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Paclitaxel delivery from PLGA foams for controlled release in post-surgical chemotherapy against glioblastoma multiforme

Benjamin Y.S. Ong [a], Sudhir H. Ranganath [a], Lai Yeng Lee [a], Fan Lu [b], How-Sung Lee [b], Nikolaos V. Sahinidis [c], Chi-Hwa Wang [a,*]

[a] Department of Chemical and Biomolecular Engineering, National University of Singapore, 4 Engineering Drive 4, Singapore 117576, Singapore
[b] Department of Pharmacology, National University of Singapore, Clinical Research Center Bldg MD11, Level 5, #05-9, 10 Medical Drive, Singapore 117597, Singapore
[c] Department of Chemical Engineering, Carnegie Mellon University, 5000 Forbes Avenue, Pittsburgh, PA 15213-3890, USA

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ABSTRACT

Paclitaxel loaded biodegradable poly-(DL-lactic-*co*-glycolic) acid (PLGA) foams with microporous matrix were fabricated by a novel pressure quenching approach to provide a sustained paclitaxel release. The foams with micropores provided increased surface area to volume ratio and were also implantable for post-surgical chemotherapy applications. The two formulations 5% (w/w) paclitaxel loaded PLGA 85:15 foam (F1) and 10% (w/w) paclitaxel loaded PLGA 50:50 foam (F2), were evaluated in vitro and in vivo. Both the foams were found to provide a paclitaxel release beyond a month in vitro with a near zero-order kinetics and with minimum burst release. Furthermore, apoptosis of C6 glioma cells in vitro demonstrated the benefits of sustained paclitaxel release by the foams in comparison to acute Taxol® exposure. Both the foams exhibited continuous paclitaxel release in an in vivo (subcutaneous) environment up to a month which correlated well with the in vitro release profiles. Bio-distribution results in the rat brain showed paclitaxel penetration at therapeutic levels up to 3 mm into the tissue from the site of foam implantation. Hence these foams could be employed as potential implants for post-surgical chemotherapy against malignant glioma.

1. Introduction

Over the last three decades, there has been a rise in brain cancers. In particular, glioblastoma multiforme (GBM), oligodendroglioma, anaplastic astrocytoma, medulloblastoma, and mixed glioma have been on the rise. Of these, GBM is the most frequent, accounting for 16,797 cases out of 38,453 cases of malignant brain tumors between 1973 and 2001 in the United States alone [1]. The conventional clinical treatment for glioma involves surgical debulking of the accessible tumor from the patient's brain. The amount of tumor removed is often limited by proximity to critical regions for brain function, thus resulting in risk of tumor re-growth from residual tumor. Cancer remission can be limited by conventional systemic post-surgical chemotherapy and radiotherapy courses. Unfortunately, these have resulted in limited clinical effectiveness, due to restricted transport of chemotherapy agents across the blood brain barrier (BBB) and significant P-glycoprotein (P-gp) efflux barrier effects [2]. To overcome barriers and achieve effective drug transport, biodegradable controlled release polymeric implants could be surgically located at the site of tumor removal during the debulking surgery. Commercial implants, such as the Gliadel® wafer delivering BCNU (Carmustine), have enjoyed limited successes in improving patient survival rates. Clinical trials with Gliadel® wafer vs. placebo wafers have shown that Gliadel® prolongs survival in people with newly diagnosed high-grade malignant gliomas (in addition to surgery and radiation) from a median survival of 11.6 months to 13.9 months. In the case of recurrent GBM, using Gliadel® after surgery increased the median survival time to 6.4 months from 4.6 months [3,4]. Since only one third of GBM patients are responsive to BCNU [5], with other Gliadel® wafer associated complications like cerebral edema [4], several groups have been working on controlled release of other drugs such as doxorubicin [1] and paclitaxel.

Paclitaxel, a chemotherapeutic drug originating from the pacific yew taxus brevifolia, and other members of the taxaceae family [6] is commonly used as a chemotherapeutic agent for ovarian and breast cancer. Paclitaxel functions through promotion of the assembly and stabilization of microtubules inhibiting cellular

* Corresponding author. Tel.: +65 6874 5079; fax: +65 6779 1936.
E-mail address: chewch@nus.edu.sg (C.-H. Wang).

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division. It also prevents de-polymerization of the assembled microtubules and thereby halts mitosis or cell division and binds to Bcl-2 [7,8] which normally blocks the process of apoptosis, allowing apoptosis to proceed. Unfortunately, paclitaxel is hydrophobic and exhibits a fast plasma clearance when administered by infusion [9]. Absorption across the BBB is also poor due to P-gp efflux effects [10–12]. However, studies have showed that prolonged exposure to paclitaxel for more than 24 h can provide significant clinical efficacy [13].

Several studies have been carried out using different materials to achieve controlled release of paclitaxel from surgical implants of various forms. Walter et al. evaluated poly[bis(p-carboxy-phenoxy propane anhydride) sebacic acid] (PCPP-SA) microsphere discs loaded with paclitaxel and reported that the fast degrading polymer matrix resulted in a large initial burst causing sporadic toxicity among experimental animals [14]. Wang et al. reported the in vitro release profiles of paclitaxel released from poly-(DL-lactic-*co*-glycolic) acid (PLGA), PLGA 50:50 (MW 45,000–75,000) microspheres fabricated by spray-drying followed by 2 ton compression that exhibited a delay of 15 days before drug release was observed [15]. Li et al. used implants based on polyphosphoester p(DAPG-EOP) polymer which released paclitaxel close to a 100 days in vitro. Brain tissue concentrations in rats after 30 days showed increasing drug concentrations and enhanced survivability [16]. Von Eckardstein et al. used a nitrosoureas liquid crystalline cubic phase encapsulating carboplatin and paclitaxel and reported reduction in tumor sizes in F98 rat brains. Brain tissue concentration of paclitaxel showed little or no drug in the vicinity of 3 mm beyond 7 days. Clinical observations using the same formulations have suggested feasible and safe usage if $<$15 mg paclitaxel was used [17,18]. Elkharraz et al. worked with injectable paclitaxel loaded implants made of glycerol tripalmitate which released 73–87% of the encapsulate drug within 7 days [19]. Recently, PLGA-based paclitaxel loaded implants such as microspheres and compressed microsphere discs [20] and microfiber discs [21] have been shown to inhibit subcutaneous C6 glioma tumor better than intratumoral Taxol® injection. However, most of these devices have very high initial drug burst, or delayed release followed by a burst, or not suitable for post-surgical implantation in the resection cavity. Hence, in this study we use PLGA-based microporous foams loaded with paclitaxel which exhibit a minimal initial burst, near zero-order release kinetics, and efficient implantability.

The objectives of this study are four. First, we study the in vitro release of paclitaxel from paclitaxel loaded PLGA implantable foams fabricated by a high pressure quenching technique. Second, we evaluate the drug release sustainability and cytotoxicity of the foams against C6 glioma cells by in vitro apoptosis study. Third, we evaluate the paclitaxel release from the foams in an in vivo environment and biocompatibility. Finally, we investigate the in vivo bio-distribution of paclitaxel in the rat brain from these foams. Due to uniform pore structures in the foams, we hypothesize that it facilitates diffusion of the drug out of the matrix into surrounding tumor tissue over a prolonged period of time.

2. Materials and methods

2.1. Materials

Poly-(DL-lactic-*co*-glycolic) acid, PLGA 50:50 and PLGA 85:15 (MW 40,000–74,000 Da and 50,000–75,000 Da, respectively) were purchased from Sigma–Aldrich. Paclitaxel was a generous gift from Bristol-Myers Squibb, New Brunswick, NJ. Dichloromethane (DCM) and acetonitrile (ACN), purchased from TEDIA (Fairfield, OH, USA), were of HPLC grade. Phosphate buffered saline (PBS) was bought from Sigma–Aldrich containing 0.1 M sodium phosphate and 0.15 M sodium chloride, pH 7.4.

2.2. Fabrication of PLGA-paclitaxel foams

Two formulations were used in this work, a 5% (w/w) paclitaxel loaded foam with PLGA 85:15 (F1) and a 10% (w/w) paclitaxel loaded foam with PLGA 50:50 (F2). Fabrication of the foams was done using supercritical CO_2 foaming process as explained by Zhu et al. [22]. Briefly, paclitaxel was incorporated into the polymer matrix by dissolution in dichloromethane and spray-drying the solution to form microparticles using a Buchi Spray Drier with inlet air flow-rate of 700 L/min, inlet temperature at 70 °C, aspirator setting of 100% and a pump rate of 30%. The microparticles were collected and freeze dried for 72 h to remove any residual solvent before being subjected to a high pressure of 70 bar for 45–60 min under supercritical CO_2 within a chamber. The high pressure depresses the glass transition temperature of the polymer allowing dissolution of polymer melt into CO_2 gas. Upon rapid decompression at 15 bars per minute, CO_2 loses its solubility and the glass transition temperature of the polymers rise forming gas pockets which escape leaving interconnecting and uniform micropores in the foam.

Two dosage forms in the form of disc (3 mm diameter and 1 mm thickness) and rod (7 mm length and 1 mm diameter) were fabricated. The foams were set on a mold holding the polymer melt in the compression chamber and then punched to form discs, whereas the foams were cut into rods of desired dimensions. Discs were used for the in vitro release and apoptosis studies while rods were used for intracranial bio-distribution studies. Blank placebo foams were fabricated in the same way without paclitaxel. The foam discs and the rods were sterilized by gamma irradiation at a dose of 15 kGy before being used for cell culture and animal studies. Total weight of the foam discs and rods were approximately 3 mg and 2 mg, respectively.

2.3. Foam morphology and pore size

Investigation of foam morphology, pore size and distribution were performed using scanning electron microscope (SEM) (Jeol JSM 5600LV, Tokyo, Japan). The foams were sectioned and mounted onto copper studs using double-sided adhesive tape and then vacuum coated by thin layer of platinum using Auto Fine Coater (JFC-1300, Jeol, Tokyo, Japan) with a current of 40 mA for 40 s. Diameters of the internal pores were measured by the SmileView software.

2.4. In vitro release of paclitaxel in PBS

The foam discs and rods were incubated in 5 ml of PBS (pH 7.4) at a temperature of 37 °C and 120 rpm in a shaking water bath to simulate physiological conditions. At specified time intervals, 5 ml of the release medium was withdrawn from the samples (triplicates) and an equal volume of the release medium was replaced to maintain constant sink conditions. Paclitaxel was extracted from the withdrawn solution by adding 2 ml of DCM and allowing mass transfer for one day. A mixture of acetonitrile/water (50:50, v/v) was added to the extracted paclitaxel after DCM had evaporated. The resulting solution was analyzed using HPLC using a C-18 column and the mobile phase acetonitrile/water (50:50, v/v) was delivered at a rate of 1 ml/min. 100 μl of sample was injected by an auto-sampler and the column effluent was detected at 227 nm using an ultra violet (UV) detector. The detection data were corrected according to the extraction efficiency. Standard paclitaxel concentration samples were used to produce calibration plot.

2.5. Cell culture and maintenance

The cell line used was a rat C6 glioma cell (ATCC® Number: CCL-107™), established by Benda et al. [23] and reported to be derived from *N*-methyl-nitrosourea-transformed rat astrocytes. The cells were grown in DMEM (Dulbecco's Modified Eagle Medium, Sigma) supplemented with 10% bovine fetal serum (Gibco, Invitrogen) and 1% penicillin/streptomycin (Gibco, Invitrogen) in a humidified incubator under the conditions of 37 °C, 5% CO_2 and 90% relative humidity. After reaching confluence, the cells were prepared by washing in PBS and detached from the T-flask with trypsin–EDTA (Gibco, Invitrogen). The cells were re-suspended at a speed of 1500 rpm for 6 min to obtain a concentration of 3×10^5 cells/2.5 μL before inoculation into a 75 cm^3 T-flask containing 15 ml of fresh media.

2.6. Cell growth, viability and apoptotic activity studies

Investigation of the cellular response to the foams was carried out in comparison with three control groups (namely blank, 5050_Taxol & 8515_Taxol). All groups (experimental and controls) were inoculated at a density of 1×10^6 C6 glioma cells into 175 cm^3 T-Flask with 50 ml of DMEM culture medium on day 0 and cell density was counted on days 4 and 8. The cells in all the flasks were allowed 2 days to attach and grow before the respective foams F1 and F2 were administered into the flask.

Two control flasks, 5050_Taxol and 8515_Taxol, received 49 μg and 7 μg of paclitaxel respectively administered in the form of the commercial Taxol® (obtained from Bristol-Myers Squibb) on day 2. The two paclitaxel concentrations were calculated to give an equivalent dosage of 2 $\times$ area under the curve (AUC) in the in vitro release curve over 24 h corresponding to the drug release by the foam implants over 6 days of exposure. After 24 h, the paclitaxel laden medium was removed and

the cells washed twice with PBS before replacing it with paclitaxel-free medium. These controls were used to simulate acute exposure to paclitaxel to cells over a 24 h window and observing cellular recovery on days 4 and 8.

Growth curves of the groups were generated from separate flasks (in triplicates) and counted by conventional trypan blue dye exclusion method in a haemocytometer to obtain cell densities and cell viability on days 2, 4 and 8. 3×10^6 cells were collected for caspase-3 activity level measurement using a caspase-3/CPP32 fluorometric assay kit from Biovision. Caspase-3 activity levels were determined by re-suspending cells in 50 μl of chilled cell lysis buffer. The cells were then incubated on ice for 10 min before adding 50 μl of 2× reaction buffer (containing 10 mM DTT) to each sample. 5 μl of the 1 mM DEVD-AFC substrate was then added before incubating at 37 °C for 2 h. Then the samples were read in a fluorometer equipped with a 405 nm excitation filter and 485 nm emission filter. Upon cleavage of the substrate by CPP32 or related caspases, free AFC emitted a yellow-green fluorescence ($\lambda_{max} = 505$ nm). Absorbances were then compared with controls for comparison on caspase activity levels.

2.7. Animal care and maintenance

All experiments were carried out with approval from the National University of Singapore's Institutional Animal Care and Use Committee (IACUC) and housing and care of animals were provided in accordance with the National Advisory Committee for Laboratory Animal Research (NACLAR) guidelines (guidelines on the care and use of animals for scientific purposes) in facilities licensed by the Agri-Food and Veterinary Authority of Singapore (AVA). A total of 55 wistar rats (obtained from Centre for Animal Resources, CARE, Singapore) were used in the present study. The rats were housed in cages and given free access to standard laboratory food and water. The tranquilization, anesthetic induction and maintaining agents administered during surgical procedures involving wistar rats contained 100 mg ketamine/kg body weight and 10 mg xylazine/kg body weight. For *BALB/c* mice (total of 18), the dose was 75 mg ketamine/kg body weight and 1 mg medetomidine/kg body weight.

2.8. In vivo release of paclitaxel (in BALB/c male mice)

The objective of this study was to verify the paclitaxel release profile from the foams in an in vivo environment and to check for safety for use against implant failure. An in vivo release study of paclitaxel for the F1 and F2 foams was carried out by implanting the foam disc subcutaneously in *BALB/c* male mice (beginning at an average weight of 30 g). The animals were anesthetized with ketamine (75 mg/kg) and medetomidine (1 mg/kg), shaved and scrubbed down with 70% alcohol, dilute Hibiscrub (chlorhexidine) followed with a final scrub with betadine. A small 1 cm incision was made on the lateral flank of the animal and the foam disc inserted. The wound was then sutured up and allowed to heal before removing stitches after 1 week. Weights of the animals were monitored weekly to evaluate paclitaxel related toxicity and polymer biocompatibility. At fixed time points such as days 7, 14, and 28, the animals were sacrificed ($n = 3$ mice) and the foams recovered from the cadaver. The foam was re-dissolved in DCM and analyzed for residual paclitaxel by HPLC as described in the Section 2.4.

2.9. In vivo intracranial bio-distribution study

Bio-distribution studies were carried out for F1 rods ($n = 5$) and F2 rods ($n = 5$) which were used over three separate time points (14, 21 and 28 days after implantation). The rods were approximately 1 mm in diameter × 7 mm in length and weighing 2 ± 0.2 mg each. Total number of rats for bio-distribution studies was 60. Each wistar rat was anaesthetized, shaved and scrub down with 70% alcohol and dilute Hibiscrub (chlorhexidine) followed by a final scrub with betadine. An incision was made to the scarp of the rat and a 3 mm diameter burr hole was made at 1.5 mm posterior of the bregma and 2 mm left from the midline under stereotaxic control. To create a path for the insertion of the foam rod into the brain, an 18 Gauge needle was inserted to a depth of 7 mm from the brain surface and retracted. The foam rod was then inserted completely into the incision created. The scarp of the rat was then closed by suturing and the animal was allowed to recover. The weights of the animals were monitored daily. At the respective time points, the rats were sacrificed and their brain harvested. The brains were immediately frozen, kept at −80 °C before being sectioned coronally in a rat brain matrix yielding 1.0 mm thick brain slices. Each slice was carefully weighed, homogenized and analyzed for paclitaxel concentration by liquid chromatography–tandem mass spectrometry method (LCMSMS) in Dept. of Pharmacology, NUS.

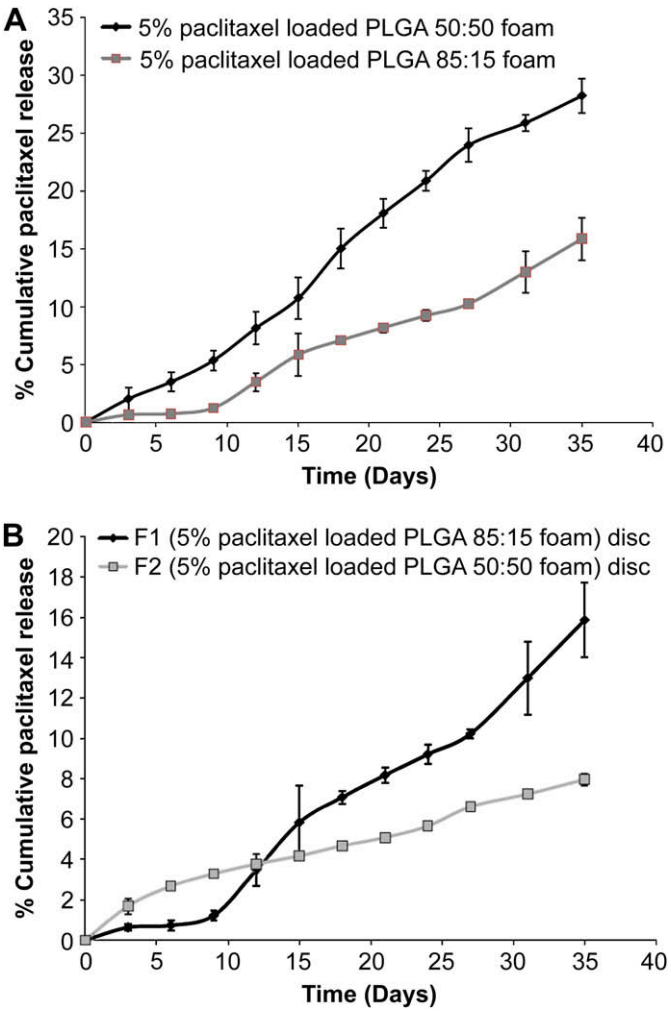

Fig. 2. In vitro release profiles of (A) 5% paclitaxel loaded PLGA 85:15 and PLGA 50:50 foam discs; (B) 5% paclitaxel loaded PLGA 85:15 (F1 foam) disc and 10% paclitaxel loaded PLGA 50:50 (F2 foam) disc. Each data point represents the average of triplicate samples and error bars represent standard deviation.

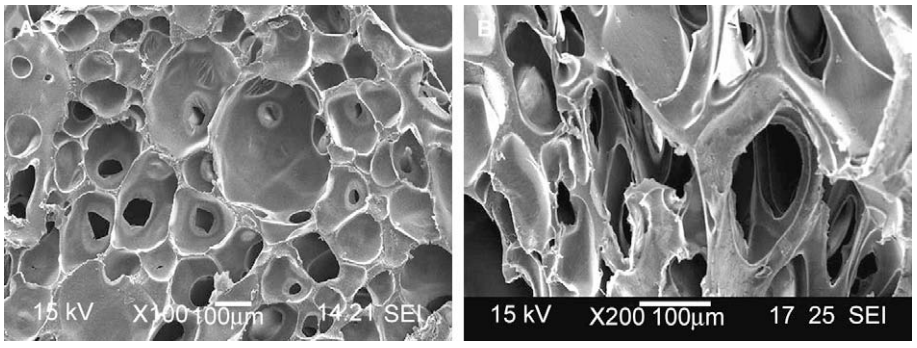

Fig. 1. SEM image of (A) 5% paclitaxel loaded PLGA 85:15 microporous foam; (B) 10% paclitaxel loaded PLGA 50:50 microporous foam. (Bars represent 100 μm).

2.10. Statistical analysis

In the in vitro apoptosis study discussed in Section 2.6, all the fluorescence intensity data were presented as the average and the error bars represents the standard deviation. The data obtained from the fluorescence measurements were statistically analyzed using single-factor analysis of variances (ANOVA) test. Differences between any two study groups were considered statistically different when $p < 0.05$ (95% confidence interval).

3. Results and discussion

3.1. Foam morphology and pore size

SEM image of cross-section of the foams shown in Fig. 1A and B reveals non-uniform pores of different diameters. The pores were well interconnected inside the foam structure, thus enhancing the surface area to volume ratio available for drug diffusion and polymer degradation. The pore size varied from about 400 μm to 50 μm.

3.2. In vitro paclitaxel release profile

Initially, two foam formulations (5% paclitaxel loaded PLGA 50:50 foam discs and PLGA 85:15 foam discs) were evaluated in vitro in PBS. Fig. 2A shows a comparison between the release of paclitaxel from the two copolymers having 5% paclitaxel loading. The data highlights the fact that the release rate of PLGA 50:50 is much higher than PLGA 85:15. This could be possibly due to the higher hydrophilic poly-glycolate content in PLGA 50:50, which results in faster molecular weight drop in aqueous PBS than PLGA 85:15 which has higher hydrophobic poly-lactate content. This understanding formed the basis for selecting these PLGA copolymers. We wanted to offer two options: fast releasing foam to arrest fast growing tumors besides bringing up the surrounding tissue to a therapeutic concentration; and a slow releasing foam to maintain the tissue's paclitaxel concentration over a prolonged period of time. Paclitaxel loading in the PLGA 50:50 foams was doubled to 10% to increase the absolute amount of paclitaxel release while PLGA 85:15 drug loading was kept at 5% to offer a slow release implant. It is anticipated that these two options or the combination of both would provide the neurosurgeon with the room for decision making for patient-specific glioma chemotherapy.

For all the subsequent studies, F1 and F2 foams were used. The in vitro release profiles of the F1 foam (5% paclitaxel loaded PLGA 85:15 foam) and F2 foam (10% paclitaxel loaded PLGA 50:50 foam) in the form of discs are presented in Fig. 2B. Both foams exhibited minimum initial drug burst with near zero-order release kinetics and the data shows a cumulative 16% and 8% drug release after 35 days in PBS for F1 and F2 foams, respectively. In terms of total mass of paclitaxel released, by day 35, 15.2 μg was released out of 96.3 μg encapsulated in F1 foam discs and 24.1 μg was released out of 305 μg encapsulated in F2 foam discs. Also there was no obvious difference in the release profiles of rods and discs of the same formulation (data not shown). This confirms that the release variabilities between discs and rods are not significantly different and allows the use of rods for bio-distribution studies as a model (rods are easy to insert into the brain and improve survivability from surgery). Furthermore, the comparison between 5% and 10% paclitaxel loaded PLGA 50:50 discs revealed the reduction in release rate as the drug loading was increased. This phenomenon could probably be the result of slower polymer degradation rate when the drug loading was increased.

3.3. In vitro cell proliferation and apoptotic activity study

This study was aimed at evaluating the controlled release of paclitaxel from the foams resulting in sustained paclitaxel induced cellular apoptosis in C6 glioma cells in vitro. Cell proliferation response and caspase-3 apoptotic activity of C6 glioma cells were quantified for experimental groups (F1 and F2 foams) and compared with that of blank and Taxol® control groups. Cell proliferation response was compared using the cell growth ratio which is defined as the ratio of cells/flask on day 8 to cells/flask on day 4 of treatment.

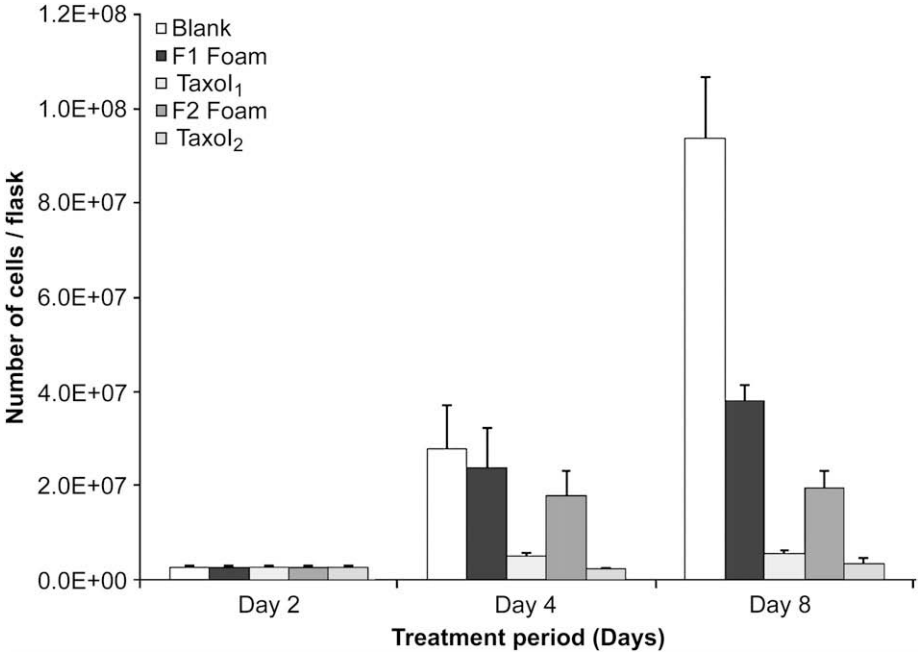

Fig. 3. In vitro cell proliferation response in C6 glioma cells treated for 4 and 6 days with blank control, 5% paclitaxel loaded PLGA 85:15 (F1 foam) discs, Taxol1 control group, 10% paclitaxel loaded PLGA 50:50 (F2 foam) discs and Taxol2 control group. The Taxol control groups are based on a paclitaxel concentration based on 2 × AUC with 6 days release of F1 foams and F2 foams respectively. Each data point represents the average of triplicate samples and error bars represent standard deviation.

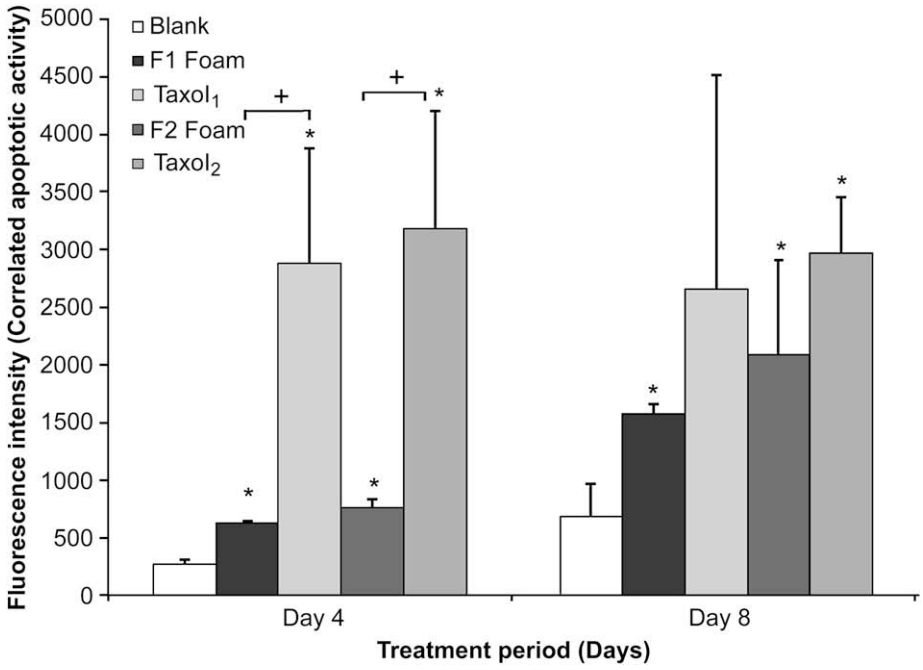

Fig. 4. Extent of apoptosis (fluorescence intensity) in C6 glioma cells in vitro treated for 4 and 6 days with Blank control, 5% paclitaxel loaded PLGA 85:15 (F1 foam) discs, Taxol1 control group, 10% paclitaxel loaded PLGA 50:50 (F2 foam) discs and Taxol2 control group. The Taxol control groups are based on a paclitaxel concentration based on 2 × AUC with 6 days release of F1 foams and F2 foams respectively. Each data point represents the average of three samples and error bars represent standard deviation. (*represents $p < 0.05$ in comparison to control by ANOVA; + represents $p < 0.05$ for comparison between two groups by ANOVA).

It is evident from Fig. 3 that the cell proliferation response to the F1 foams showed a reduction of 14.6% and 61.8% in cells/flask on day 4 and day 8 respectively over the blank control (without treatment). On the other hand, the 8515_Taxol control significantly arrested cell proliferation in comparison to the blank control. However, it showed a marginal recovery after the 24 h acute exposure from day 2 onwards which is reflected in the cell growth ratio of 1.12 which was smaller than the ratio for F1 group which proliferated by 1.52 times over 4 days.

All the groups (barring F1 foam on day 8) exhibited significantly higher apoptosis compared to control group (represented by * in Fig. 4) on days 4 and 8. In comparison to respective Taxol® groups, F1 and F2 foams had significantly lower apoptotic activity on day 4. However, the apoptotic activity of F1 and F2 foams increased and the difference in comparison to Taxol® groups was non-significant. The apoptotic activity data in Fig. 4 suggests that between day 4 and 8, the extent of apoptosis seems to have plateaued for 8515_Taxol group indicating a recovery trend while the activity increases for the F1 group which indicates increasing toxicity due to sustained drug release.

Similar trends were seen with F2 foams which showed a reduction of 35.3% and a 78.9% reduction in cell densities over the blank control groups on days 4 and 8 (Fig. 3). Recovery in cell growth was noticed for 5050_Taxol group after significant cell growth arrest from acute exposure. Cell growth ratio for 5050_Taxol control group was 1.47. On the contrary, F2 foam treated cells showed a ratio of 1.11 indicating stronger suppression of proliferation by the foam. In terms of apoptotic activity, the 5050_Taxol group showed a decreasing trend while the F2 foams exhibit an increase. Also, the higher apoptotic activity exhibited by F2 compared to F1 could be attributed to the higher amount of paclitaxel released as seen in the in vitro release profile in Fig. 2.

This study shows that acute exposure to Taxol® (similar to systemic drug administration which results in drug clearance from the body and tumor recurrence) and subsequent drug clearance resulted in tumor cell recovery and decreased apoptotic activity. Encouragingly, both the foam formulations (F1 and F2) exhibited increase in caspase-3 (apoptotic) activity and low cellular recovery. This demonstrates the benefits of sustained release of paclitaxel from the foams over acute paclitaxel exposure (systemic treatment as observed in conventional IV infusion chemotherapy treatment) at over two times a similar AUC as the foams over 8 days. Hence, sustenance of a therapeutic concentration level of paclitaxel within the vicinity of the tumor is critical to prevent cellular recovery and to ensure a commitment to the apoptosis pathway.

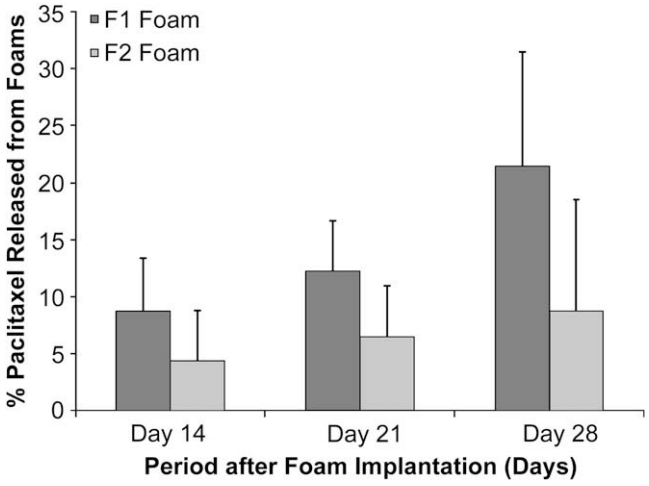

Fig. 5. In vivo release profiles of 5% paclitaxel loaded PLGA 85:15 (F1 foam) discs and 10% paclitaxel loaded PLGA 50:50 (F2 foam) discs over four weeks after subcutaneous implantation. Each data point represents the average of five animals and error bars represent standard deviation.

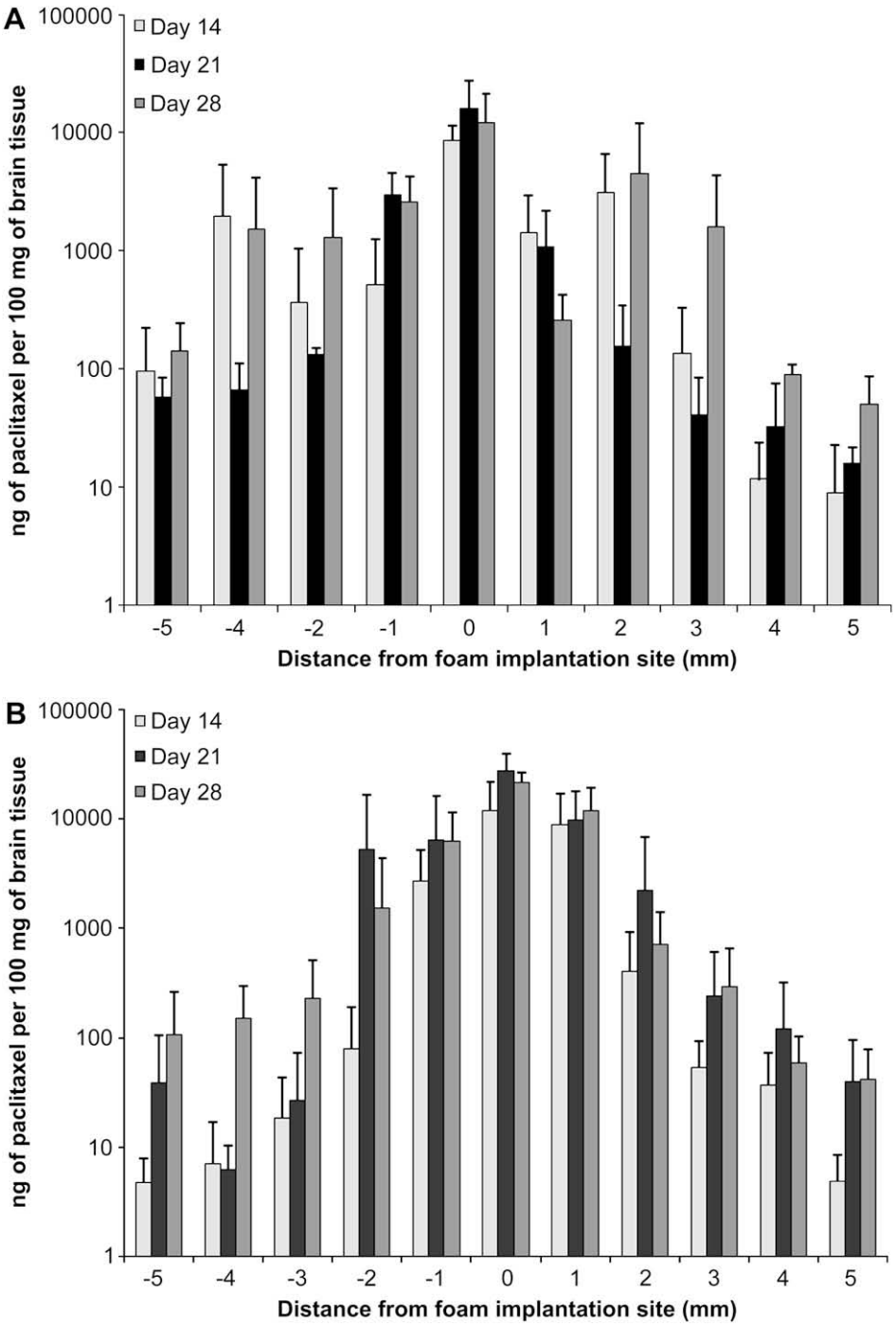

Fig. 6. In vivo bio-distribution of paclitaxel in the rat brain from the site of implantation of (A) 5% paclitaxel loaded PLGA 85:15 (F1 foam) rods; (B) 10% paclitaxel loaded PLGA 50:50 (F2 foam) rods. Each data point represents five samples ($n = 5$ animals). Error bars represent the standard deviation.

3.4. In vivo paclitaxel release (in BALB/c mice)

The paclitaxel release rate was evaluated in vivo to determine the actual rate of drug release within the body's environment. This was used to observe the rate of diffusion of paclitaxel from the pores of the foam into the body fluids and to evaluate degradation of the polymer matrix. Fig. 5 shows the in vivo cumulative % release of paclitaxel from F1 and F2 discs implanted in *BALB/c* mice ($n = 3$ mice) over 3 weeks respectively as described in Section 2.8. This experiment was carried out to evaluate the safety and drug release behavior of the foam disc implants in the in vivo environment. It was observed that, in the F1 foams, 22 wt% of paclitaxel was released after 28 days while for the F2 foams, 9 wt% of paclitaxel was released. By normalizing both foams to an initial drug loading of 10%, we get about 18 wt% drug release for F1 foams and this suggests no significant difference in the in vivo release profiles between F1 and F2. Considering the safety in using these implants, weights of the animals were regularly monitored and it was observed that the weights consistently rose throughout the experiments and did not show signs of paclitaxel related toxicity or

polymer nonbiocompatibility (data not shown), indicating minimal burst release over the 3 weeks post implantation.

3.5. In vivo bio-distribution study

The bio-distribution of paclitaxel in brain tissue was measured in vivo over 3 time points on days 14, 21 and 28 after intracranial implantation. This was carried out to evaluate the penetration of paclitaxel and sustainability of paclitaxel concentration over time. The foams were cut into a $1 \times 1 \times 7$ mm rod weighing approximately 2 mg each (paclitaxel concentration at 100 μg) and inserted vertically into the brain on day 0. At the specific time points, the rat brain was harvested and sectioned coronally into slices of 1 mm thickness and each slice was measured for its paclitaxel concentration.

The results for the F1 and F2 foams shown in Fig. 6A and B, respectively, suggest that paclitaxel concentrations in the tissue could be sustained even up to 28 days after implantation. It is noteworthy here to mention the fact that a multitude of processes would constitute the drug transport from the implant to the target tissue. Two most important processes would be the diffusion of paclitaxel in the extracellular space (ECS) and paclitaxel elimination (due to enzymatic hydrolysis or transcapillary diffusion) [24]. These results indicate that the rate of drug elimination is lower than the release of paclitaxel from the foam and could be justified by the fact that paclitaxel degradation half-life in the brain is reported to be 17,000 min [25]. Also after 28 days, paclitaxel levels beyond 3 mm from the site of implantation were observed to drop off by 500 times. Nonetheless, the paclitaxel concentrations observed were in the desired range of 1 ng/mg tissue ($\sim$1 μM) similar to the mean peak serum concentration for cancer chemotherapy reported [26,27]. No visible signs of necrotic tissue were observed. The LCMSMS assay determined the total paclitaxel concentrations and not the free paclitaxel. It is believed that the tissue-bound paclitaxel (which would account for a larger portion of the drug) would serve as a depot for future continuous paclitaxel release. Over the duration of the experimental groups, no sign of weight loss or indication of toxicity with paclitaxel was observed. Furthermore, while the paclitaxel bio-distribution from the F1 foams seem to be well spread out from the implantation site, the distribution from the F2 foams seemed concentrated at the implantation site. This difference can be attributed to the different drug release rates by the two dosage forms in vitro and in vivo. The F1 foams have slightly higher release rate compared to the F2 foams and could possibly result in slightly farther penetration of paclitaxel.

Hence, the F1 and F2 foams can provide an approximate paclitaxel penetration depth of 3 mm which is consistent with the depth reached by Li et al. (5–7 mm) [16] due to the tissue-binding nature of paclitaxel. Nevertheless, the results suggest that sustained release of paclitaxel up to a month is achievable and thus the foams could be potentially utilized as implants for glioma chemotherapy in the future.

In comparison to other paclitaxel delivery devices used to treat glioma, the PLGA microporous foams have advantages on three terms. The fabrication method (supercritical CO_2 foaming) prevents the need for using organic solvents and hence these foams are preferable. In comparison, other fabrication methods [like EHDA (20), electrospinning (21)] use organic solvents such as dichloromethane and hence need freeze drying to remove residual organic solvent. Also, other devices, such as liquid crystalline cubic phases (18) and polilactofate microspheres (16), use ethanol and ethyl acetate respectively and hence need to be removed. The PLGA microporous foams exhibits low but near zero-order release with minimum initial burst and such a release could be ideal to treat recurrent glioma after surgery. But other devices such as paclitaxel delivering microspheres (16; 20) show a large initial burst which may cause sporadic neurotoxicity. Also, they exhibit very high release rates which could result in faster clearance of the drug from the system, thus negating the advantage of sustained release. In contrast, PLGA compressed discs (15) exhibit almost no release for the first few days followed by a huge burst. In our study, the in vivo release profile confirms the sustained release for up to 28 days. The liquid crystalline cubic phases show undetectable paclitaxel levels in the brain just before seven days (18) and polilactoface microspheres exhibit paclitaxel levels until 30 days with a therapeutic penetration distance of $\sim$5 mm (16). The PLGA foams in this study also exhibit similar bio-distribution compared to the polilactofate microspheres and due to lower release rates are better suited for glioma treatment.

4. Conclusions

The present study demonstrates that the PLGA microporous foams fabricated by supercritical CO_2 gas foaming were able to provide sustained paclitaxel release with near zero-order kinetics and minimal initial burst over one month in vitro. A good correlation was observed between the in vitro and in vivo performances in terms of paclitaxel release. In the evaluation of foams, this paper has demonstrated that in vivo release of paclitaxel for F1 foams was about 13% within the first 2 weeks of implantation and released about 0.35% of the encapsulated drug per day over the range of 14–28 days. The release of the F2 foams was linear over one month at a rate of 0.273% per day. Sustained release from both foams in cell culture experiments induced an increasing apoptotic activity as opposed to an acute exposure over 24 h, which showed tumor cell recovery from decreasing apoptotic activity over time thereby presenting a strong case for sustained release from foams over periodic intravenous systemic chemotherapy. In support of this, sustained levels of paclitaxel were achievable in brain tissue (without tumor) up to 28 days with significant concentration levels (mean peak serum concentration needed for cancer chemotherapy) up to 3 mm from the site of implantation. Nevertheless, further investigation on the anti-tumor efficacy in intracranial glioma bearing mouse models is warranted.

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