [95].

The O^6-BG mediated AGT inhibition enhances sensitivity to BCNU, but when given in combination with systemic BCNU, it was discovered that maximal tolerated doses of BCNU was reduced up to sixfold in animal models due to bone marrow toxicity [96]. Rhines et al. [97], postulated that by combining systemic O^6-BG with intracranial local-delivered BCNU polymers instead of systemic BCNU, the systemic side-effects of the combined treatment could be significantly reduced. Using the established rat intracranial F98 glioma model, they

showed that by giving systemic O^6-BG with BCNU-loaded PCPP:SA polymers, median survival was improved in the combination therapy to 34 days over animals receiving O^6-BG alone (22 days, $P = 0.0002$) or BCNU polymer alone (25 days, $P = 0.0001$). They also discovered that it was not necessary to reduce the BCNU polymer load when systemic O^6-BG was introduced. The animals did not exhibit any bone marrow or gastrointestinal toxicity.

These results suggested that the concurrent use of O^6-BG and BCNU polymers may be an important addition to the treatment of malignant brain tumors. In fact, preliminary Phase I clinical trials investigating O^6-BG as an adjuvant to BCNU treatment have just been completed. In addition, current ongoing efforts include incorporating O^6-BG within polymers for local delivery, and dose-escalation studies for systemic and local-delivered O^6-BG.

4.2.2. Taxol

Taxol (paclitaxel) is a naturally occurring microtubule-binding agent, which has been shown to have tumoricidal activity against several human neoplasms, including non-small cell lung cancer, breast cancer, and ovarian cancer. In vitro studies have shown Taxol to be potent against rat and human glioma cell lines [98], but it does not cross the BBB [99]. By circumventing the BBB, local polymeric delivery of Taxol could potentially avoid systemic toxicity and show considerable clinical potential.

Using in vitro kinetics studies, Walter et al. [100] demonstrated that 20–40% (w/w) Taxol-loaded PCPP:SA (20:80) polymers released for up to 1000 hours. In vivo biodistribution studies revealed tumoricidal concentrations of Taxol in the rat brain for >30 days after implantation. In the rat intracranial 9L gliosarcoma model, median survival was improved from 19.5 days in rats treated with placebo polymers to 61.5 days with 20% Taxol-loaded polymers ($P < 0.001$).

Clinical trials using a Taxol-loaded PACLIMER [101] microsphere delivery system are currently in progress for ovarian cancer. Toxicity trials on dogs for the treatment of intracranial tumors are ongoing; the initial results show no early mortality or significant morbidity that can be attributed to the Taxol-loaded polymer [102]. Phase I clinical trials are being planned for implementation as soon as these canine toxicity studies demonstrate safety.

4.2.3. Cyclophosphamide and 4-hydroxyperoxycyclophosphamide

Cyclophosphamide (Cytoxan) is an alkylating agent widely used for the treatment of systemic malignancies. Because its active metabolite, hydroxycyclophosphamide, poorly crosses the BBB, the drug has not been widely used against malignant gliomas; the necessary high doses for efficacy are systemically intolerable. Unfortunately, its use in local delivery is also limited; cyclophosphamide requires enzymatic activation by the p450 cytochrome oxidase system in the liver [103].

A derivative of cytoxan, 4-hydroxyperoxycyclophosphamide (4-HC) spontaneously converts in vivo into the active metabolite [104], thus making it a more attractive candidate for polymeric local delivery. Because of 4-HC's hydrophilic properties, the FAD:SA matrix was chosen for delivery. In vitro and in vivo pharmacokinetic studies demonstrated both favorable release kinetics and biodistribution [105]. In the rat brain, cerebral drug levels peaked between 5 and 20 days after drug implantation, in contrast to a rapid fall in drug level 48 h after systemic dosing. After the maximum tolerable polymer dose of 20% (w/w) was established, efficacy was measured by comparing survival in the established rat intracranial 9L gliosarcoma and F98 glioma model [74]. Animals treated with placebo 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 ($P = 0.004$).

Recently, it was demonstrated that L-buthionine sulfoximine (BSO) potentiates the antitumor effects of 4-HC in the rat 9L gliosarcoma model [106]. The glutathione *S*-transferase (GST) enzyme system appears to play an important role in the inactivation of alkylating agents by catalyzing their reaction with glutathione to produce stable, nontoxic conjugates [107]. Both increased cellular GST activity and glutathione content have been shown to increase resistance to alkylating agents in a variety of human and animal malignancies. BSO modulates tumor resistance to some alkylating agents by inhibiting

glutathione synthesis. Co-release of both 4-HC and BSO from the FAD:SA polymer boosted median survival 4.6 times greater than rats treated with empty polymer (61.5 vs. 13 days, $P < 0.001$) in the established rat intracranial 9L gliosarcoma model, while release of 4-HC alone improved median survival to only 2.3 times control ($P < 0.001$). Systemic delivery of BSO in conjunction with polymer-delivered 4-HC did not impact survival. In addition to its advantages in efficacy, a separate experiment in this study showed that local delivery of BSO may be safer than systemic administration. BSO-loaded EVAc polymer implanted in a rat brain depleted intracranial glutathione levels without impacting liver levels, while systemic BSO delivery was shown to reduce hepatic glutathione levels without impacting cerebral levels.

4.2.4. 5-Fluorouracil

5-Fluorouracil (5-FU), a thymidine analog, blocks the conversion of deoxyuridylic acid to thymidylic acid, thus depriving the cell of one of the essential precursors for DNA synthesis. While effective in treating various metastatic gastrointestinal cancers and other malignancies, its efficacy for brain tumors is limited by its systemic toxicities, which include myelosuppression and gastrointestinal mucosal injury [108]. In fact, the results of earlier efficacy trials testing 5-FU's role in treating brain tumors were disappointing [109–111].

The earliest attempt at local delivery of 5-FU involved a 1968 study in which 5-FU was loaded into gelatin sponges or Surgicel [79]; while no evidence of toxicity was noted, no therapeutic effect was demonstrated either. In 1979, Silastic tubes loaded with 5-FU and urokinase were shown to continually release both drugs for over 5 weeks [81]. When tested against the rat flank ethylnitrosourea-induced gliomas, the 5-FU/urokinase-loaded tubes were found to be capable of inhibiting tumor growth. This led to an initial clinical trial of 14 patients with malignant gliomas or metastatic disease. Thirteen out of 14 lived for >8 months following 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 median survival of 18 months for patients with malignant gliomas, with a 3-year survival rate of 16% [112,113]. Tumoricidal levels of 5-FU were measured as long as 2 years after implantation.

In 1986, the first attempt to deliver 5-FU with polymers was made when antineoplastic agents, including 5-FU, adriamycin, and mitomycin C, were incorporated into a matrix consisting of 'glassified monomers' with 10% polymetacrylic methyl acid and tested on 55 patients [114]. A 47% 1-year survival rate in patients with malignant gliomas was noted. In 1995, 5-FU was successfully incorporated into PLGA microspheres, and pharmacokinetic studies showed good release characteristics [115]. When tested against the established rat intracranial C6 glioma model, treatment with 5-FU-loaded microspheres significantly decreased mortality ($P = 0.017$), while treatment with placebo and bolus 5-FU injections had no effect [116]. Histologic examination showed only mild tissue reaction, and no toxicities were observed in the animals.

In 1999, Menei et al. [117], loaded eight patients with newly diagnosed GBM with 5-FU-loaded PGLA microspheres following surgical debulking. The patients also underwent postoperative adjuvant external beam radiation therapy. Clinically significant concentrations of 5-FU were measured in the CSF up to 1 month after implantation, while 5-FU levels in the blood were low and transitory. At the time of publication, the median survival time was 98 weeks for the eight patients, with two patients exhibiting disease remission at 139 and 153 weeks.

Fluorodeoxyuridine, a related compound to 5-FU, has also been successfully delivered from FAD-SA polymers in vitro and in vivo [118]. This study was based on another work in which fluorodeoxyuridine was continuously infused with a Medtronic SynchroMed pump to treat a single patient with intracranial metastatic renal cell carcinoma [119]. A complete response was achieved in 3 months and maintained for 22 months.

4.2.5. Adriamycin

Adriamycin is an anthracycline antibiotic that intercalates with DNA, causing strand scission and double-stranded cross breaks. Its tumoricidal activity has been documented on breast cancer, acute leukemia, lymphoma, and others [108]. In 1983, Ommaya reservoirs were used to deliver adriamycin

to the tumor bed in 20 patients with malignant gliomas following surgical resection [120]. A total dose of 5.0–10.0 mg in daily 0.5-mg aliquots was injected into the reservoir. In addition, all patients received cobalt-60 irradiation and immunotherapy. A 1-year survival rate of 55% was achieved. In comparison to systemic administration, local delivery with Ommaya reservoir increased the local concentration of adriamycin up to 38 times, and the drug was noted to penetrate within 3 cm into the brain parenchyma.

Adriamycin has been successfully incorporated into EVAc polymers in the shape of needles [121]. Zero-order kinetic release was established in in vitro assays, and the needles significantly inhibited growth of brain tumor xenografts in nude mice. Adriamycin has also been successfully incorporated into PCPP:SA polymers [122]. In vitro assays show sustained release of adriamycin, and in vivo experiments demonstrate improved median survival (33 vs. 13 days in controls, $P < 0.0006$) in the rat intracranial 9L glioma model. Further animal studies of the adriamycin-loaded PCPP:SA polymers, including those with concurrent immunotherapy, are currently ongoing.

4.2.6. Methotrexate

Methotrexate (MTX) is a folate antagonist widely used against a number of malignancies. Its use against malignant gliomas is limited again due to its lack of permeability across the BBB and its systemic side effects, including myelosuppression and gastrointestinal necrosis [108]. In animal models, once introduced to the brain parenchyma via direct injection, MTX disseminates widely in the brain [123]; its dissemination is comparatively poor with ventriculocisternal or intraventricular infusion [20,124].

The first large-scale trials of intratumoral MTX were reported in 1987, where 269 patients were divided into five treatment groups, each receiving a different postoperative strategy following surgical resection [125]. One of the groups received 'local chemotherapy' as part of its strategy; this involved the implantation of a Spongistan matrix soaked with 50 mg MTX. No side effects or complications of the local MTX delivery were observed. While the results of the studies showed that local MTX had no significant effect on overall survival, there were more long-term survivors in the group receiving local MTX than in the other groups.

In an effort to improve stability and inhibit degradation, MTX has also been modified by covalent linking to dextran [126]. The resulting MTX-dextran conjugate was found to be as potent as unmodified MTX in in vitro studies against the human H80 glioma line. In a three-dimensional collagen matrix designed to mimic extracellular matrix, the MTX–dextran conjugate had superior penetration compared to unmodified MTX. When incorporated into the FAD:SA polymer, the conjugate offered modest but significant improvement over controls in the rat intracranial 9L gliosarcoma model.

MTX has also been incorporated into a polymethylmethacrylate pellet [80] and a PLGA copolymer matrix [127]. Despite release kinetics revealing 96–99% of the drug was released within 2 days, the MTX-loaded pellets significantly improved median survival by 69% compared to controls in the rat intracranial ethylnitrosourea-induced tumor model. The MTX-loaded PLGA polymer inhibited glioma growth in the rat flank. A fibrin glue-based system also reported inhibition of glioma growth in the rat flank when used to deliver MTX [78].

Although early clinical trials [125,128] have shown minimal toxicity in intratumoral MTX, other case reports have documented associated toxicity. One patient with meningeal carcinoma reportedly developed an abulic-hypokinetic syndrome and left hemiparesis after receiving intraventricular MTX [129]. Two patients have been reported to develop large cysts at the site of intratumoral therapy with MTX administered through an Ommaya reservoir [130].

4.2.7. Platinum drugs

Carboplatin, a second-generation platinum analog, causes myelosuppression when administered systemically, but is less neurotoxic than its parent compound, cisplatin [131]. Being water soluble, carboplatin was optimally released by the FAD:SA polymer [73]. Using the rat intracranial F98 glioma model, a maximum nontoxic dose of 5% (w/w) was determined, and then tested for efficacy. Median survival was increased from 16 days in control animals (with all animals dead by day 19) to 52 days

in the carboplatin-loaded polymer-treated animals. Carboplatin polymers were also assessed against various mouse metastatic brain tumors in the study described previously [86]. In combination with radiation therapy, carboplatin-loaded polymers prolonged survival against the CT26 colon carcinoma (median survival 33 vs. 20.5 days for controls, $P = 0.013$) and RENCA renal cell carcinoma (15 vs. 12 days, $P < 0.01$). The carboplatin-loaded polymers alone demonstrated efficacy against the B16 melanoma (27 vs. 16.5 days, $P = 0.043$), while combination therapy was ineffective.

In an attempt to further the efficacy of carboplatin-loaded polymers, a separate endeavor was made to couple carboplatin with α-cyclodextrin to delay decomposition [132]. Both agents were incorporated into ethylcellulose microcapsules at a 2.2% (w/w) loading. In vitro kinetics assays demonstrated that 56% of the loaded carboplatin was released over 110 days. When tested against the rat intracranial F98 glioma, median survival was 20 days for microcapsules loaded with only α-cyclodextrin, 34 days with microcapsules loaded with only carboplatin ($P < 0.001$), and 51 days with microcapsules loaded with both ($P < 0.01$).

In addition, the role of local delivery for carboplatin's parent compound cisplatin has also been investigated by incorporating it in a biodegradable polylactic acid polymer. Local polymer-delivered cisplatin was compared to local bolus infusion and systemic administration after maximum nontoxic doses of systemic and polymeric cisplatin were established. In the rat intracranial 9L gliosarcoma model, median survival was 24 days in the control group, 32 days for the systemic cisplatin group, 39 days for the local bolus delivery group, and > 60 days for the polymer delivery group ($P < 0.00006$ vs. systemic group; $P < 0.001$ vs. bolus group). Furthermore, histopathological 'cure' was seen in eight of 12 cisplatin-loaded polymer animals versus three of 13 local bolus infusion animals ($P < 0.01$). No cures were seen in the other groups. Biocompatibility was confirmed in a follow-up study [38].

4.2.8. Camptothecin

The camptothecins are a family of inhibitors of DNA-replicating enzyme topoisomerase I [133]. While in vitro and in vivo trials of systemic treatment showed great promise, unexpected toxicities with systemic administration were discovered in clinical trials [134]. This prevented its use as a systemic agent for gliomas. Because of encouraging in vitro data against brain tumor cell lines, camptothecin was considered for local polymer delivery [31]. Sodium camptothecin was selected due to its its ease of loading into the polymer. Initially, the compound was loaded into the EVAc polymers, where in vitro kinetics experiments demonstrated sustained release. In the established rat intracranial 9L gliosarcoma model, the 50% (w/w) loaded polymer dramatically extended survival (> 120 days vs. 19 days for controls; $P < 0.001$). In addition, while none of the control rats survived beyond 32 days, 59% of camptothecin-treated animals survived > 120 days. Systematic camptothecin had no impact on survival. No local or systemic toxicity was observed in the polymer-implanted animals.

Sodium camptothecin was also loaded onto PCPP:SA polymers and tested both in the previously described metastatic tumor study [86] and, most recently, in the established rat intracranial 9L gliosarcoma model [135]. The metastatic study showed that PCPP:SA camptothecin-loaded polymers were effective only in combination with radiation therapy and only against the B16 melanoma (median survival 27.5 days vs. 19 days; $P = 0.043$). In the 9L gliosarcoma model, median survival with 50% (w/w) camptothecin-loaded polymers was 69 days, compared to 17 days in the control animals ($P < 0.001$). No local or systemic toxicity was noted in any of the animals. The polymers were also shown to release intact camptothecin for up to 1000 h in vitro.

Current ongoing efforts include preclinical studies examining the efficacy of various camptothecin analogs when loaded onto biodegradable polymers for local drug delivery.

4.2.9. Bleomycin

Bleomycin is a tumoricidal antibiotic used to treat testicular cancer, squamous cell carcinoma, and other malignancies. Limiting systemic side effects include mucocutaneous and pulmonary toxicities. Polymer-delivery of bleomycin for brain tumors has been limited to two reports, where the drug was loaded into a compressed form of lactose and encapsulated

with ethylcellulose [136,137]. When implanted into dog brains, the preparations demonstrated a release half-life of 11 days, with CSF bleomycin levels detected for up to 20 days. In a Wistar rat intracranial glioma model, the tablet inhibited tumor growth better than systemically delivered bleomycin. The preparation was then placed into six patients undergoing craniopharyngioma resection. The authors claimed that recurrence was prolonged in one patient.

4.3. Experience with other agents

4.3.1. Angiogenesis inhibitors

Angiogenesis is the process where new blood vessels form, and is important for the growth of tumors in humans [138]. Without angiogenesis, tumor size is limited to an asymptomatic few millimeters by diffusion of nutrients from the periphery, which results in an equilibrium between peripheral cell proliferation and central cell death. Once angiogenesis forms, nutrient delivery can reach the central cells, and tumor growth becomes exponential with potential for metastatic spread [139]. Since GBM is one of the most angiogenic of all tumors, the use of angiogenesis inhibition for the treatment of brain tumors has generated much interest.

One of the earliest attempts at local polymer-delivered antiangiogenesis agents involved heparin and cortisone [140], which in combination exhibit antiangiogenesis properties [141]. The drugs loaded in combination in EVAc copolymers were found to reduce VX2 carcinoma-induced angiogenesis in the rabbit cornea by 60% at 21 days after implantation ($P < 0.05$). In the same study, the drug combination loaded in PCPP:SA polymers inhibited growth in the rat flank 9L gliosarcoma model by 78% ($P < 0.05$).

Another antiangiogenesis agent is minocycline, a broad-spectrum antibiotic with known anticollagenase properties [142]. When loaded onto the EVAc polymer, it inhibited neovascularization in the rabbit cornea VX2 carcinoma model by a factor of 4.5, 4.4, and 2.9 on days 7, 14, and 21, respectively ($P < 0.05$ at all time points) [143]. In a follow-up study, the polymers were found to release minocycline in a sustained fashion for up to 90 days, with 55% of the drug released at that time point [30]. When minocycline was loaded up to 50% (w/w), the polymers were found to improve median survival from 13 to 69 days ($P < 0.001$) in rats treated simultaneously with tumor implantation in the rat intracranial 9L gliosarcoma model. When treated in the established rat intracranial 9L gliosarcoma model, minocycline-loaded polymers alone did not impact survival, but they did extend median survival by 43% when used in conjunction with surgical resection ($P < 0.002$), and by 90% with surgical resection and systemic BCNU. Recent work with 40–50% (w/w) minocycline-loaded PCPP:SA polymers suggests that their efficacy is significantly better than that of the EVAc polymers.

Squalamine, an aminosterol isolated from the dogfish shark, has been shown to exhibit antiangiogenesis properties by inhibiting tumor mitogen-induced endothelial cell proliferation [144]. EVAc copolymers loaded with 20% (w/w) squalamine have been shown to inhibit vascular ingrowth in the rabbit cornea VX2 tumor model. Currently, squalamine is being evaluated in clinical trials for a variety of advanced cancers [145]. Further work examining its efficacy in local polymer deliver is ongoing.

4.3.2. Dexamethasone

Vasogenic edema, a major source of morbidity in brain tumors, is induced by malignant gliomas secondary to breakdown of the BBB [108]. High dose corticosteroid therapy can significantly alleviate the edema [146], but systemic exposure lead to significant side effects, including diabetes mellitus, skin atrophy, ‘Cushingoid’ features, weight gain, hemorrhagic gastrointestinal ulcers, myopathies, osteoporosis, and pathological fractures [147]. When loaded 35% (w/w) onto the EVAc copolymer, clinically significant dexamethasone delivery was demonstrated both in vitro and in vivo in the rat brain for up to 21 days [148]. Concurrent plasma levels were noted to be low.

To assess efficacy, both systemic and local polymer-delivered dexamethasone was tested in the rat intracranial 9L gliosarcoma-induced model [29]. By assessing edema as percentage water weight, it was demonstrated that both intracranial dexamethasone-loaded polymer (79.15%; $P < 0.05$) and intraperitoneal injections (79.16%; $P < 0.05$) were more efficacious compared to controls (79.45%) and intraperitoneal polymer treatment (79.39%).

4.3.3. Immunotherapy

The role of local delivery of immunotherapy to combat malignant gliomas has mainly involved the use of cytokines. Cells of the immune system produce cytokines to generate and maintain an immune response. Examples include interleukins (IL), interferons (IFN), and colony-stimulating factors. Because of their genetic mutations, tumor cells express proteins that are foreign to the host, thereby rendering them vulnerable to an immune response. Efforts have been made in using local delivery of cytokines to brain tumors, in part because local extension is the main source of tumor morbidity and mortality, and in part because cytokines are largely not permeable to the BBB and carry with them systemic toxicity.

Two general strategies have been made in providing for local delivery of cytokines. The first is the sustained release of cytokine at the tumor site using irradiated tumor cells transduced to secrete the cytokine in a paracrine fashion. In one study testing the C57BL/6 mouse intracranial melanoma model, IL-2-transduced tumor cells were shown to generate an immune response to wild-type tumor via direct injection to the tumor site, but not in the flank; GM-CSF-transduced cells generated an immune response via flank injection, but not when directly injected intracranially [149]. Synergy was noted when intracranial IL-2-transduced cells and subcutaneously-injected flank GM-CSF-transduced cells were used together.

A follow-up study using the same animal model demonstrated several interesting findings [150]. First, rats with intracranial implantation of IL-2-transduced cells demonstrated increased survival compared to controls when challenged with tumor both intracranially and in sites distal to the brain. Second, after successful rejection of an initial tumor challenge, these animals also exhibited immunological memory by mounting an immune response and increasing survival in the face of a second tumor challenge, both intracranial and in sites distal to the brain. Conversely, identical or 10-fold larger doses of subcutaneously injected IL-2-transduced cells failed to elicit such memory responses. Finally, the study demonstrated with gene-knockout mice that the cells most responsible for the anti-tumor immune response were natural killer (NK) cells and not CD4+ T cells. Because flank injected IL-2 paracrine cells did not elicit a similar response, the study postulated that immune cells within the CNS had different cytokine requirements than their counterparts in the periphery.

Another study identified IL-12 as a potential candidate for local paracrine delivery [151]. In addition to its immune regulatory effects, IL-12 also exhibits antiangiogenesis properties. By challenging the rat intracranial 9L gliosarcoma model, tumor cells transduced with IL-12 were implanted intracranially. In vivo expression of IL-12 was confirmed by reverse transcriptase-polymerase chain reaction. Furthermore, in addition to prolonging survival, local paracrine delivery of IL-12 also induced immunological memory to the animals. A second injection of wild-type 9L gliosarcoma tumor cells also elicited an immune response.

Several experiments have recently examined the interaction between local paracrine immunotherapy and local polymer-delivered chemotherapy. Using the mouse intracranial F16-B10 melanoma model, an experiment demonstrated synergy between 10% (w/w) BCNU PCPP:SA polymers and local paracrine IL-2-transduced cells [152]. Seventy percent of the animals receiving combination therapy survived >72 days, compared to none (with a median survival of 15.8 days) in controls ($P = 0.0023$). When the local paracrine IL-2 therapy was combined with 1% (w/w) carboplatin PCPP:SA polymers, 80% of the animals survived >72 days, compared to none (with a median survival of 20.6 days) in controls ($P = 0.0001$) (Table 1). Histological examination of animals receiving combination therapy revealed rare degenerating tumor cells with a marked mixed inflammatory reaction on post-implantation day 14, and no tumor cells and resolution of the inflammatory reaction on day 72.

Another strategy for sustained delivery of cytokines involves their incorporation into polymers. In 1998, murine IFN-α/β were loaded onto EVAc polymers. In vitro and in vivo experiments demonstrated that the released IFNs were biologically active. In vitro assays determined most of the activity was released within the first 4 days. In vivo trials demonstrated most of the activity was released within the first 24 h with a gradual decrease over the next 3 days.

Recently, attention has shifted to incorporation of

Table 1
Efficacy of BCNU-loading polymers and carboplatin-loaded polymer with or without local IL-2 immunotherapy

Experiment[a]	Median survival (days)	Range (days)	LTSs[b] (%)	*P*-value[c]
1				
Group 1 (control, $n = 10$)	25.7	19–54	0	
Group 2 (3.8% BCNU, $n = 10$)	19	18–20	0	0.06 versus control
Group 3 (IL-2 alone, $n = 10$)	34.5	22–70	0	0.18 versus control
Group 4 (combination, $n = 10$)	41.9	27–90	10	0.05 versus control
				0.14 versus IL-2
				0.02 versus BCNU
2				
Group 1 (control, $n = 10$)	20.6	19–27	0	
Group 2 (20% BCNU, $n = 10$)	125.4	14–160	30	0.0023 versus control
Group 3 (IL-2 alone, $n = 10$)	39.4	14–160	20	0.034 versus control
Group 4 (combination, $n = 10$)	118.7	42–160	30	0.0018 versus control
				0.55 versus IL-2
				0.86 versus BCNU
3				
Group 1 (control, $n = 9$)	15.8	13–18	0	
Group 2 (10% BCNU, $n = 10$)	26.4	22–39	0	< 0.001 versus control
Group 3 (IL-2 alone, $n = 9$)	33.8	13–70	11	0.077 versus control
Group 4 (combination, $n = 10$)	Not reached	18–70	70	0.0023 versus control
				0.042 versus IL-2
				0.0033 versus BCNU
4				
Group 1 (control, $n = 10$)	20.6	19–27	0	
Group 2 (1% carboplatin, $n = 10$)	23.3	13–40	0	0.26 versus control
Group 3 (IL-2 alone, $n = 10$)	38.4	14–160	30	0.035 versus control
Group 4 (combination, $n = 10$)	Not reached	22–160	80	0.01 versus control
				0.017 versus IL-2
				0.002 versus carboplatin

[a] In each experiment, combination therapy included drug–polymer with IL-2 treatment. Experiment 1, 3.8% BCNU-loaded polymer; experiment 2, 20% BCNU-loaded polymer; experiment 3, 10% BCNU-loaded polymer; experiment 4, 1% carboplatin-loaded polymer.
[b] Long-term survivors.
[c] Using nonparametric (Kruskal–Wallis) statistical analyses.

cytokines into gelatin-chondroitin sulfate (GCS) microspheres [77] and delivered by an injectable mixture. For example, a recent study [153] encapsulated IL-2 into GCS and confirmed that the mixture maintained a sustained release of activity over 2 weeks in vitro and up to 3 weeks in vivo. Using the rat intracranial 9L gliosarcoma model, the mouse intracranial B16-F10 melanoma model, and the mouse liver CT26 carcinoma model, the study then demonstrated statistically increased effectiveness in generating a protective immune response when injecting the GCS–IL-2 mixture into tumor, when compared to controls or local paracrine delivery. In the B16-F10 model, 42% of animals exhibited protection on a second tumor challenge.

Local immunotherapy is an area of active research. Continued work is ongoing.

4.3.4. Other agents

Radiosensitizers, such as 5-iodo-2′-deoxyuridine (IudR), which act by replacing thymidine in replicating DNA, have been incorporated into PCPP:SA polymers and tested in animal models as adjuvants to radiation therapy [154,155]. Anticonvulsants, such as phenytoin, have been successfully incorporated into the EVAc copolymer, and shown to decrease cobalt-induced seizures in Sprague–Dawley rats [156]. Neither class of drug has seen clinical use in conjunction with local-polymer drug delivery.

5. Future directions

Polymer-based delivery systems represent a 'proof of principle' that controlled drug delivery improves the treatment of brain tumors. Active investigation is ongoing in several other exciting approaches to sustained drug delivery in the brain.

5.1. Convection-enhanced delivery systems

An area of active current research is fluid convection. In tissue, compounds travel by diffusion, which is dependent solely on the free concentration gradient and the diffusivity of the compound in the tissue. Convection, which can be used to supplement diffusion, relies on a simple pressure gradient, and is independent of molecular weight. When a drug is infused into the cerebral white matter, a pressure gradient is created, and can be used to introduce high concentrations of drug throughout the brain without structural or functional side-effects [157–159]. Primate trials have been conducted using convection-enhanced drug delivery (CEDD) to treat Parkinsonian symptoms [160]. Recently, a study used CEDD of Taxol to treat three brain tumor patients [161]. The study focused on the use of diffusion-weighted MRI (DWMRI) to monitor the effects of drug delivery.

5.2. Microchip drug delivery

A novel method of drug delivery with significant clinical potential is the use of newly developed microchips (Fig. 7A) [162]. Based on a solid-state silicon microchip that provides controlled release of multiple microreservoirs, this powerful system provides for single or multiple agent delivery. The release mechanism is based on the electrochemical dissolution of a thin anode membrane covering each microreservoir, which can be filled with solids, liquids, or gels. The time each reservoir is scheduled to deliver its contents can be programmed independently, thus providing for a seemingly endless array of release profiles and therapy combinations. The device is an integrated circuit, thus capable of providing its own microbattery, memory, and multiplexing circuitry. It can be implanted surgically, mounted on a tip of a small probe, or even swallowed. Alternative biodegradable 'passive chips' (Fig. 7B) are also being developed, where the release mechanism of each microreservoir is based on slow degradation of a thin polymeric membrane covering each reservoir of drug. As ongoing work continues, this 'pharmacy-on-a-chip' may be used to deliver up to 1000 different drugs on demand.

6. Conclusions

Tumors of the CNS have represented a significant pharmacologic and clinical challenge. The physiological barriers that sequester the CNS from the rest of the body often makes difficult the goal of delivering high concentrations of antineoplastic agents within the tumor bed without causing unacceptably toxic systemic levels of drug. The development of biodegradable polymers has allowed a new approach to treating brain tumors. Gliadel®, the BCNU-loaded PCPP:SA polymer, represents not only the first successful drug developed from this technology, but also the first new treatment approved by the FDA for malignant gliomas in 23 years. Multiple clinical trials have demonstrated its safety and efficacy.

With the development of Gliadel® as a model, many other new drug–polymer combinations are currently undergoing active study. Other investigations are focusing on combinations of local-delivery approaches, such as the promising mix of immunotherapy and local-delivered chemotherapy. Still newer technologies, such as the microchip, are being studied and developed, which hopefully may supplant the contributions that polyanhydride polymers have made in local drug delivery.

The potential for this technology to change the future neurosurgical treatment of malignant gliomas is significant. In the near future, perhaps when a patient diagnosed with a brain tumor undergoes an operative resection/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 dexamethasone to treat cerebral edema. 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. The microch-

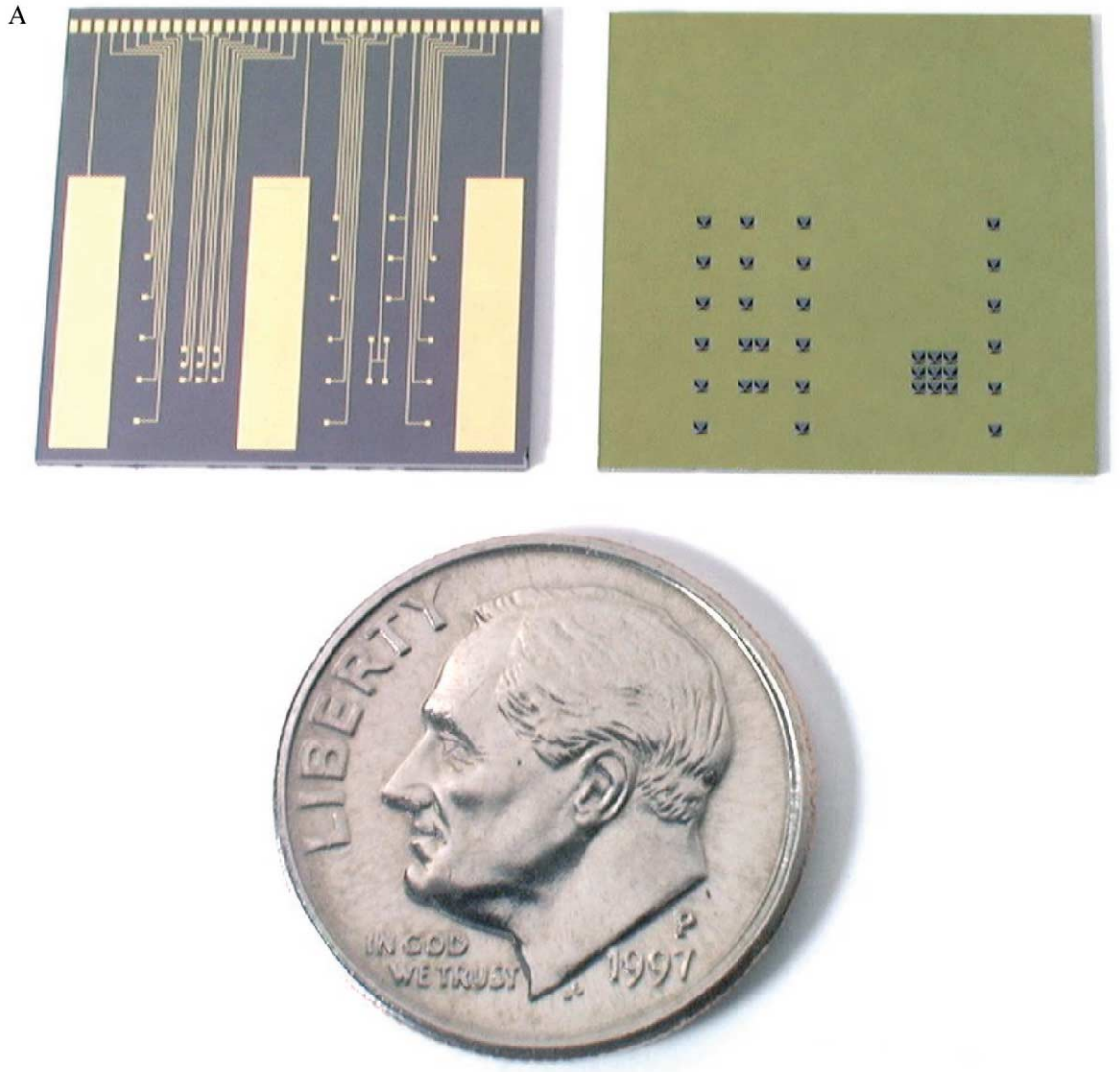

Fig. 7. (A) Microchip with dime caption. Front (left) and back views of a new microchip for controlled local release of chemicals. The dots between the three large bars (cathodes) on the front are the caps (anodes) covering the reservoirs holding the chemicals. Electrical voltage applied between the 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.) Photograph by Paul Horwitz, Atlantic Photo Service, Inc. (B) Schematic of the passive microchip. Initial models will use PLGA and other existing polymer matrices for the substrate. The entire chip will be biodegradable.

ip(s) would then be implanted in the tumor cavity intraoperatively. Other portions of the tumor could be saved for in vitro testing to determine sensitivity to a wide battery of chemotherapy agents. The most promising drugs could be loaded onto polymer microspheres and injected into the tumor site in a separate stereotactic procedure to augment treatment. Should there be a recurrence, stereotactic biopsy for diagnosis could be followed by the implantation of angiogenesis inhibitors and further chemotherapy agents in microspheres. If chip technology and miniaturization continues to improve, perhaps the

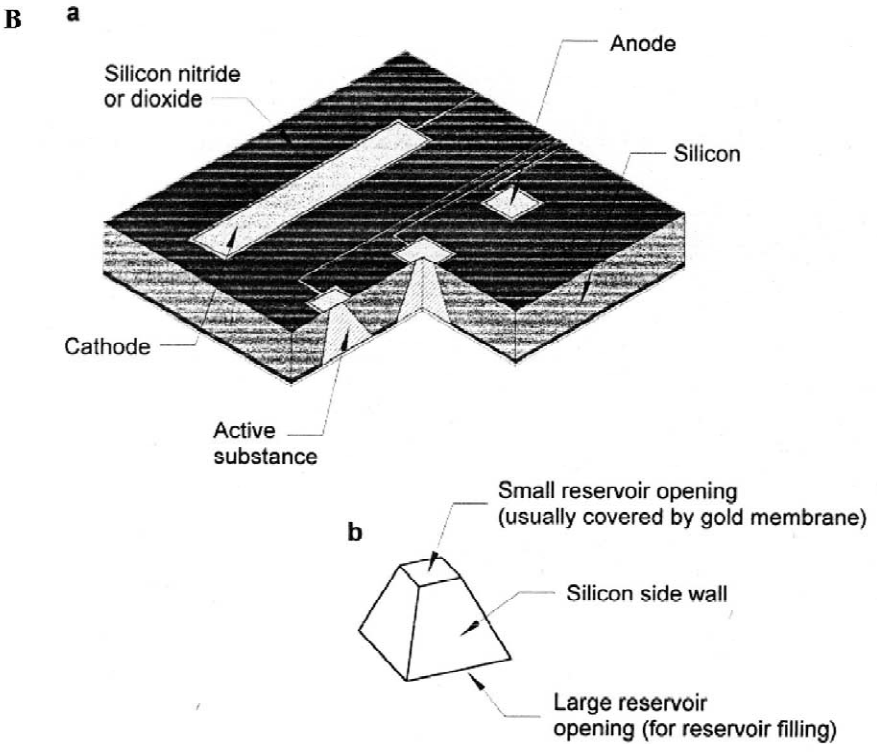

Fig. 7. (*continued*)

functionality of the microchip could be incorporated into nanotechnology; minute devices that can be implanted stereotactically with the microchip's functionality may be part of a neurosurgeon's arsenal. Such exciting possibilities for both the patient and physicians have been made possible by the development of local and controlled drug delivery to the central nervous system.

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