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Intracranial microcapsule drug delivery device for the treatment of an experimental gliosarcoma model

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ABSTRACT

Controlled-release drug delivery systems are capable of treating debilitating diseases, including cancer. Brain cancer, in particular glioblastoma multiforme (GBM), is an extremely invasive cancer with a dismal prognosis. The use of drugs capable of crossing the blood–brain barrier has shown modest prolongation in patient survival, but not without unsatisfactory systemic, dose-limiting toxicity. Among the reasons for this improvement include a better understanding of the challenges of delivery of effective agents directly to the brain tumor site. The combination of carmustine delivered by biodegradable polyanhydride wafers (Gliadel®), with the systemic alkylating agent, temozolomide, allows much higher effective doses of the drug while minimizing the systemic toxicity. We have previously shown that locally delivering these two drugs leads to further improvement in survival in experimental models. We postulated that microcapsule devices capable of releasing temozolomide would increase the therapeutic capability of this approach. A biocompatible drug delivery microcapsule device for the intracranial delivery of temozolomide is described. Drug release profiles from these microcapsules can be modulated based on the physical chemistry of the drug and the dimensions of the release orifices in these devices. The drug released from the microcapsules in these experiments was the clinically utilized chemotherapeutic agent, temozolomide. *In vitro* studies were performed in order to test the function, reliability, and drug release kinetics of the devices. The efficacy of the temozolomide-filled microcapsules was tested in an intracranial experimental rodent gliosarcoma model. Immunohistochemical analysis of tissue for evidence of DNA strand breaks via terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay was performed. The experimental release curves showed mass flow rates of 36 μg/h for single-orifice devices and an 88 μg/h mass flow rate for multiple-orifice devices loaded with temozolomide. *In vivo* efficacy results showed that localized intracranial delivery of temozolomide from microcapsule devices was capable of prolonging animal survival and may offer a novel form of treatment for brain tumors.

1. Introduction

Recent advances in brain tumor therapy have increased the median survival for patients with glioblastoma multiforme (GBM) from 9 months to approximately 20 months [1]. GBM is the most common and aggressive form of glioma, accounting for 12–15% of all brain tumors, and 60–75% of astrocytic tumors [2]. Despite recent advances in the treatment of GBM that increased the median survival from 9 months to 21 months when patients were given the maximal treatment of surgery, radiation and chemotherapy, advances in treatment are required [3–6]. There are few reports of complete GBM regression; resulting in an overall 5-year survival rate of less than 10% [4]. Glioblastoma multiforme and related gliomas present devastating challenges to patient survival and demand improved treatment strategies.

Efficacious delivery of drug to the brain has long been a challenge. Systemic delivery methods are often hindered by the low

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permeability of the blood–brain barrier to non-lipophilic drugs, which requires high systemic doses to be administered and increases the risk of systemic toxicity [7–10]. Some nanoparticle based, targeted drug delivery methods have been shown to successfully cross the blood–brain barrier, but issues due to toxicity and unsatisfactory clearance by the immune system of these materials have hindered their translation from the laboratory to the clinic [11]. One method of circumventing the blood–brain barrier is to place polymers that can release drug in a time-controlled manner at the site of tumor resection [12–17]. Localized drug delivery devices provide high drug concentrations directly to the tumor site while avoiding systemic toxicity and protecting the drug from degradation and clearance by the immune system until its release [18–24]. Studies performed in this work were designed to develop a polymeric, diffusion-based drug delivery microcapsule for the localized treatment of GBM.

Two types of drug delivery devices were investigated in this work. One device was fabricated from poly(L-lactic) acid (PLLA), a biodegradable polymer, and the other from liquid crystal polymer (LCP). Previous experimental studies in our laboratory found that PLLA drug delivery devices loaded with carmustine failed to prevent partitioning of drug through the polymer housing (unpublished data). This led to unsatisfactory drug release profiles and unacceptable drug leakage. The hermiticity of these devices was compromised, which resulted in the need to re-evaluate the material choices for use in the current microcapsule delivery devices. LCP is non-biodegradable, but is biocompatible, chemically inert, and its liquid crystal structure along with polymer chain rigidity directly address past issues related to drug partitioning through thin PLLA polymer membranes [25].

Fick's First Law of Diffusion was used to estimate the rate of drug delivery from microcapsule devices for a given device design. Drug delivery kinetics was controlled by the diameter of the drug release orifices, the number of orifices, and the solubility of the drug. The inner volume of the reservoir, which stored the drug, measured 15 μL, which allowed for higher drug loading per device volume when compared to previous diffusion drug delivery devices [24,26]. The higher drug loading capability facilitated the local delivery of large drug payloads to tumors, which was critical for effective chemotherapeutic treatments. One important innovation in this work was the use of the injection molding process for high precision device production, which gave reliable and consistent results, including very small tolerances in payload volume. These devices can be intelligently engineered to achieve desired release kinetics that fit the need of disease treatment.

We chose to incorporate the chemotherapeutic drug, temozolomide (TMZ), into the microcapsule devices, since it is one of the primary drugs used for the treatment of GBM. The primary anti-tumor effect of TMZ is methylation of tumoral DNA [27]. Methylation induces DNA damage, and in turn activates apoptotic pathways in the cell cycle that eventually lead to cell death. The current standard for systemic chemotherapeutic treatment of GBM is Temodar®, the oral version of temozolomide. Brem et al. demonstrated that localized delivery of TMZ from a polymer-matrix wafer significantly improved the survival of 9L gliosarcoma-challenged F344 rats [24]. Studies detailing the physical chemistry of this drug, along with the engineering principles of small molecule diffusion, allowed for the fabrication of this microcapsule drug delivery device that can deliver TMZ over a determined period of time. *In vitro* drug release studies were performed in order to test the function, reliability, and drug release kinetics of the devices prior to the *in vivo* efficacy studies which used an intracranial gliosarcoma rodent model. These *in vivo* experiments were conducted to test the efficacy of TMZ delivery from microcapsule devices.

2. Materials and methods

2.1. Chemicals

Temozolomide (TMZ) was provided by the National Cancer Institute (Drug Synthesis and Chemistry Branch, Developmental Therapeutics Program, Division of Cancer Treatment and Diagnostics, National Cancer Institute, Bethesda, MD). HPLC-grade water, ammonium acetate, and acetonitrile were purchased from Sigma–Aldrich.

2.2. Device fabrication

Liquid crystal polymer, LCP, (Vectra MT1300) was purchased from microPEP (East Providence, RI). Poly(L-lactic) acid, PLLA, (100 L) was purchased from Lakeshore Biomaterials (Birmingham, AL). PLLA and LCP polymer microcapsule devices were injection molded into their final form at microPEP (East Providence, RI).

2.3. Temozolomide characterization

2.3.1. High pressure liquid chromatography

TMZ was quantified using high pressure liquid chromatography (HPLC). 20 μL of sample was quantified at 37 °C on an Agilent 1200 Series HPLC using a Synchropak SCD-100, 5 μm, 150 × 4.6 mm column (Synchrom, Lafayette, IN, USA), a flow rate of 0.4 ml/min, 0.01 M Ammonium Acetate (aqueous):Acetonitrile (92:8) mobile phase, and UV absorption at 316 nm. A standard curve was constructed to quantify *in vitro* TMZ release from the polymer microcapsule devices.

2.4. In vitro drug release kinetics

2.4.1. TMZ-loaded drug delivery devices

TMZ (12 mg) was loaded into LCP single and multiple-hole polymer microcapsules for *in vitro* drug release characterization. Single-hole devices had an orifice diameter of 889 μm in the device cap. Multiple-hole devices had a single 889 μm-diameter cap orifice and four 403 μm-diameter orifices around the circumference of the device reservoir. After TMZ was loaded into each device, caps were sealed to the reservoir with UV-curable epoxy (1-20542 UV Curing Cationic Epoxy, Dymax Corp, Torrington, CT). Devices were exposed to UV light for a cure time of 90 s. Control devices with epoxy-sealed orifices were fabricated for leak tests to examine the hermeticity of the UV epoxy and polymer reservoir. A vacuum pump was used to remove residual air from the devices. Devices were placed into 2 ml of HPLC/MS-grade water to begin release studies. The sample bath was removed and refilled periodically. Samples were stored in liquid nitrogen until analysis. Interpolation of the readings with a standard curve of TMZ was used to quantify the amount of drug released over time.

2.5. In vivo rodent experiments

2.5.1. Tumor and device implantation

9L gliosarcoma cells were implanted in the flank of F344 rats (Harlan Sprague Dawley, Indianapolis, IN) and allowed to proliferate for two weeks until the tumor was approximately 2 cm in diameter. The tumor was harvested after euthanasia of the rat and cut into pieces approximately 1 mm^3 . Female F344 rats weighing 125–175 g were anesthesized with intraperitoneal injections of a xylazine/ketamine stock solution, at a concentration of 3–5 ml/kg. The heads were shaved and the skin prepped using a betadine scrub. A mid-line incision 1 cm in length was made followed by removal of the underlying fascia. A 3.5 mm diameter hole was drilled using a stainless steel drill bit. The hole was drilled 2–3 mm lateral to the midline suture and 5 mm posterior from the coronal suture. Excess CSF fluid and white matter were lightly suctioned to make space for the tumor and device. A microscope was used to assist in implanting the tumor and drug delivery microcapsule, cap side down, into the intracranial space. The device was placed either simultaneously with tumor or 5 days following tumor implantation. The skin was closed using autoclips once the implantation was completed, and animals were returned to their cages.

2.5.2. Efficacy of locally delivered TMZ

Two experiments were conducted in order to determine the effectiveness of locally delivered TMZ in intracranial tumor-bearing rats. The first pilot experiment included nine experimental groups and a total of 68 F344 rats: 1) Control animals that received no treatment ($n = 8$). 2) Animals given TMZ (Temodar®, Schering Plough) by gavage at a dose of 50 mg/kg on days 5–9 after tumor implantation ($n = 8$). 3) Animals given two 50%wt TMZ-polymer wafers (5 mg TMZ payload per wafer) 5 days after tumor implantation ($n = 8$). 4) Control animals that were given a blank LCP microcapsule (no drug payload) ($n = 6$). 5) Control animals that were given a blank PLLA microcapsule (no drug payload) ($n = 7$). 6) Animals given a TMZ-filled LCP microcapsule on the day of tumor implantation (Day 0) ($n = 8$). 7) Animals given a TMZ-filled PLLA microcapsule on the day of tumor implantation (Day 0) ($n = 8$). 8) Animals given a TMZ-filled LCP microcapsule 5 days after tumor implantation (Day 5) ($n = 7$). 9) Animals given a TMZ-filled PLLA microcapsule 5 days after tumor implantation (Day 5) ($n = 8$). All microcapsule devices used in this experiment had a total TMZ payload of

12 mg with a single 889um-diameter orifice cap. Overall animal survival was compared to that of the device-free untreated control group.

The results of the first *in vivo* efficacy experiment were used to design a second *in vivo* experiment with modified drug delivery devices. This second study included 30 F344 rats that received a 9L tumor piece and were then divided into the four following experimental groups: 1) Control animals receiving no treatment ($n = 8$). 2) Control animals that received a blank LCP microcapsule (no drug payload) on Day 0 ($n = 6$). 3) Animals given Temodar® by gavage at a dose of 50 mg/kg on days 5–9 after tumor implantation ($n = 8$). 4) Animals given a TMZ-filled LCP microcapsule on the day of tumor implantation (Day 0) ($n = 8$). All drug delivery microcapsules used in this experiment had a total TMZ payload of 12 mg, a single 889um-diameter orifice in the cap, and four 403um-diameter drug release orifices around the reservoir of the device. Overall animal survival was compared to that of the device-free untreated control group.

2.5.3. Animal care

All animals were housed in standard facilities and given free access to food and water. Rats were treated in accordance with the policies and guidelines of the Johns Hopkins University Animal care and Use Committee. Each study was terminated at Day 120, and the surviving rats were deemed long-term survivors (LTS). Following death, all excised brains were either flash frozen and stored at −80 °C for further immunohistochemical analysis or removed and placed in formalin for histological studies.

2.5.4. Statistical analysis

Death was the primary endpoint for all *in vivo* efficacy studies. The method of Kaplan and Meier was used to determine the distribution of intervals until death. Statistical analysis was performed using the Prism GraphPad software package and included Kaplan–Meier survival analysis and a log rank scoring test. In addition, we performed an unpaired *t*-test statistical analysis for comparison with the log rank test. For clarity, the *p* values reported in our results are those from the log rank test.

2.6. Immunohistochemical analysis

2.6.1. TUNEL stain

Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL staining) was performed on rat brain tissue samples in order to quantify the effect of TMZ on 9L glioma nuclear DNA. The protocol was specifically used for detection and quantification of TMZ-induced DNA damage. The TUNEL method works by labeling DNA strand breaks that are caused by exposure to cytotoxic agents such as TMZ. These single or double stranded breaks are identified by labeling the free 3′-OH terminal with modified nucleotides [28]. Counting of cellular nuclei with TUNEL-positive stains allows for quantitative determination of apoptosis. The rat brain tissue used in this study was paraffin-embedded and cut through the coronal plane before TUNEL analysis. The multi-step staining protocol developed by Heatwole et al. was used [29].

3. Results

3.1. Tailoring drug release kinetics

The appropriate dimensions of the microcapsule orifice(s) and drug formulation depend on the drug and treatment characteristics. Drug solubility and its desired release rate were critical to determining the final design of the devices. Fick's First Law of Diffusion was used to calculate the theoretical release rate of drug from microcapsule devices.

3.1.1. TMZ-releasing microcapsules

3.1.1.1. Single-hole devices. The TMZ-impregnanted polymer wafers developed by Brem et al. were chosen for comparison with our first generation microcapsule devices [24]. An average release rate of 1.17 mg per day from these wafers was shown to prolong rodent survival [24]. Software renderings of single and multiple-orifice devices are shown in Fig. 1. The theoretical release rate of TMZ from single, 889 μm diameter orifice microcapsules was calculated by calculation of the flux, J, from Fick's First Law of Diffusion and the area, A, over which drug release occurs. The theoretical release rate of TMZ delivered from an 889 μm diameter orifice device is $1.4 \times 10^{-5}\frac{mg}{s}$, or $1.2096\frac{mg}{day}$. This theoretical release rate was used for comparison with the experimentally determined TMZ release rates from the microcapsules.

3.1.1.2. Multiple-hole devices. Multiple-orifice microcapsules were fabricated in order to increase the release rate of TMZ. Four additional orifices were laser-drilled around the circumference of the microcapsules to achieve multi-directional drug release. These second-generation microcapsule devices were manufactured with a total of five orifices for drug release: One, 889 μm diameter orifice in the cap and four 403 μm diameter orifices around the circumference of the microcapsule reservoir. The release rate of TMZ from these devices was calculated using the same assumptions and approximations as in the single-orifice case, and was found to be $2.5 \times 10^{-5}\frac{mg}{s}$, or $2.16\frac{mg}{day}$. The multiple-orifice release rate was nearly twice as fast as the single-orifice release rate.

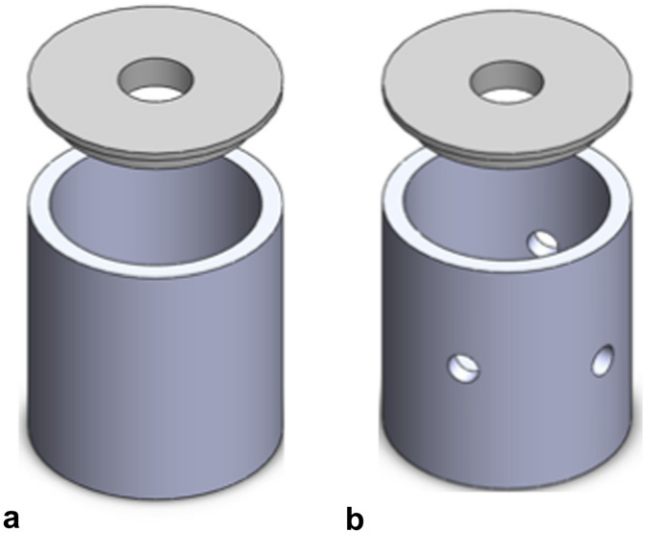

Fig. 1. Microcapsule device design. a) Single-orifice device. b) Multiple-orifice device.

3.2. In vitro TMZ drug release kinetics

3.2.1. Single-hole devices

The *in vitro* release of TMZ from single 889 μm diameter orifice LCP drug delivery microcapsules into HPLC-grade water was assayed by HPLC. Control devices with completely sealed orifices were also fabricated in order to test the hermeticity of the UV-curable epoxy and polymer housing. A theoretical release curve based on Fick's First Law of Diffusion was constructed for comparison with experimental drug release data. The mass flow rate calculated from Fick's 1st Law was 50.4 μg/h. The experiment was completed in triplicate. Results were reported as average values with standard deviations for each time point. The experimental release curves were in close agreement with the theoretical release curve, especially at times before 80 h. The experimental TMZ release rate during the first 100 h of release was 36 μg/h ± 2.87 μg (Fig. 2). At this time, 40% of the total 12 mg was released. The release rate decreased after 100 h, with a final drug release of 50% after 175 h. The devices fabricated for drug leak tests demonstrated that the UV-curable epoxy was capable of sealing the devices and preventing leakage of drug from the devices. No drug partitioning through the polymer housing was detected, suggesting that LCP was capable of storing the drug throughout the length of therapy.

3.2.2. Multiple-hole devices

LCP devices with multiple drug release orifices were tested *in vitro* to examine the effect of increasing the number of release orifices on drug release rate. A theoretical release curve based on Fick's First Law of Diffusion was constructed for comparison with experimental drug release data. The mass flow rate calculated from Fick's 1st Law was 90 μg/h. The experiment was completed in

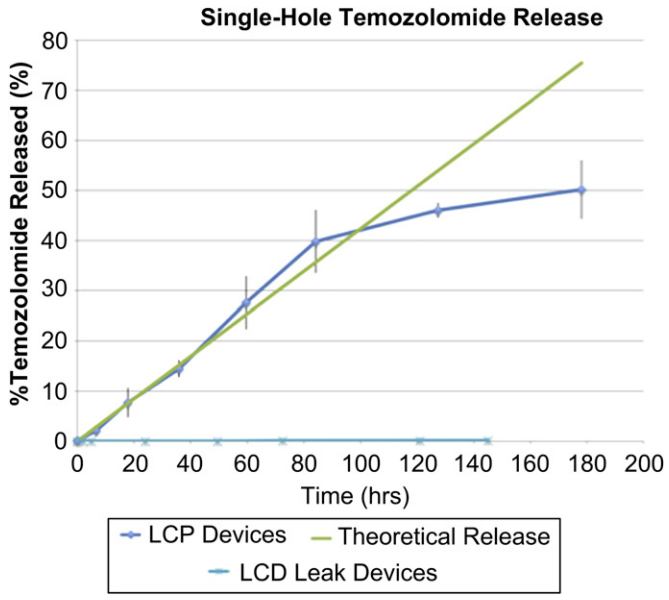

Fig. 2. Single-hole TMZ release into water.

triplicate. Results were reported as average values with standard deviations for each time point. The experimental release curves were in agreement with the theoretical release curve. The experimental TMZ release rate during the first 100 h of release was 88 μg/h ± 6.75 μg (Fig. 3). At this time, 67% of the total 12 mg was released. The release rate decreased after 100 h, with a final drug release of 71% after 190 h.

3.3. *In vivo* rodent experiments

3.3.1. *Efficacy of locally delivered TMZ*

3.3.1.1. Single-hole devices. The primary metric for drug delivery device efficacy was animal survival in the first *in vivo rodent* study. Untreated control animals had a median survival of 17 d (Fig. 4). The two groups that received either blank LCP or blank PLLA microcapsules both had median survivals of 11 d. Rats given TMZ-loaded LCP and PLLA devices 5 d after tumor implantation also showed high death rates and median respective survival times of 17 and 18 days.

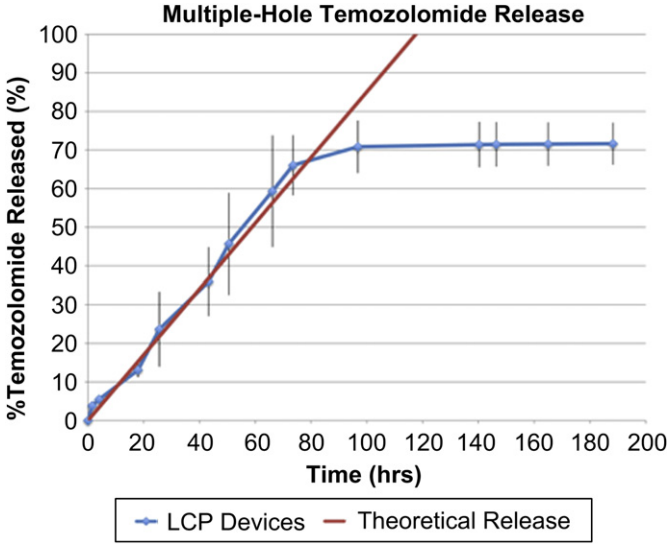

Fig. 3. Multiple-hole TMZ release into water.

Rats from the oral TMZ, TMZ wafer, and day 0 TMZ microcapsule groups exhibited longer survival rates than the previously mentioned experimental groups. Animals given oral TMZ had a median survival time of 25 d with all of the animals dying by day 61. Increased rodent survival was achieved with the day 0 TMZ-loaded LCP and PLLA groups, with median survival times of 31 and 50 d, respectively. The group that received two TMZ-impregnated polymer wafers 5 d after tumor implantation had a median survival of 65 d.

Animals that survived 120 d or later were deemed long-term survivors (LTS). Three experimental groups contained long-term survivors: TMZ wafers, with 50% LTS, LCP-TMZ day 0 with 37.5% LTS and PLLA-TMZ day 0 with 25% LTS. No long-term survivors were present in the group that received oral TMZ treatment.

Statistical analysis of the survival data was performed and significant differences were observed between the control/TMZ wafer ($p = 0.0001$), control/LCP-TMZ day 0 ($p = 0.0009$), oral/TMZ wafer ($p = 0.0011$), and oral/LCP-TMZ day 0 groups ($p = 0.0401$). There was no statistically significant difference between the day 0 TMZ-filled LCP and PLLA devices. The only difference between the unpaired *t*-test and the log rank test analysis was in the statistical significance between the wafer/LCP-TMZ day 0 group and the wafer/PLLA-TMZ day 0 group. The unpaired *t*-test resulted in statistically significant differences between these groups, while the log rank test showed that there was no statistical difference between the groups. The differing results of these methods have no effect on the conclusions described below.

3.3.1.2. Multiple-hole devices. The second *in vivo* TMZ experiment was performed in order to test the effectiveness of multiple-orifice LCP microcapsule devices implanted on the day of tumor administration. The median survival time for untreated control animals was 14 d (Fig. 5). Animals that had received blank LCP had a median survival time of 13 d. Animals receiving oral gavage of TMZ had a median survival time of 26 d. Multiple-orifice TMZ-filled LCP devices showed a median survival time of 62 d. This survival advantage was two weeks greater than seen with single-orifice LCP devices used in the first *in vivo* experiment. Additionally, the group that received TMZ-filled devices had 37.5% LTS.

Statistically significant differences were observed between control/oral TMZ ($p < 0.0001$), control/LCP-TMZ day 0 ($p < 0.0001$), and oral/LCP-TMZ day 0 groups ($p = 0.0014$). These results are consistent with statistical analysis performed with both the unpaired *t*-test and log rank test. The LCP devices have again demonstrated improved rodent survival rates as compared to oral delivery of TMZ, the current clinical standard of care.

3.4. *Immunohistochemical TUNEL stain*

TUNEL staining was conducted to assess the cytotoxicity of TMZ *in vivo*. Brain tissue samples were exposed to staining reagents and the number of TUNEL-positive cells in each sample was counted. Brain tissue sections were cut ipsilateral and contralateral to the location of the implanted devices in order to understand the significance of device location and intracranial drug distribution. TUNEL staining of brain sections from both *in vivo* efficacy experiments showed that the LCP-TMZ treatment groups had higher ipsilateral TUNEL-positive cell counts than all other experimental groups (Fig. 6). LCP-TMZ and TMZ-polymer wafer groups had similar contralateral TUNEL-positive cell counts that were larger than control, blank LCP, and oral TMZ TUNEL-positive cell counts. Control and blank LCP microcapsule groups showed the lowest total number of TUNEL-positive cells (Fig. 7). Averages of the total TUNEL-positive cell counts (ipsilateral + contralateral) showed that tissue samples from the LCP-TMZ treated group had the largest

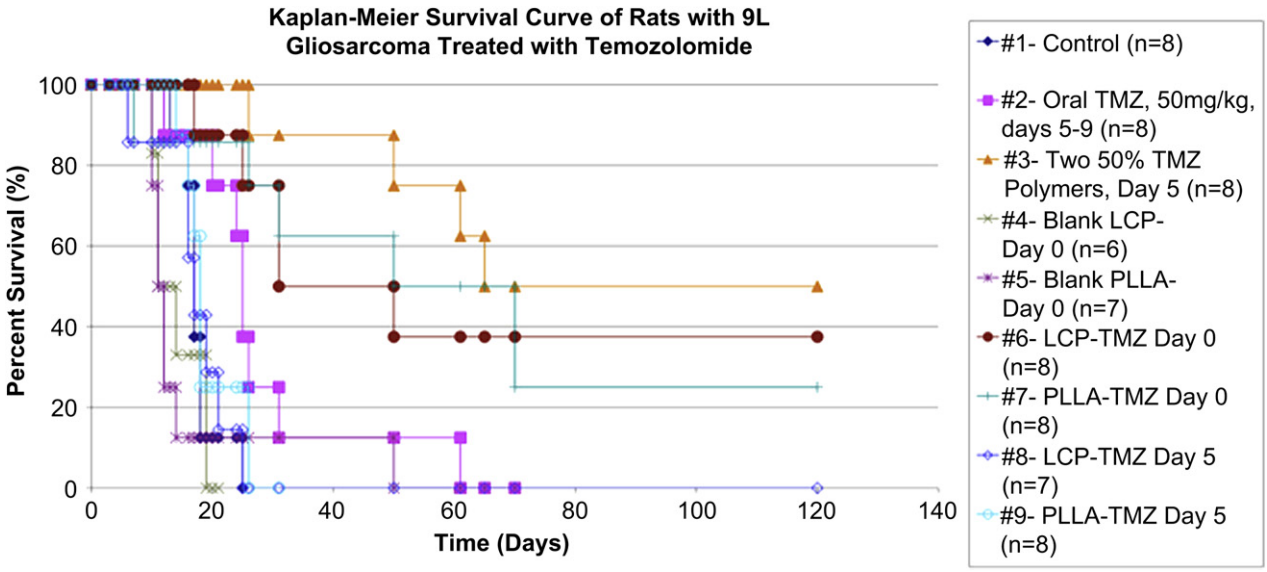

Fig. 4. Localized TMZ efficacy experiment #1.

number of TUNEL-positive cells in both *in vivo* experiments. This number was higher than both the oral TMZ and TMZ-polymer treatment groups.

4. Discussion

4.1. In vitro TMZ drug release kinetics

4.1.1. TMZ-loaded microcapsule devices

Single and multiple-hole drug delivery microcapsules showed similar *in vitro* drug release behavior. Both types of devices exhibited close agreement with theoretical mass flow rate calculations for the first 100 h of drug release. The release rates of the single and multiple-hole microcapsules began to plateau after this time. This plateau is due to a decrease in the chemical driving force for diffusion and drug degradation. The concentration profile between the reservoir and outside environment begins to approach the same value as drug is released and the mass flow rate across the device orifice decreases. This results in slower drug diffusion than theoretically predicted from Fick's First Law.

The stability of TMZ also has a profound impact on the *in vitro* release kinetics of drug delivery microcapsules. TMZ is a prodrug that quickly degrades into the bioactive MTIC molecule under basic conditions and in high ionic strength solvents [27]. *In vitro* release profiles of TMZ into water from microcapsule devices highlight the importance of the release buffer environment. The half-life of TMZ in water is considerably greater than its half-life in PBS, FBS, and *in vivo* [27]. This accounts for the close agreement with theory that is seen during the first 100 h of the TMZ-water release profiles. Degradation of TMZ due to its instability in solution accounts for the plateau in drug release over long periods of time and also explains why 100% drug release is not seen experimentally. Stability characteristics of TMZ in approximate *in vivo* conditions are beneficial in interpreting *in vitro* results and designing drug delivery devices for *in vivo* studies.

4.2. In vivo rodent experiments

4.2.1. Efficacy of locally delivered TMZ

The *in vivo* efficacy of localized TMZ therapy was investigated against the 9L gliosarcoma model in rodents. Results showed that the implantation of two 50% (w/w) TMZ-polymer wafers most effectively prolonged animal survival rates. Microcapsule devices (single and multiple-orifice) implanted on the same day as 9L

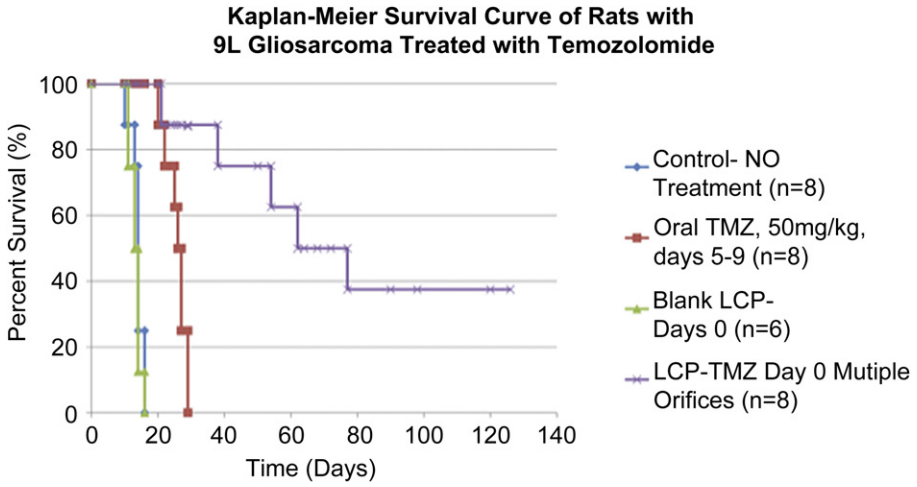

Fig. 5. Localized TMZ efficacy experiment #2.

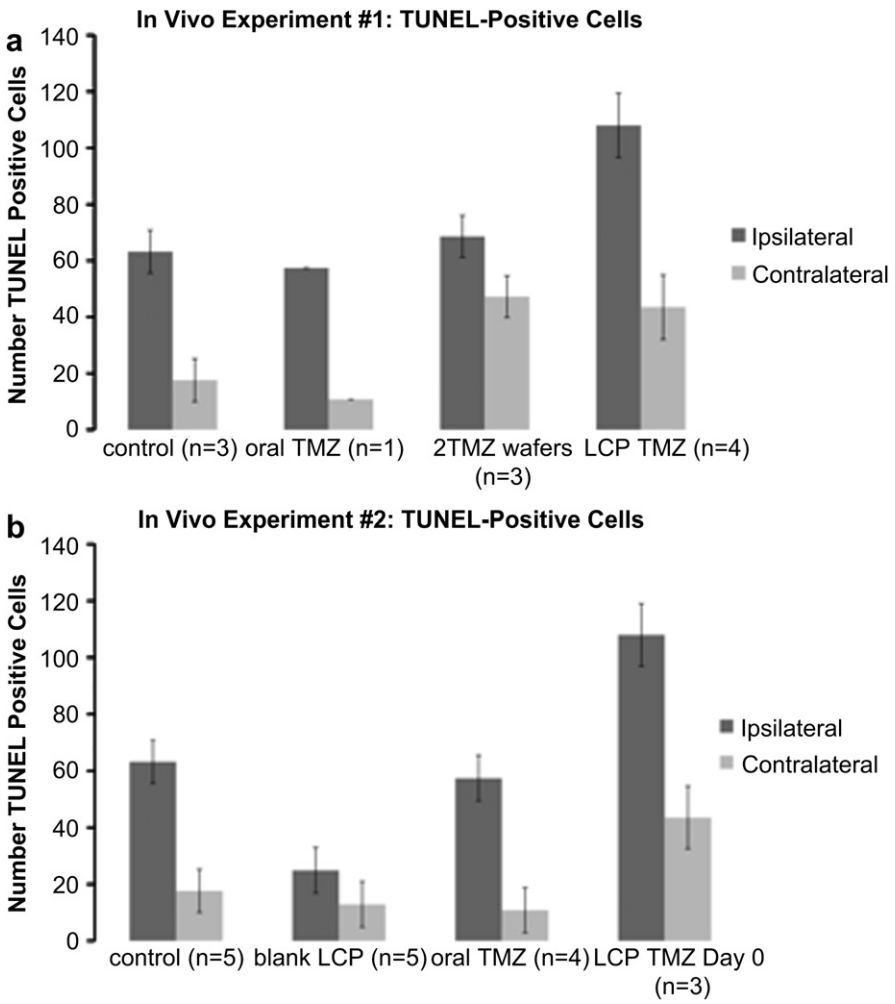

Fig. 6. TUNEL stain of rodent brain tissue a) Ipsilateral and contralateral brain section TUNEL stain from experiment #1. b) Ipsilateral and contralateral brain section TUNEL stain from experiment #2.

tumor showed the second-best survival benefit in tumor-challenged rats. Both localized drug delivery systems out-performed Temodar®, one of the clinically utilized treatments for GBM.

TMZ-polymer composite wafers are capable of multi-directional drug diffusion because the entire device surface is exposed to the tumor bed and is able to release drug. Microcapsule devices, alternatively, release drug from either one orifice or five orifices. Multiple-orifice microcapsules extended median animal survival by two weeks when compared with single-orifice devices, and both groups demonstrated significant improvement in survival when compared to controls. The faster drug release kinetics provided by the multiple-orifice microcapsules (shown by *in vitro* drug release) deliver a larger drug payload to the tumor than single-orifice devices over a shorter period of time. This relatively quick release is important to maintaining the bioactivity of the inherently unstable TMZ molecule. Additional release orifices can be fabricated in microcapsule reservoirs in order to approach the truly multi-directional drug release achieved by the wafer system.

The device implantation procedure also has important ramifications on the efficacy of localized treatment. The microcapsule dimensions were chosen for delivery of a large drug payload from a device that is still small enough for implantation in the rodent brain cavity. The tight dimensional tolerances between the rodent skull and the microcapsule device along with the introduction of a growing 9L tumor mass could cause brain damage due to increased intracranial pressure (mass effect). This increase in pressure is a common manifestation in GBM patients and is one of the primary reasons for tumor resection surgery. The detrimental effect of increased intracranial pressure is shown by the relatively short survival seen in the blank microcapsule device groups.

The effect of decreased free volume between the microcapsule drug release orifice and brain tissue due to growing tumor also explains why multiple-orifice devices performed better than single-orifice devices, and why day zero microcapsule devices performed better than day five microcapsules devices. Day zero microcapsules out-performed day five devices because the tumor mass on day zero was not as large as the tumor mass present on day five. The hypothesis is that growing tumor causes increased intracranial pressure, as noted in the previous paragraph, and this tissue mass also occludes the drug-releasing orifice in single-hole devices. This occlusion occurs because the devices are implanted with the orifice facing the tumor. Multiple-orifice devices and polymer wafers are capable of multi-directional drug release that is able to avoid the occlusion caused by the growing tissue mass. Explanted single-orifice devices were found to have residual TMZ left in the reservoir and multi-orifice devices were empty. With the payload of drug being equal in all of the microcapsule treatment groups, the efficacy of localized drug delivery devices is directly related to the ability for multi-directional drug release. The progressive improvement in animal survival from single-orifice microcapsules

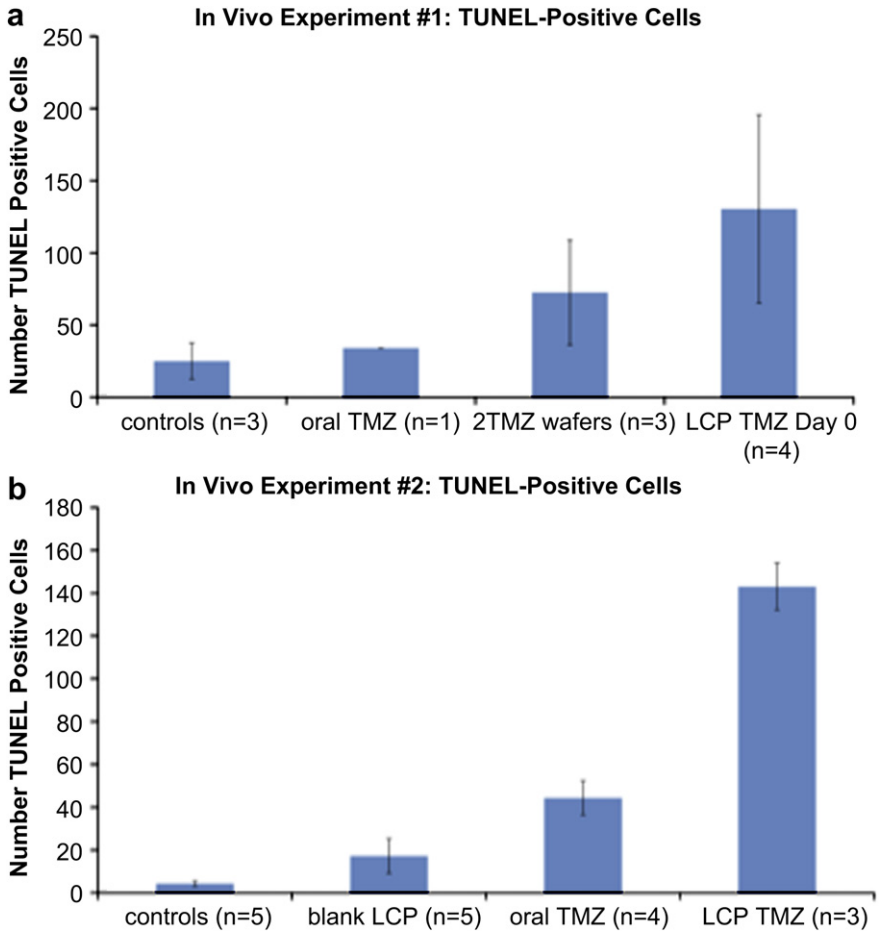

Fig. 7. TUNEL stain of rodent brain tissue a) Average Total (ipsilateral + contralateral) TUNEL-positive cell count from experiment #1. b) Average total (ipsilateral + contralateral) TUNEL-positive cell count from experiment #2.

to multiple-orifice microcapsules confirms this. It may also be beneficial to move to larger animal models where there is more intracranial space for microcapsule implantation and lower probability of orifice occlusion.

It is possible that the microcapsule design described here can provide high local concentration of drug within brain tumors which could improve upon what is observed with Gliadel® polymer-composite wafer [5,6,15]. The microcapsule system delivers more than twice the drug payload as the TMZ polyanhydride wafers (12 mg and 5 mg, respectively) in a similar volume that is suitable for intracranial implantation [5,15–17,22–24]. Microcapsule devices are also capable of delivering solid or liquid drug formulations, an ability that polymer-composite wafer systems do not currently possess. This allows for highly versatile and tunable drug delivery with the possibility of delivering a wide range of therapeutic compounds to brain tumors as well as other human diseases.

4.3. Immunohistochemical TUNEL stain

Animals treated with TMZ-loaded LCP microcapsules showed the largest number of TUNEL-positive cells when compared to both untreated control groups (tumor only and blank LCP). This result was expected because TMZ is known to cause single and double stranded DNA breaks, or "nicks," and animals that were not exposed to TMZ (both control groups) showed very low levels of TUNEL-positive cells. The relatively low TUNEL staining present in the oral TMZ group is probably due to low partitioning of TMZ from systemic circulation to the brain, with only an estimated 35% of the oral TMZ dosage reaching the brain [30]. The higher drug payload in the LCP microcapsule devices (12 mg) may explain why more TUNEL-positive cells were found in this group versus the TMZ wafer group (10 mg).

5. Conclusion

This work has demonstrated that polymer microcapsule devices are capable of releasing chemotherapeutic molecules *in vitro* at rates similar to theoretical values calculated from Fick's First Law. The polymers used in the manufacture of these devices are biocompatible (LCP and PLLA) and biodegradable (PLLA) when implanted intracranially in a rodent animal model. Molecular histochemical analysis confirms the ability of TMZ to cause cytotoxic DNA damage in cell nuclei. We have also shown that LCP implantable drug delivery devices releasing TMZ are capable of significantly prolonging survival in 9L gliosarcoma-challenged rodents in comparison with systemic delivery methods. Microcapsule devices offer versatile localized delivery of molecules without the requirement for intensive drug formulation necessary for liposomal, nanoparticle, or solvent drug-loading methods.

Appendix

Figures with essential colour discrimination. Certain figures in this article, particularly Figs. 1–5 and 7 are difficult to interpret in

black and white. The full colour images can be found in the on-line version, at doi:10.1016/j.biomaterials.2010.12.020.

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