Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Controlled Release 106 (2005) 138–145
journal of controlled release
www.elsevier.com/locate/jconrel

In vivo delivery of BCNU from a MEMS device to a tumor model

Yawen Li[a], Hong Linh Ho Duc[a], Betty Tyler[b], Tiffany Williams[b], Malinda Tupper[a], Robert Langer[c], Henry Brem[b], Michael J. Cima[a,*]

[a]Department of Materials Science and Engineering, Massachusetts Institute of Technology, Cambridge MA 02139, USA
[b]Departments of Neurological Surgery and Oncology, Johns Hopkins University School of Medicine, Baltimore MD 21205, USA
[c]Department of Chemical Engineering, Massachusetts Institute of Technology, Cambridge MA 02139, USA

Received 14 February 2005; accepted 12 April 2005
Available online 23 May 2005

Abstract

A drug delivery micrcoelectromechanical systems (MEMS) device was used to locally deliver a chemotherapeutic agent (BCNU) to an experimental tumor in rats. This MEMS device consists of an array of reservoirs etched into the silicon substrate. The drug release is achieved by the electrochemical dissolution of the gold membranes covering the reservoirs. A new Pyrex package was developed to improve the BCNU release kinetics and enhance device capacity. Co-formulation of BCNU with polyethylene glycol (PEG) led to complete and rapid release of drug in vivo. BCNU delivered from the MEMS device showed dose-dependent inhibiting effect on the tumor growth in the BCNU dosage range of 0.67~2 mg. BCNU delivered from the activated devices was as effective as equipotent subcutaneous injections of BCNU in inhibiting tumor growth. Further optimization using this MEMS device to deliver BCNU in combination with other therapeutic agents against the tumor challenge is possible because of the unique capability of the device to precisely control the temporal release profiles of multiple substances.

Keywords: Microelectromechanical systems (MEMS); Drug delivery; BCNU; Tumor; Release kinetics

1. Introduction

1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU), also called carmustine, is a member of the most extensively used chemotherapeutic agents for brain tumors [1]. Clinical use of BCNU by systemic delivery is dose limited because high systemic BCNU concentration can cause serious side effects such as myelosuppression, hepatic toxicity, and pulmonary fibrosis [2]. Local delivery by BCNU-impregnated biodegradable polymer wafers (GLIADEL®) has provided a novel approach to circumvent the systemic toxicity and has demonstrated in Phase III clinical trials, a statistically significant improvement in 6-month survival with human primary brain tumors, both at recurrence [3] and at initial presentation [4,5].

* Corresponding author. Tel.: +1 6172536877; fax: +1 6172586936.
E-mail address: mjcima@mit.edu (M.J. Cima).

0168-3659/$ - see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.jconrel.2005.04.009

Microelectromechanical systems (MEMS) devices represent an alternative to polymer-based systems and offer the advantage of extremely precise control of the drug delivery regimen. One example is a silicon-based drug delivery MEMS device developed in this laboratory [6]. This MEMS device exhibits the ability to locally deliver multiple substances with uniquely precise control of the temporal release profiles [7]. As some recent studies are aiming to deliver BCNU in combination with some immunotherapeutic agents in order to expand the potential benefits of polymeric delivery for brain tumors [8,9], the challenge is to precisely control the dosages and delivery profiles of multiple drug combinations using the biodegradable polymer systems. The MEMS device, on the other hand, may provide a better platform to directly deliver multiple drug combinations and to optimize the delivery regimen for optimum efficacy. Furthermore, the device could be left in place at the first opening and activated at a later, appropriate time. This degree of control is not feasible with a simple polymer system.

The release of ^{14}C labeled BCNU delivered from the MEMS device has been demonstrated in a rat flank model using a mechanically sealed package design. The in vivo ^{14}C-BCNU release kinetics have been found to be slightly slower compared to subcutaneous injections and in vitro controls [10]. Only 40% of the payload was recovered from activated devices in that initial study. Subsequent examination of the explanted devices demonstrated that the small molecule BCNU (MW 214) had permeated into the neoprene gasket used in the stainless steel frame package (unpublished).

In this study, a new packaging method for the MEMS device was introduced that significantly improved the percentage of payload release. The effect of drug formulation on the release kinetics of BCNU was studied in vitro. The new package and drug formulation were then used to examine efficacy of BCNU delivered from the MEMS device to an experimental tumor model in rats.

2. Materials and methods

2.1. Device fabrication and packaging

The devices were fabricated using a process described previously [6]. The package design is shown schematically in Fig. 1. A Pyrex plate (500 μm thick) was machined in a sandblasting system (Micro Jet 200, Hunter Products, Bridgewater, NJ) using aluminum oxide as abrasive media to form two rectangular through-thickness macroreservoirs. Each macroreservoir covered 10 microreservoirs on either side of the cathode on the MEMS device. Channels leading to the macroreservoirs were cut into another Pyrex plate with machined macroreservoirs to allow filling of the packaged devices. One Pyrex plate with machined macroreservoirs was anodically bonded to the bottom side of the MEMS device (300 °C for 25 min under − 1000 V DC potential) in a custom-built fixture. UV epoxy (1-20542 cationic epoxy, Dymax Corporation, Torrington, CT) was used to bond the Pyrex plates. Wirebonds were used to connect the bond pads on the device to a connector board and Teflon coated cables, and were mechanically protected and electrically isolated with UV epoxy.

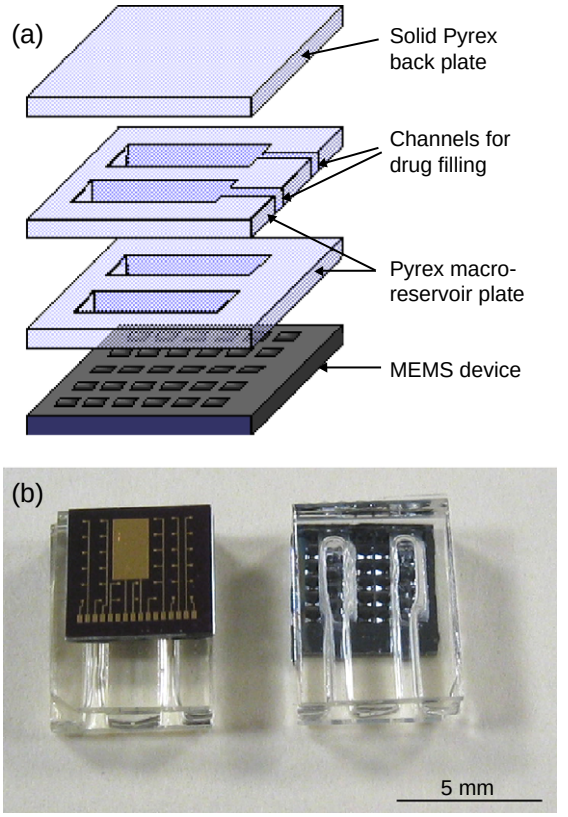

Fig. 1. (a) Schematic showing the Pyrex package design. (b) Photograph showing the top and bottom of an assembled device.

Devices were filled with mixed solutions of BCNU (Bristol-Myers Squibb Co., Evansville, IN), ^{14}C-BCNU (Moravek Biochemicals, Brea, CA), and polyethylene glycol (PEG, MW 400), using a microinjector (UltraMicroPump II, World Precision Instruments, Sarasota, FL) and 32 gauge microneedles (Hamilton Company, Reno, NV).

2.2. Leak test

One Pyrex packaged device was filled with a 2 μl mixed solution of BCNU and PEG at 80:20 volume ratio and with a total radioactivity of 0.25 μCi ^{14}C-BCNU. The device was immersed in deionized water at room temperature. One milliliter samples were taken at predetermined intervals, and the testing medium replenished with 1 ml water after removal of each aliquot. The sample ^{14}C content was analyzed by scintillation counting on a Packard Tri-Carb liquid scintillation analyzer (Model 2200CA, Perkin-Elmer Life Sciences, Downers Grove, IL). Raw DPM (disintegrations per minute) data were converted to activity in microCuries (μCi) by a conversion factor of 2.2×10^6 DPM/μCi. The percentage of the total payload (determined by scintillation counting of identically filled, unpackaged devices) leaked into the release medium was calculated for each aliquot.

2.3. In vitro release study

Devices filled with BCNU and PEG at different volume ratios were activated in the phosphate buffered saline (PBS) solution (0.15 M Cl^-, pH 7.2) using a potentiostat (Gamry PC4-300, Warminster, PA). The applied voltammetry included a 4-cycle cathodic cleaning from -1.0 to -1.5 V, a 4-cycle diagnostic scan from 0 to 1.5 V, and a 10-min square wave voltammetry between 0 and 0.8 V. All potentials were relative to a blackened Pt wire placed approximately 5 mm away from the device in the same PBS-containing glass vial. One milliliter samples were taken at predetermined intervals and 1 ml PBS was added to the release medium after removal of each aliquot. Detection of the sample ^{14}C content and calculation of the payload release percentage followed the same method described in the leak test.

2.4. Tumor effect study

The animal study protocol was approved by the Animal Care and Use Committee of the Johns Hopkins University School of Medicine before implementation of any procedure.

2.4.1. Animals

Female rats (Fisher 344, weight 150~200 g) were purchased from the Charles River Laboratories (Wilmington, MA). Some rats were housed in normal cages, while others were housed individually in metabolic cages (Nalgene model # 650-0100, Braintree Scientific Inc., Braintree, MA). The metabolic cage features a unique funnel and cone design that effectively separates feces and urine into tubes outside the cage. Animals were given standard rat chow and water ad libitum. Animals housed in metabolic cages were given sugar water (4 tsp/500 ml), which encouraged the animals to drink more and allowed more frequent collection of urine samples.

2.4.2. Tumor implantation

The 9L glioma, an experimental gliosarcoma syngeneic to the Fisher 344 rat, was obtained from the Brain Tumor Research Center, University of California, San Francisco, and maintained in the flank of carrier rats. The tumor in carrier rats was passaged every 2–3 weeks. Animals were anesthetized prior to implantation using an intra-peritoneal injection of a stock solution containing ketamine hydrochloride (25 mg/ml), xylazine (2.5 mg/ml), and 14.25% ethyl alcohol and diluted with normal saline. Solid 9L tumor masses removed from the anesthetized carrier rat were cut into 2 mm^3 pieces. Each rat in the tumor effect study was implanted with one piece of tumor in the flank.

2.4.3. Animal grouping and treatment

Forty-eight rats were randomized into 8 groups ($n=6$) 10 days after tumor implantation. Three groups received drug delivery devices implanted adjacent to the tumor. A mixed solution of BCNU and PEG (at 50:50 volume ratio) was used to fill these devices with three BCNU dosages (0.67, 1.2, and 2 mg). The surgical site for each rat was shaved and prepared with a 70% ethanol- and iodine-containing solution. Ster-

ilized devices were implanted subcutaneously next to the established tumor with the electrodes facing the tumor and the cable exiting through the incision. The incision was closed with autoclips.

Animals were re-anesthetized on scheduled days (Days 11 and 16 post tumor implantation) and devices were activated to release half of the filled BCNU dosage. The original incision was prepped and opened to allow electrical connection to the potentiostat. Device activation was performed using the same protocol established earlier [10], which included four cathodic cleaning cycles from −1.0 to −1.5 V, four diagnostic scan cycles from 0 to 1.8 V, and 10-min square wave voltammetry between 0 and 1.2 V, all relative to a blackened Pt wire reference electrode. Following activation, the cable was cut flush at the end and returned to the subcutaneous pocket and the incision was closed with autoclips.

The other 5 groups of animals served as controls, including one group that received no treatment, one group that received the drug delivery devices filled with 2 mg BCNU but not activated, and 3 groups that received subcutaneous injection of the same BCNU/PEG mixture (at 50:50 volume ratio) with three target BCNU dosages (0.67, 1.2, and 2 mg). Because of the difficulty in precisely controlling the injected volume manually using a 10 μl syringe, the actual dosages that the injected control rats received were higher than the target values. The actual amount of the injected dosage was estimated by scintillation counting of the manual injections.

2.4.4. Tumor size evaluation

The tumor size for different treatment groups was evaluated using calipers three times a week and the tumor volume calculated using the approximation of an ellipsoid:

$$V_{\text{tumor}} = \text{Length} \times \text{Width} \times \text{Height} \times \pi/6 \quad (1)$$

Animals were checked daily for physical or behavioral evidence of toxicity, such as decreased alertness, impaired grooming, or gait disturbances. If any deficits developed, the animals would be euthanized by CO_2 inhalation and all organs removed for histological examination. The end point of the tumor effect study was set at 22 days post tumor implantation. Animals were euthanized after experiments were completed by either CO_2 inhalation or a sodium pentobarbital overdose (200 mg/kg, to effect). Devices were explanted and examined under an optical microscope (Olympus BH-2).

The final tumor size data of all device groups were compared to those of the control groups. Statistical analysis was performed using the unpaired, two-tailed Student *t*-test. A *p*-value of less than 0.05 was considered statistically significant.

Excreted radioactivity from each rat housed individually in metabolic cages was also analyzed to correlate with the tumor size measurement. Only urine samples were taken after an initial trial showed no radioactivity recovered in fecal samples. One ml urine sample was mixed with 5 ml scintillation fluid (Ready Gel, Beckman Coulter Inc., Fullerton, CA) in a 7 ml scintillation vial and measured on a liquid

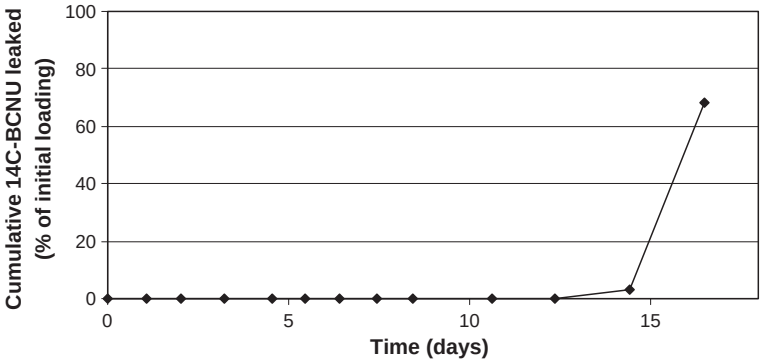

Fig. 2. Leak test of one Pyrex packaged device filled with 2 μl mixed solution of BCNU and PEG at 80:20 volume ratio and with a total radioactivity of 0.25 μCi ^{14}C-BCNU. Test performed at room temperature in deionized water.

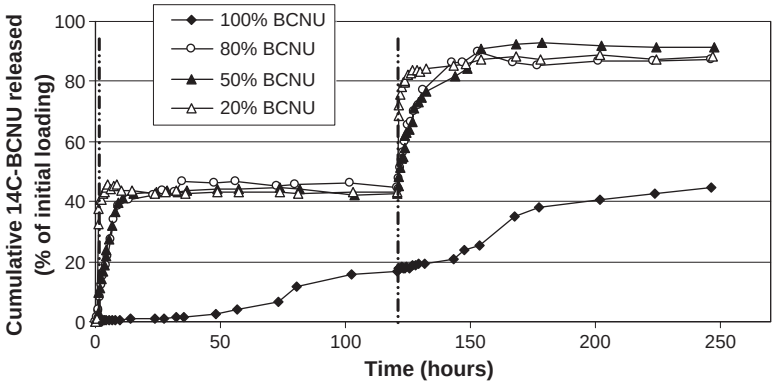

Fig. 3. Cumulative percentages of ^{14}C-BCNU released from four Pyrex packaged devices. Each activation (denoted by the dashed line) corresponds to opening of 10 microreservoirs with half of the initial loading. Each device was filled with a mixed solution of BCNU/PEG with different BCNU/PEG volume ratio. The BCNU loading was 1.2 mg in each of the three devices (100% BCNU, 80% BCNU and 50% BCNU) and 0.96 mg in the device with 20% BCNU. Test performed at room temperature in PBS.

scintillation counter (Beckman Coulter Inc., Fullerton, CA).

3. Results

3.1. Leak test

Fig. 2 shows the leak test result. The Pyrex packaged device showed good hermeticity up to 12 days soaking in water. The abrupt increase in the leakage percentage with prolonged soaking was accompanied by the observation of small bubbles evolving from the interface between the Pyrex plates on one side of the package.

3.2. In vitro release kinetics of BCNU

Fig. 3 shows the cumulative release of ^{14}C-BCNU from four Pyrex packaged devices in vitro. Approximately 40% payload release and sluggish kinetics were observed for the device filled with 100% BCNU. In contrast, the addition of PEG to the BCNU formulation significantly enhanced the BCNU release kinetics. The three devices filled with mixed solutions of BCNU and PEG showed close to 100% recovery of the total radioactivity. The time to reach equilibrium ^{14}C level was fastest for the device filled with 80% PEG (approximately 6 h), and slightly longer for the other two devices filled with 50% and 20% PEG (15~20 h).

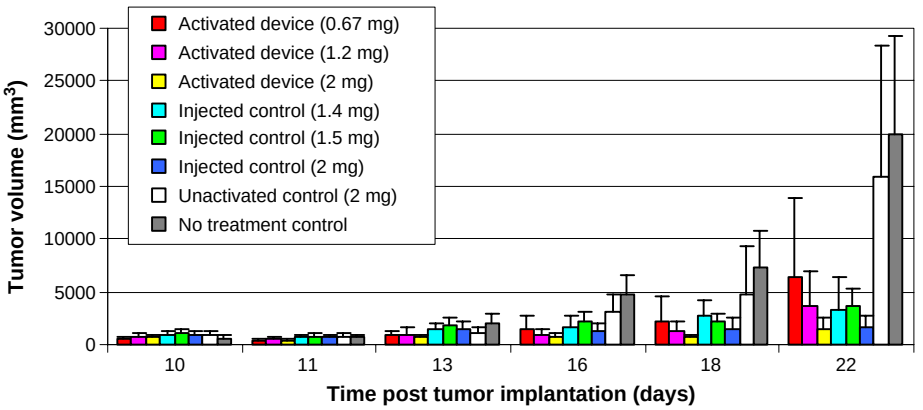

Fig. 4. Tumor size measurement for different treatment groups. Values represent mean ± SD of measurements from 6 rats. Numbers in parenthesis represent BCNU dosages.

Table 1
Urinary recovery of ^{14}C from rats with activated devices and subcutaneous injections

	BCNU dosage (mg)	Urinary recovery of ^{14}C (% of initial loading)
Activated device groups	0.67	49 ± 12
	1.2	50 ± 12
	2	54 ± 16
Injected control groups	1.4	45 ± 1
	1.5	53 ± 4
	2	71 ± 12

3.3. Tumor growth

The tumor growth data for different treatment groups are shown in Fig. 4. The groups that received activated devices or subcutaneous injections showed significantly smaller tumor sizes ($p < 0.05$) than the untreated control group, and almost significantly smaller tumor sizes (p close to 0.05) than that of the control group receiving unactivated devices. A dose-dependent inhibiting effect on the tumor growth was also observed for both the activated groups and injected control groups. A higher dosage of BCNU delivered led to a stronger inhibiting effect on the tumor growth, although quantitatively the tumor size difference between different dosage groups was not significant ($p > 0.05$). It is also interesting to note comparable tumor size between the activated groups and injected control groups that received the same dosage of BCNU.

Table 1 compares the percentage of ^{14}C excreted for the activated device groups and injected control groups. The urinary recovery of ^{14}C for the three injected control groups was $57 \pm 13\%$ of the total injected loading. This amount was slightly lower than the reported value (78%) in literature [11], but was consistent with the urinary recovery data (60% of total ^{14}C loading) in our previous release kinetics measurements (unpublished). The excreted radioactivity from the rats with activated devices reached $51 \pm 12\%$, which was very close to the value obtained for the injected control groups.

4. Discussion

Results from this study have demonstrated the efficacy of BCNU released from the MEMS drug delivery device in an experimental flank tumor model. Improvements in the device packaging and drug formulation were critical to the success of this tumor effect study.

The in vitro release results in Fig. 3 clearly showed that co-formulation with PEG greatly enhanced the BCNU release kinetics, in terms of both the percentage of payload release and the time to reach equilibrium ^{14}C levels. The sluggish release kinetics from the device filled with 100% BCNU was most likely due to the high lipophilicity of BCNU. The transport of this oily compound through the 50 μm opening could be slow in the aqueous medium. On the other hand, the presence of PEG, a highly water soluble polymer, helped to drive the outward diffusion of BCNU when the membranes were opened, leading to more complete and rapid release of payload. This explanation is also consistent with the observed faster release kinetics for the device filled with 80% PEG compared to the other devices filled with less PEG. The closeness in the urinary recovery of ^{14}C between the activated device groups and the injected control groups indicates that the payload release from the Pyrex packaged devices filled with a mixture of BCNU and PEG (at 50 : 50 volume ratio) was complete in vivo.

Compared to the stainless steel frame package, the Pyrex package design eliminated the need for a gasket and achieved complete release of payload. Moreover, the capacity of the Pyrex packaged devices (approximately 9 μl) was increased tenfold with respect to the stainless steel frame packaged devices (0.85 μl), due to the addition of the two Pyrex macroreservoirs. Interestingly, the overall volume of the Pyrex package (approximately 100 mm^3) was smaller than the stainless steel frame package (approximately 300 mm^3). It is generally desirable to reduce the size of the packaged device to decrease the invasiveness and trauma caused by implantation. One limitation of this Pyrex package is that only two release events can be achieved with the current design. Such a design was compatible with the tumor efficacy study reported in this paper with two independent BCNU releases. However, it is possible to modify the design and increase the number of macroreservoirs on one Pyrex plate if more independent release events are desired. Another limitation of the current Pyrex pack-

age is its inability to achieve long term hermeticity. The abrupt increase in the leakage percentage after 12 days soaking in water (Fig. 2) suggests that the bond formed by the UV epoxy may have been compromised by the prolonged exposure to BCNU. Thermal bonding is currently being investigated to replace the UV epoxy in order to achieve long-term hermeticity of the package.

Rats with activated devices showed comparable tumor growth data to rats with subcutaneous injections, suggesting that the device packaging, shipping and gold membrane corrosion processes did not reduce the therapeutic benefit of BCNU delivered from the activated devices. The antitumor activity of BCNU arises from its alkylating decomposition products that induce DNA crosslinking and kill cells [12]. Sealing of the MEMS device after BCNU filling was achieved with 20-s UV exposure. Our analysis using a colorimetric assay [13] showed that up to 1-min UV exposure did not change the activity of the BCNU (unpublished). It is unclear whether the electrochemical corrosion process had an effect on the decomposition of BCNU contained inside the reservoirs. Nevertheless, the similar antitumor activity observed for BCNU delivered from the activated drug delivery devices as compared to that delivered by subcutaneous injection indicates that the device packaging and activation were not detrimental in this case. Appropriate in vitro assays will be necessary to evaluate these effects for other therapeutic agents in order to ensure that the drugs remain active prior to release.

The BCNU release from the MEMS device after each activation in this study followed a pulsatile temporal profile similar to the subcutaneous injection. The delivery of BCNU from a biodegradable polymer matrix follows a sustained release pattern [14,15]. Sustained delivery of BCNU from both the GLIADEL® wafer and poly (L-lactide co-glycolide) (PLG) microspheres have been shown to be statistically more effective than the equipotent bolus injection of BCNU to enhance survival in rat glioma models [16–18]. Further experiments are needed to determine if there is any statistically significant difference in the efficacy between the pulsatile release from the MEMS device and the sustained release from the polymer systems in this flank tumor model. On the other hand, sustained delivery of BCNU is also possible using the MEMS device through modifying the packaging to allow more independent release events, whose release profiles can superimpose to approach a sustained pattern. Modification of the drug formulation provides additional flexibility to alter the rate at which BCNU is delivered from the MEMS device to the tumor site. Further study will evaluate the efficacy of delivering BCNU combined with other therapeutic agents to the rat flank tumor, and exploit the complex release capabilities of this MEMS device for development of an efficacious drug regimen against the malignant tumor challenge.

5. Conclusions

BCNU delivered from the MEMS device was efficacious in inhibiting the 9L flank tumor growth in the rats. Co-formulation with PEG greatly enhanced the release kinetics of BCNU in vitro and in vivo. The inhibiting effect of BCNU on tumor growth was dose-dependent in the range of 0.67~2 mg investigated in this study. Rats receiving activated devices showed similar retarded tumor growth to rats receiving subcutaneous injections. These results provided preliminary efficacy validation of the drug delivery device as well as important dosage information for further efficacy evaluation of the combination therapy using the MEMS device.

Acknowledgements

This work was funded by The U.S. National Institute of Health grant No. 1 R24 AI47739-01 and U19-CA52857. All microfabrication work was carried out at the Microsystems Technology Laboratory at MIT.

Henry Brem and the Johns Hopkins University own Guilford Pharmaceutical stock, which is subject to certain restrictions under University policy. Henry Brem is also 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 policies. Michael Cima and Robert Langer are shareholders in MicroCHIPS, Inc., Bedford, Massachusetts.

References

[1] E.R. Barnhart, B.B. Huff, Physicians' Desk Reference, edition 48, Medical Economics Company, Oradell, NJ, 1988.

[2] P.L. Kornblith, M. Walker, Chemotherapy for malignant gliomas, Journal of Neurosurgery 68 (1) (1988) 1–17.

[3] H. Brem, S. Piantadosi, P.C. Burger, M. Walker, R. Selker, N.A. Vick, K. Black, M. Sisti, S. Brem, G. Mohr, Placebo-controlled trial of safety and efficacy of intraoperative controlled delivery by biodegradable polymers of chemotherapy for recurrent gliomas. The Polymer-Brain Tumor Treatment Group, Lancet 345 (8956) (1995) 1008–1012.

[4] S. Valtonen, U. Timonen, P. Toivanen, H. Kalimo, L. Kivipelto, O. Heiskanen, G. Unsgaard, T. Kuurne, Interstitial chemotherapy with carmustine-loaded polymers for high-grade gliomas: a randomized double-blind study, Neurosurgery 41 (1) (1997) 44–48.

[5] M. Westphal, D.C. Hilt, E. Bortey, P. Delavault, R. Olivares, P.C. Warnke, I.R. Whittle, J. Jaeaeskelaeinen, Z. Ram, A phase 3 trial of local chemotherapy with biodegradable carmustine (BCNU) wafers (gliadel wafers) in patients with primary malignant glioma, Neuro-Oncology (Durham, NC, United States) 5 (2) (2003) 79–88.

[6] J.T. Santini Jr., M.J. Cima, R. Langer, A controlled-release microchip, Nature 397 (6717) (1999) 335–338.

[7] J.T. Santini Jr., A.C. Richards, R. Scheidt, M.J. Cima, R. Langer, Microchips as controlled drug-delivery devices, Angewandte Chemie, International Ed. 39 (14) (2000) 2396–2407.

[8] L.D. Rhines, P. Sampath, F. DiMeco, C. Lawson, B.M. Tyler, J. Hanes, A. Olivi, H. Brem, Local immunotherapy with interleukin-2 delivered from biodegradable polymer microspheres combined with interstitial chemotherapy: a novel treatment for experimental malignant glioma, Neurosurgery 52 (4) (2003) 872–879.

[9] P. Sampath, J. Hanes, F. DiMeco, B.M. Tyler, D. Brat, D.M. Pardoll, H. Brem, Paracrine immunotherapy with interleukin-2 and local chemotherapy is synergistic in the treatment of experimental brain tumors, Cancer Research 59 (9) (1999) 2107–2114.

[10] Y. Li, R.S. Shawgo, B. Tyler, P.T. Henderson, J.S. Vogel, A. Rosenberg, P.B. Storm, R. Langer, H. Brem, M.J. Cima, In vivo release from a drug delivery mems device, Journal of Controlled Release 100 (2) (2004) 211–219.

[11] V.T. DeVita, C. Denham, J.D. Davidson, V.T. Oliverio, The physiological disposition of the carcinostatic 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU) in man and animals, Clinical Pharmacology and Therapeutics 8 (4) (1967) 566–577.

[12] K.W. Kohn, Interstrand cross-linking of DNA by 1,3-bis(2-chloroethyl)-1-nitrosourea and other 1-(2-haloethyl)-1-nitrosoureas, Cancer Research 37 (5) (1977) 1450–1454.

[13] L.T. Loo, R.L. Dion, Colorimetric method for the determination of 1,3-bis(2-chloroethyl)-1-nitrosourea, Journal of Pharmaceutical Sciences 54 (5) (1965) 809–810.

[14] H. Brem, Polymers to treat brain tumors, Biomaterials 11 (9) (1990) 699–701.

[15] A.B. Fleming, W.M. Saltzman, Pharmacokinetics of the carmustine implant, Clinical Pharmacokinetics 41 (6) (2002) 403–419.

[16] K.G. Buahin, H. Brem, Interstitial chemotherapy of experimental brain tumors: comparison of intratumoral injection versus polymeric controlled release, Journal of Neuro-Oncology 26 (2) (1995) 103–110.

[17] D.F. Emerich, S.R. Winn, Y. Hu, J. Marsh, P. Snodgrass, D. LaFreniere, T. Wiens, B.P. Hasler, R.T. Bartus, Injectable chemotherapeutic microspheres and glioma: I. Enhanced survival following implantation into the cavity wall of debulked tumors, Pharmaceutical Research 17 (7) (2000) 767–775.

[18] D.F. Emerich, S.R. Winn, P. Snodgrass, D. LaFreniere, M. Agostino, T. Wiens, H. Xiong, R.T. Bartus, Injectable chemotherapeutic microspheres and glioma: II. Enhanced survival following implantation into deep inoperable tumors, Pharmaceutical Research 17 (7) (2000) 776–781.