Available online at www.sciencedirect.com
SCIENCE DIRECT®
International Journal of Pharmaceutics 251 (2003) 1–12
international journal of pharmaceutics
www.elsevier.com/locate/ijpharm

BCNU-loaded poly(D, L-lactide-co-glycolide) wafer and antitumor activity against XF-498 human CNS tumor cells in vitro

Hasoo Seong [a], Tae Kun An [b], Gilson Khang [b], Sang-Un Choi [c], Chong Ock Lee [c], Hai Bang Lee [a,*]

[a] *Biomaterials Laboratory, Korea Research Institute of Chemical Technology, P.O. Box 107, Daejon 305-340, South Korea*
[b] *Department of Advanced Organic Materials Engineering, Chonbuk National University, 664-14, Dukjin Ku, Chonju 561-756, South Korea*
[c] *Toxicology and Pharmaceutic Screening Center, Korea Research Institute of Chemical Technology, P.O. Box 107, Daejon 305-340, South Korea*

Received 3 July 2002; received in revised form 26 August 2002; accepted 14 September 2002

Abstract

Implantable polymeric device that can release chemotherapeutic agent directly into central nervous system (CNS) has had an impact on malignant glioma therapy. The purpose of our study was to develop an implantable polymeric device, which can release intact 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU) for long-term period over 1 month, and to evaluate its cytotoxicity against XF 498 human CNS tumor cells in vitro. BCNU was incorporated into biodegradable poly(D,L-lactide-co-glycolide) (PLGA), by using spray-drying method. BCNU-loaded PLGA microparticles were characterized by scanning electron microscopy (SEM), powder X-ray diffraction, and differential scanning calorimetry. SEM observation of the microparticles showed that the microparticles were spherical, i.e. microspheres. Homogeneous distribution of BCNU in PLGA microsphere was confirmed by significant reduction of crystallinity of BCNU. Microspheres were fabricated into wafers with flat and smooth surface by direct compression method. In vitro release of BCNU in pH 7.4 phosphate buffered saline was prolonged up to 8 weeks after short initial burst period. Antitumor activity of BCNU-loaded PLGA wafer against XF 498 human CNS tumor cells continued over 1 month and, PLGA only did not affect the growth of the cells. Meanwhile, the cytotoxic activity of BCNU powder disappeared within 12 h. These results strongly suggest that the BCNU/PLGA formulations increase release period of carmustine in vivo and also be useful in the development of implantable polymeric device for malignant glioma.

Keywords: Malignant glioma; 1,3-Bis(2-chloroethyl)-1-nitrosourea; Poly(D,L-lactide-co-glycolide) microparticle; Human tumor cell line

* Corresponding author. Tel.: +82-42-860-7220; fax: +82-42-861-4151
E-mail address: hblee@krict.re.kr (H.B. Lee).

0378-5173/02/$ - see front matter © 2002 Elsevier Science B.V. All rights reserved.
PII: S0378-5173(02)00543-4

1. Introduction

A major obstacle in the successful use of cytotoxic chemotherapeutic agents against brain tumors is the presence of the blood-brain barrier (BBB) that restricts permeability of certain drug molecules within the brain and prevents diffusion of these agents into the brain tumor (Kornblith and Walker, 1998). 1,3-Bis(2-chloroethyl)-1-nitrosourea (BCNU, also called as carmustine) is an important chemotherapeutic agent used for brain tumors, partially due to its ability to cross the BBB (Paoletti, 1984). However, BCNU must be administered in high systemic doses to achieve therapeutic brain levels due to its short half-life of about 20 min in plasma (Loo et al., 1966). Furthermore, prolonged systemic administration is associated with severe side effects such as bone marrow suppression, pulmonary fibrosis and hepatic toxicity (Hochberg et al., 1985).

Development of implantable polymers that release chemotherapeutic agents directly into the central nervous system (CNS) has had an impact on glioma therapy (Chasin et al., 1990; Yang et al., 1989; Reinhard et al., 1991). This technology makes it possible to achieve very high local concentrations of drugs while minimizing systemic toxicity and circumventing the need of a drug to cross the BBB. To date, one of the most outstanding results is BCNU-loaded polyanhydride wafer and recently won approval from FDA adjunct therapy for treatment of brain tumors (Dang et al., 1996; Sampath and Brem, 1998). Clinical trials with this controlled delivery polymer, Gliadel®, have shown an increase of median survival rate of patients, all of whom had failed prior therapy (Brem et al., 1995). Despite the clinical benefits achieved with Gliadel® are significant, improvement in survival was modest (Sipos et al., 1997). Therefore, a clinical dose escalating study was recently carried out and proved that up to 20% loaded BCNU wafer was safe in recurrent malignant glioma (Engelhard, 2000).

One possible approach to release BCNU over expanded period is development of biodegradable polymer system, which has longer degradation period than that of polyanhydride used in the implantable BCNU polymeric device (Langer, 1990; Leong et al., 1985). Modulation of BCNU release period could be achieved by increasing the ratio of carboxyphenoxypropane (CPP) to sebacic acid (SA) in the polyanhydride (Tamada and Langer, 1993; Göpferich and Langer, 1993; Göpferich et al., 1995). However, the maximum release period using 50:50 CPP:SA copolymer was 18 days after a rapid initial burst of BCNU release within the first 24 h (Akbari et al., 1998; Dang et al., 1996; Sipos et al., 1997; Domb et al., 1999). Poly(D, L-lactide-co-glycolic acid) (PLGA) is a well-known biodegradable polymer, which has long history of safe use in pharmaceutical and medical applications (Hutchison and Furr, 1990; Wu, 1995). Furthermore, PLGA is biocompatible and biodegradable in brain tissue (Menei et al., 1993). We therefore designed BCNU loaded PLGA implant for long-term delivery of BCNU over 1 month period due to slowly degrading property of PLGA. Based on these good properties, injectable PLGA microspheres that can release chemotherapeutics, cisplatin and BCNU, were prepared and their therapeutic efficacy was evaluated in the cavity wall of debulked tumors (Emerich et al., 2000).

In this study, to investigate the utility of PLGA as biodegradable polymeric carrier for long-term delivery of BCNU, the release characteristics of BCNU from PLGA wafers were studied. BCNU-loaded PLGA microparticles were prepared by spray-drying method and were fabricated into wafers. The physical characteristics of BCNU-loaded PLGA microparticles were studied using powder X-ray diffraction (XRD), and differential scanning calorimetry (DSC). The in vitro antitumor activity of BCNU released from the wafer was assessed against human CNS tumor cell line in comparison with that of BCNU powder.

2. Materials and methods

2.1. Materials

BCNU was purchased from Sigma Chemical Co. (St. Louis, MO) and stored at −20 °C until use. PLGA (75:25 mole ratio of lactide to glyco-

lide) having molecular weight of 20 000 g/mole (Resomer®, RG 752) and 90 000 g/mole (Resomer®, RG 756) were purchased from Boehringer Ingelheim (Germany). Methylene chloride (MC, Tedia, Japan) and methanol (Junsei, Japan) were used as received. All other chemicals were reagent grade. Deionized water was prepared by a Milli-Q purification system from Millipore (Molsheim, France). RPMI 1640, fetal bovine serum (FBS) and trypsin were purchased from Gibco BRL (Grand Island, NY). Sodium bicarbonate, amphotherin, gentamycin, and 1,2-cyclohexanediaminetetraacetic acid (CDTA) were purchased from Sigma Chemical Co. Sulforhodamine B (SRB), trisma base, tricholoroactic acid (TCA) were also purchased from Sigma Chemical Co. T-25, T-75, 6- and 96-well plates were purchased from Falcon Co. (Lincoln Park, NJ).

2.2. Preparation of BCNU-loaded microparticles and wafers

BCNU-loaded PLGA microparticles were prepared using spray-drying method. BCNU and PLGA were co-dissolved in MC at room temperature. Concentration of polymer solutions was varied from 3 to 30%. Amount of BCNU against PLGA was also varied from 3.85 to 20%. The solution was spray-dried by fluidized bed coater (Uniglatt, Glatt Co., Germany) under the conditions such as 7 ± 2 ml/min of pump speed, 30 ± 4 psi of spraying air pressure, and 50 ± 2 °C of inlet air temperature. The spray-dried microparticles were freeze-dried at 35 mTorr, -50 °C for 48 h and stored at 0 °C until use. Hundred milligram of microparticles were compression molded into wafers using Carver Press (MH-50Y CAP 50 tons, Japan) at 20 kgf/cm^2 for 5 s at room temperature. The wafers were 10×1 mm^2 in size with a flat surface and stored at 0 °C until use.

2.3. Characterization of microparticles

BCNU-loaded PLGA microparticles were observed by scanning electron microscopy (SEM, model S-2250N, Hitachi, Japan) in order to examine the morphology and size of the microparticles. Samples for SEM were mounted on metal stub double-sided tape and coated with platinum for 30 s under argon atmosphere using plasma sputter (SC 500K, EMscope, UK). Crystalline state of BCNU was analyzed by powder XRD (D/MAX-III B, Rigaku, Japan). A Ni filter at 35 kV and 15 mA generated the radiation. The samples were placed in a quartz sample holder and scanned from 0 to 80° at a scanning rate of 5°/min. Thermal characteristics such as melting temperature (T_m) and glass transition temperature (T_g) of BCNU, PLGA, and BCNU-loaded PLGA microparticles were determined by DSC (DSC 3100, TA Instrument, USA). The endothermic heats associated with the melting of BCNU and glass transition of PLGA were analyzed. DSC analyses were carried out at a nitrogen flow of 50 ml/min and a heating rate of 10 °C/min from 0 to 80 °C. The endothermic energy was derived by gravimetrically measuring the peak areas.

2.4. Encapsulation efficiency and in vitro release study

BCNU encapsulated in PLGA microparticles were assayed by a high-performance liquid chromatography (HPLC) in order to determine encapsulation efficiency (EE) associated with the preparation conditions. The EE was calculated from the equation.

$$EE = D_m \times 100/D_t \quad (1)$$

where, D_t is the amount of BCNU loaded to PLGA solution for spray drying and D_m is the amount of BCNU in the spray-dried microparticles. Hundred milligram of BCNU-loaded PLGA microparticles were dissolved in 2 ml of MC. PLGA was precipitated by adding 18 ml of methanol and after centrifugation, 100 μl of aliquots of supernatant were analyzed by HPLC. All samples were analyzed using HPLC system equipped with a Model P-2000 pump, a Model AS-3000 autosampler and a Model UV-1000 UV detector at 237 nm (Thermo Separation Products, Fermont, CA). The column used was μ-Bondapak™ C18 (3.9×300 mm^2, Waters, Milford, MA). Mobile phase was deionized water/methanol

(6:14 v/v) mixture and flow rate was adjusted 1.0 ml/min.

Release of BCNU from PLGA wafer was monitored for a period of incubation in phosphate buffered saline (PBS), pH 7.4 at 37 °C. Wafers were individually placed in 20 ml of PBS with constant shaking at 60 rpm. At specific time following incubation wafers were taken out and freeze-dried for 48 h. After precipitation of PLGA with methanol, 20 μl of aliquots of supernatant were analyzed by HPLC. Due to the unstability of BCNU in the release test condition the amount of BCNU released into PBS was calculated by the amount of BCNU remained in the wafer after specific release test period.

2.5. Cell culture

The human CNS cancer cell line XF498 was provided by National Cancer Institute (MD, USA) and maintained in our laboratory. The cells were grown in RPMI 1640 medium supplemented with 5% (v/v) FBS. The cells were cultured in a 95% air/5% CO_2 atmosphere at 37 °C in a humidified incubator, and were dissociated with 0.25% trypsin and 3 mM CDTA in PBS in case of transferring or dispensing before experiment.

2.6. Cytotoxicity test

For the preparation of testing solution, the BCNU-loaded PLGA or PLGA only wafer was put in cell culture medium (2, 4 and 8 ml), and incubated at 37 °C in the CO_2 incubator. The BCNU powder was also dissolved in cell culture medium at various concentrations (0, 0.3, 1.0, 3.0, 10.0, 30.0 and 100 μg/ml), and incubated at 37 °C in the CO_2 incubator.

The cytotoxicity assay was conducted by using SRB assay, as described previously (Skehan et al., 1990). Briefly, XF498 cells were inoculated over a series of standard 96-well flat bottom microplates (Falcon) and were then preincubated for 24 h to allow attachment to the microtiter plate. Then, 100 μl of the testing solutions were added to the each well according to the time course schedule, and incubated additional 72 h. In the case of wafer-containing solution, the same volume of fresh medium for the subtracted sample solution was added to the test-tube. After continuous exposure to the samples for 72 h, the culture medium was removed from each well, and the cells fixed with 10% cold TCA at 4 °C for 1 h. After washing with tap water, the cells were stained with 0.4% SRB dye and incubated for 30 min at room temperature. The cells were washed again, and then solubilized with 10 mM unbuffered Tris base solution (pH 10.5). The absorbance was measured spectrophotometrically at 520 and 690 nm with a microtiter plate reader (Molecular Devices E-max, Sunnyvale, CA). To eliminate the effects of non-specific absorbance, the absorbance at 690 nm was subtracted from that at 520 nm. The data were transferred and transformed into Micro Excel format (Microsoft Co., Santa Rosa, CA), and survival fractions were expressed as a percent of control. Cell survival fractions were calculated with the aid of three basic measurements; a time zero (Tz) at the beginning of drug incubation, a cell control (CC) at the end of incubation without drug, and a drug-treatment (DT) at the end of the drug incubation period. If $DT \geqslant Tz$, the net percent of cell growth inhibition was calculated by $(DT-Tz)/(CC-Tz) \times 100$. If $DT < Tz$, the net percent of cell killing activity was calculated by $(DT-Tz)/Tz \times 100$. All experiments were performed in triplicate.

3. Results and discussion

3.1. BCNU-loaded PLGA microparticles

The cytotoxic drug BCNU is an effective chemotherapeutic agent used in the treatment of meningeal leukemia and primary and metastatic tumors of the brain. BCNU shows antitumor activity by alkylating DNA or RNA of tumor cell and has no interaction with other alkylating agents. Hydrolysis of BCNU is dependent on pH of solution (Loo et al., 1966). It is most stable at pH 4, however, in acid and in solutions above pH 7, it decomposes rapidly. In plasma, BCNU has a half-life of 20 min in vitro and less than 15 min in vivo. It is also known that BCNU has low melting point of 30–32 °C and is very unstable to light.

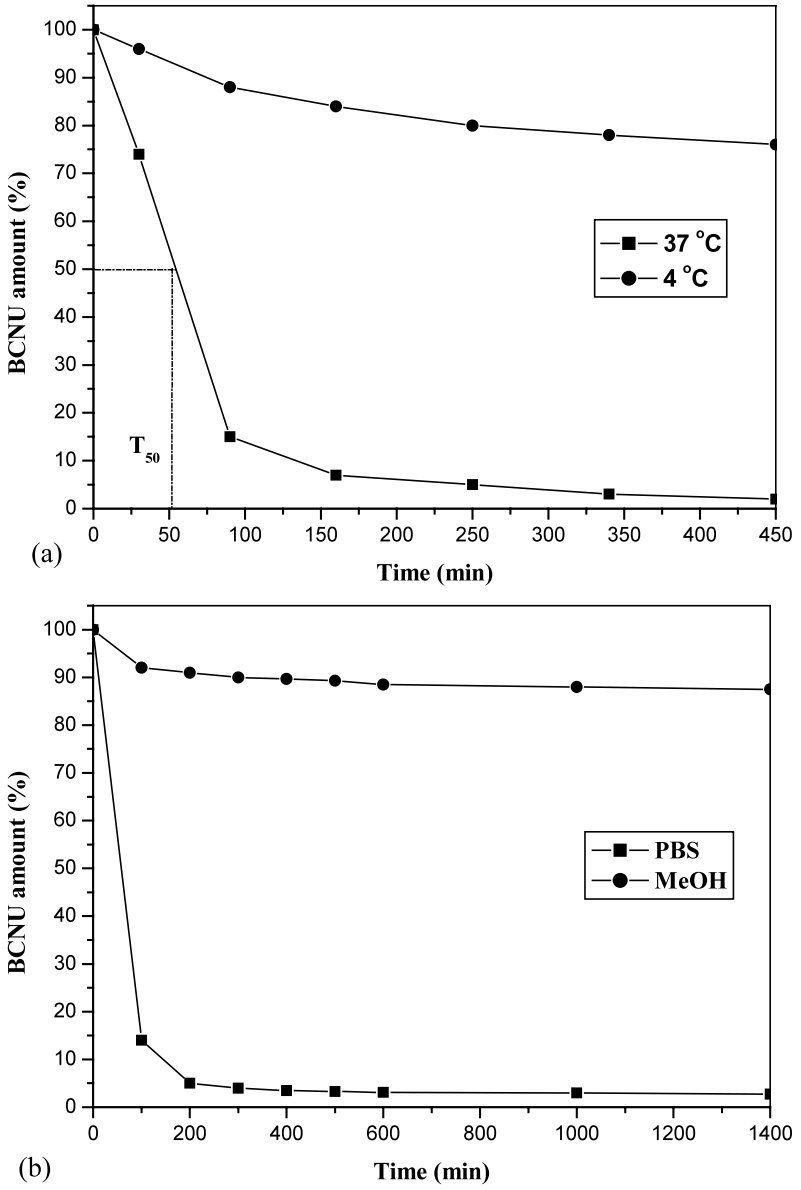

Fig. 1. Degradation behavior of BCNU: (a) BCNU in PBS (pH 7.4) at 37 °C (●) and 4 °C (■); (b) BCNU in MeOH (●) and PBS (pH 7.4) at 37 °C (■).

For the reproducible analysis of BCNU, the stability of BCNU was examined, particularly in PBS, pH 7.4 used as release test media (Fig. 1(a)) as well as methanol used as a mobile phase in HPLC analysis (Fig. 1(b)). At 37 °C (PBS, pH 7.4), half-life (T_{50}) of BCNU was 55 min and the amount of intact BCNU was below 10% after 4 h. In contrast, BCNU was degraded slowly at 4 °C (PBS, pH 7.4) and the amount of intact BCNU was over 81% even after 7 h. BCNU was more stable in methanol and over 92% of carmustine was remained even after 24 h at 37 °C. From these

results, the measurement of the amount of BCNU released into PBS would not be rational due to the instability of BCNU. Therefore, BCNU remained in wafer was measured after designated time period instead of direct measurement of BCNU amount released into PBS in the following release study.

BCNU-loaded PLGA wafers were prepared by spray-drying method. Molecular weight of PLGA, concentration of PLGA, and initial BCNU loading amount were varied and their effects on EE were summarized in Table 1. In case of PLGA having Mwt of 20 000 g/mole, EE decreased with increase of initial BCNU loading amount (Samples 2, 4, 6, and Samples 3, 5, 7). Increase of initial BCNU loading amount at the same PLGA concentration means decrease of relative amount of PLGA that could encapsulate BCNU and hence, decrease of EE could be explained by thermal degradation of BCNU that could not be encapsulated in polymer microparticles or that might be existed on surface of PLGA microparticles. However, the effect of PLGA concentration on EE (Samples 2 and 3, Samples 4 and 5, Samples 6 and 7) was much less than that of initial BCNU loading. In case of microparticles prepared with PLGA having Mwt of 90 000 g/mole, EE was not dependent on either PLGA concentration or initial BCNU loading amount. With the exception of 3.85% of BCNU, EE over 94% was obtained.

Fig. 2 shows SEM microphotographs of BCNU-loaded PLGA microparticles. Microparticles prepared with Mwt of 20 000 g/mole showed spherical shape, that is, microspheres at low BCNU loading amount, whereas the microspheres were aggregated and lost their spherical shape with the increase of BCNU loading amount. Microparticles prepared with PLGA having Mwt of 90 000 g/mole were also microspheres. Though there were some fibrous particles, the aggregated or agglomerated microspheres were not found. In this morphological observation, BCNU crystals were not found irrespective of concentration of PLGA and initial BCNU loading amount. These results suggest that BCNU exist in PLGA microspheres.

To investigate the crystallinity and thermal properties of BCNU in PLGA matrix, BCNU-loaded PLGA microparticles were characterized by XRD and DSC. Fig. 3 shows the XRD spectra of BCNU, PLGA, and BCNU-loaded PLGA microspheres. XRD patterns showed that BCNU was crystalline, showing characteristic peaks at $2\theta = 18$, 23, 27 and 28° while PLGA was amorphous. The crystalline BCNU was not detected in PLGA microspheres containing 10% of BCNU. Fig. 4 shows DSC thermograms of BCNU, PLGA,

Table 1
Spray-drying conditions and EE of BCNU-loaded PLGA microparticles ($n = 3$)

Sample	PLGA Mw (kg/mole)	Initial BCNU loading (%)	PLGA concentration (%)	EE (%)
1	20	3.85	10	92
2	20	5	10	93
3	20	5	30	94
4	20	10	10	91
5	20	10	30	91
6	20	20	10	88
7	20	20	30	89
8	90	3.85	3	89
9	90	3.85	5	91
10	90	5	3	99
11	90	5	5	99
12	90	10	3	95
13	90	10	5	99
14	90	20	3	96
15	90	20	5	94

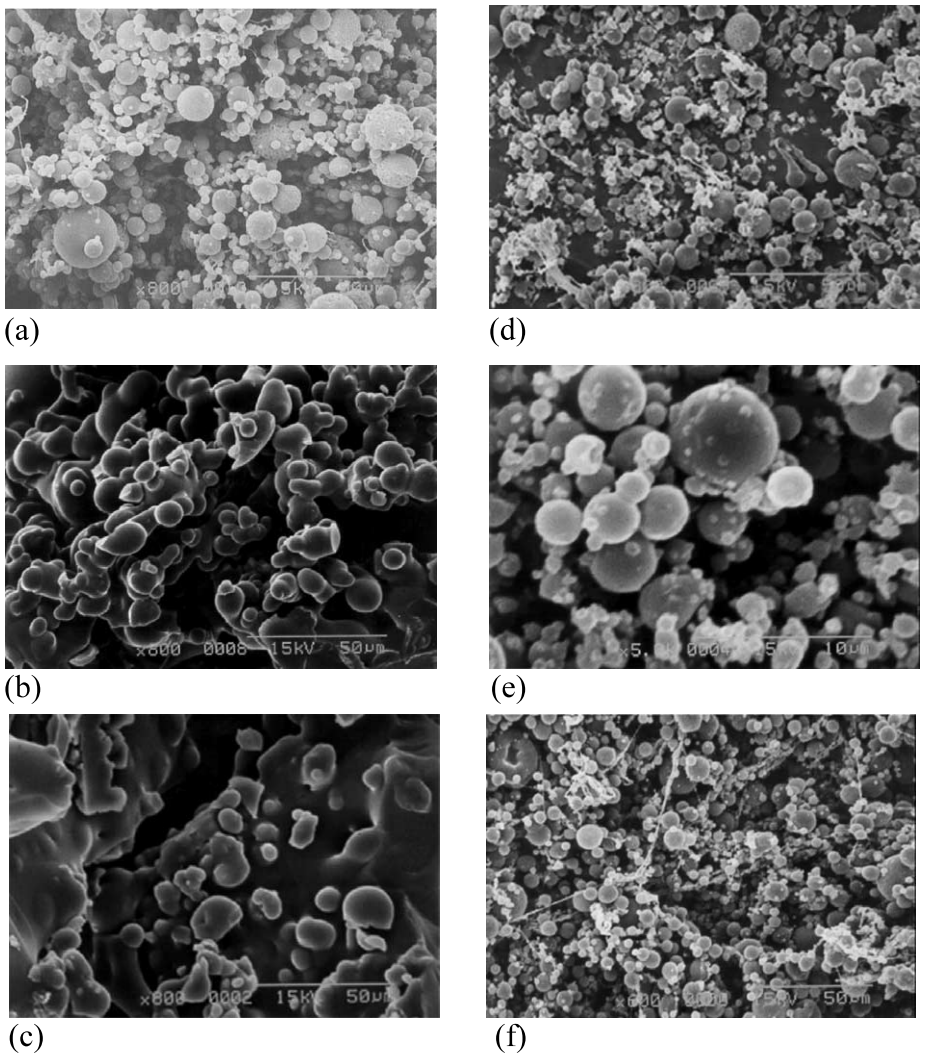

Fig. 2. SEM of BCNU-loaded PLGA microparticles: (a) PLGA 20k 30%/BCNU 5%; (b) PLGA 20k 30%/BCNU 10%; (c) PLGA 20k 30%/BCNU 20%; (d) PLGA 90k 5%/BCNU 5%; (e) PLGA 90k 5%/BCNU 10%; and (f) PLGA 90k 5%/BCNU 20%.

and BCNU-loaded PLGA microspheres. DSC thermograms indicated the T_m of BCNU at 30.6 °C and T_g of PLGA was detected at 50.6 °C. In BCNU-loaded microspheres, melting endotherm of BCNU or glass transition endotherm of PLGA was detected with very weak intensity. Decrease of crystalline domains of BCNU in the PLGA microspheres demonstrated that BCNU was molecularly dispersed in PLGA matrix (Painbeni et al., 1998). From XRD patterns and DSC thermograms, it could be suggested that BCNU existed homogeneously in amorphous form within PLGA microspheres.

3.2. *In vitro release study*

BCNU release profiles in PBS (pH 7.4), 37 °C from the wafers were shown in Fig. 5 (a and b). In case of the wafers prepared with PLGA having Mwt of 20 000 g/mole, the release rate of BCNU increased with the increase of BCNU loading amount, and thereby the period of 100% release

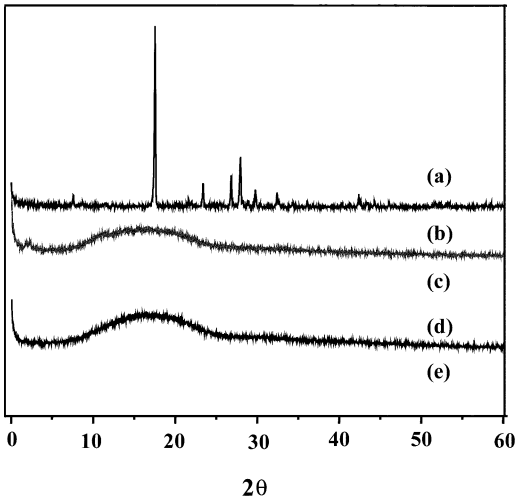

Fig. 3. XRD patterns of BCNU, PLGA, and BCNU-loaded PLGA microparticles: (a) BCNU; (b) PLGA 20k; (c) PLGA 90k; (d) PLGA 20k/BCNU 10% and (e) PLGA 90k/BCNU 10%.

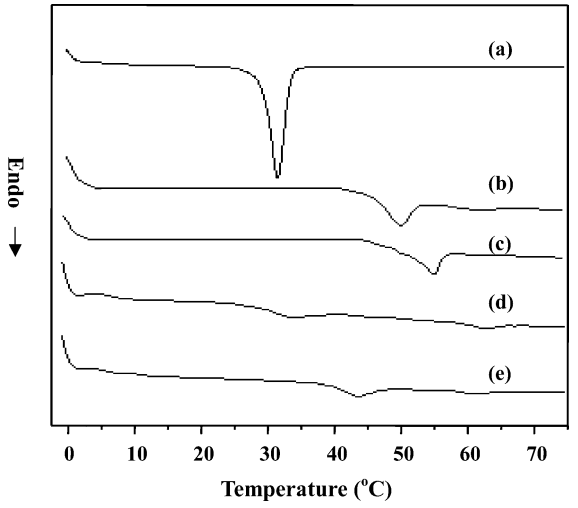

Fig. 4. DSC thermograms of BCNU, PLGA, and BCNU-loaded PLGA microparticles: (a) BCNU; (b) PLGA 20k; (c) PLGA 90k; (d) PLGA 20k/BCNU 10%; and (e) PLGA 90k/BCNU 10%.

was shorten from 60 to 50 days. Initial burst also increased with the increase of BCNU loading amount, however it could not be observed at 3.85% of BCNU loading amount. In contrast to 20 000 g/mole PLGA wafers, 90 000 g/mole PLGA wafers show no difference in initial release pattern in 5 days of incubation irrespective of BCNU loading amount. After 5 days, the release rate of BCNU increased with the increase of BCNU loading amount, and thereby the periods of 100% BCNU release were shorten from 80 to 55 days. The release rate and pattern of drug from the PLGA matrix is mainly dependent on not only diffusion of drug through the matrix but also degradation of PLGA (Batycky et al., 1997). Therefore, drug loading amount, molecular weight, and monomer ratios of PLGA copolymer are the major factors to affect the drug release rate and pattern (Alanso et al., 1993). In addition, the initial drug release pattern is governed by the hydration of polymer, which is considered as the first step in degradation of PLGA (Park, 1995). In our release study, it might be expected that low molecular weight PLGA containing high BCNU amount would be hydrated rapidly. The faster hydration and thereby the rapid chain scission of 20 000 g/mole PLGA wafers can be responsible for the remarkable differences of BCNU release patterns according to the BCNU loading amount at initial period. In contrast, similar BCNU release patterns in initial period can be attributed to less susceptibility to the hydration of the 90 000 g/mole PLGA wafers, which means that the initial drug release would be mainly dependent on the diffusion of BCNU. From these results, it could be suggested that BCNU-loaded PLGA wafers could release BCNU over 1-month period with high dose.

3.3. Cytotoxicity of BCNU-loaded PLGA wafers

In this cytotoxicity test, we selected the BCNU-loaded wafers prepared with PLGA having Mwt of 20 000 g/mole, because, in the previous in vitro releasing test, its releasing pattern was satisfied with our purpose. The cytotoxicities of the BCNU-loaded wafers are shown in Fig. 6. In the case of PLGA only wafer (without BCNU) put in culture medium, all the samples did not reveal cytotoxicity to the XF498 cells up to 48 days. The PLGA only wafer also did not effect on the XF498 cell growth and survival in the direct and continuous exposure to the cell for more than 30 days (data not shown).

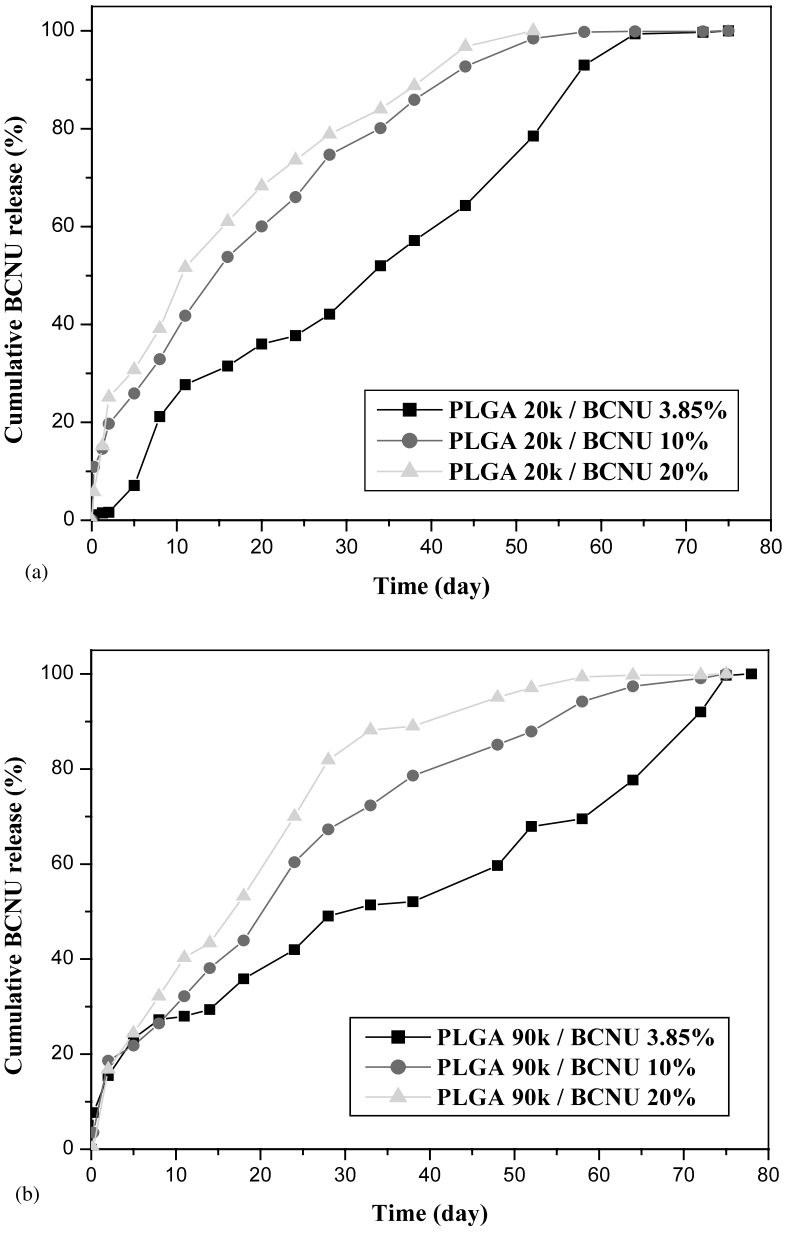

Fig. 5. Effect of BCNU loading amount on BCNU release from PLGA wafers: (a) PLGA 20k and (b) PLGA 90k.

In the case of 3.85% BCNU-loaded wafer, all the samples also did not revealed cytotoxicity to the cells up to 48 days. In 10% BCNU-loaded PLGA wafer, the samples put in 4 and 8 ml showed no effect ($<20\%$) on the cell growth for all the time course tested. Meanwhile, the sample put in 2 ml inhibited the cell growth about 40% from day 18 to day 36. After day 40, it did not effect on the cell growth ($<20\%$). In the case of 20% BCNU-loaded PLGA, the sample put in 8 ml revealed its

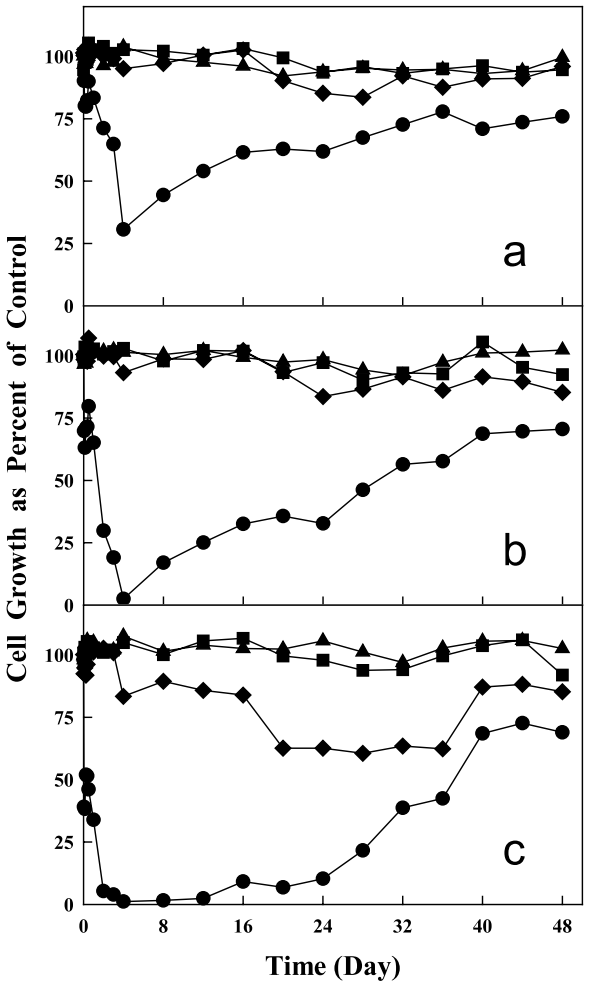

Fig. 6. Cytotoxicity of BCNU-loaded PLGA wafer to the XF498 cells in vitro. The wafers were put in cell culture medium (A, 8 ml; B, 4 ml; C, 2 ml), and the solutions of the samples were transfer to the cells after the various incubation time at 37 °C in the CO_2 incubator. Cell survival fractions were assessed after continuous exposure for 3 days by SRB assay. PLGA only (▲); 3.85% BCNU-loaded PLGA (■); 10% BCNU-loaded PLGA (◆); 20% BCNU-loaded PLGA (●).

maximum effects on the cell growth (~70% growth inhibition) at day 4, and it inhibited the cell growth more than 40% up to 24 days. The sample put in 4 ml also revealed its maximum effect on the cell growth (more than 95%) at day 4, and it inhibited the cell growth more than 70% up to 24 days. After 24 days, it continuously inhibited the cell growth more than 50% up to day 36. The

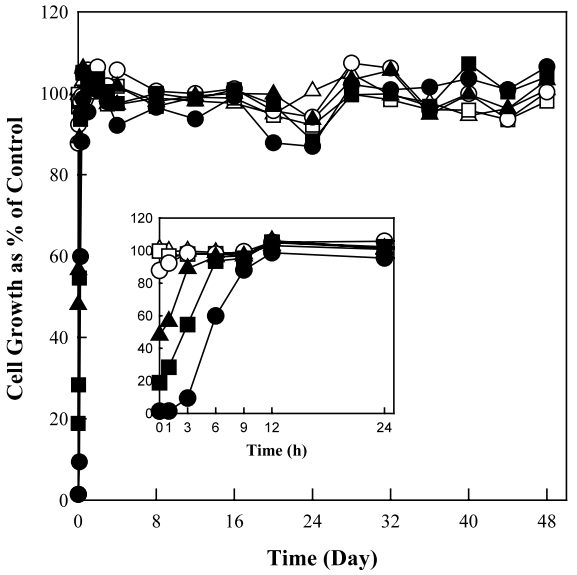

Fig. 7. Cytotoxicity of BCNU to the XF498 cells in vitro. The BCNU powder were dissolved in cell culture medium at various concentrations (Legend), and incubated for various time at 37 °C in the CO_2 incubator. Then, the solutions were added to the cells. Cell survival fractions were assessed after continuous exposure for 3 days by SRB assay. Legend: 0.3 (△), 1.0 (□), 3.0 (○), 10.0 (▲), 30.0 (■) and 100.0 (●) μg/ml.

sample put in 2 ml revealed cell growth inhibitory effect about 40–60% for 24 h. After 24 h, it perfectly inhibited the cell growth (>90%) up to day 24, and it also inhibited the cell growth more than 60% up to day 36.

In the case of free BCNU powder (Fig. 7), 10 μg/ml BCNU inhibited the cell growth more than 40% up to 1 h in comparison with control, but after 3 h, it did not reveal cytotoxicity to the cells. In the case of 30 μg/ml BCNU powder, up to 1 h, it inhibited the cell growth more than 70%, and after 3 h it inhibited the cell growth about 40%. BCNU at 100 μg/ml inhibited the cell growth perfectly (> 90%) up to 3 h, and after 6 h it inhibited the cell growth about 40%. In all the concentrations tested, BCNU powder did not reveal cytotoxicity to the XF498 cells after 9 h. From these results, we can suggest that the BCNU is continuously released from PLGA wafer as active form. In addition, these results strongly correlated with the results of in vitro release study.

4. Conclusions

BCNU-loaded PLGA microparticles were prepared by spray-drying method in reproducible manner and fabricated into wafers. Microparticles had spherical shape, i.e. microspheres. Crystals of BCNU were not detected in BCNU-loaded PLGA microspheres indicative of solid solution of BCNU and PLGA. Release rate and 100% release period of BCNU were dependent on several parameters, such as molecular weight of PLGA, concentration of PLGA, and initial BCNU loading amount. BCNU was released up to 8 weeks with close to zero-order release pattern. In contrast to loss of cytotoxic activity of BCNU due to its short half life, cytotoxic activity of BCNU released from PLGA wafer was continued over 1 month period. This long-term delivery of BCNU with higher doses may be useful in the development of new implantable polymeric device for malignant glioma.

Acknowledgements

This work was supported by KMOCIE (Grant No. B 49-990-5411-05-1-3).

References

Akbari, H., D'Emanuele, A., Attwood, D., 1998. Effect of geometry on the erosion characteristics of polyanhydride matrices. Int. J. Pharm. 160, 83–89.

Alanso, M.J., Cohen, S., Park, T.G., Gupta, R.K., Siber, G.R., Langer, R., 1993. Determinants of release rate of tetanus vaccine from polyester microspheres. Pharm. Res. 10, 945–953.

Batycky, R.P., Hanes, J., Langer, R., Edwards, D.A., 1997. A theoretical model of erosion and macromolecular release from biodegrading microspheres. J. Pharm. Sci. 86, 1464–1477.

Brem, H., Piantadosi, S., Burger, P., Walker, M., Selker, R., Vick, N., Black, K., Sisti, M., Brem, S., Mohr, G., Muller, P., Morawetz, R., Schold, S., 1995. Placebo-controlled trial of safety and efficacy of intraoperative controlled delivery by biodegradable polymers of chemotherapy for recurrent gliomas. Lancet 345, 1008–1012.

Chasin, M., Hollenbeck, G., Brem, H., Grossman, S., Colvin, M., Langer, R., 1990. Interstitial drug therapy for brain tumors: a case study. Drug Dev. Ind. Pharm. 16, 2579–2594.

Dang, W., Daviau, T., Ying, P., Zhao, Y., Nowotnik, D., Clow, C.S., Tyler, B., Brem, H., 1996. Effects of Gliadel® wafer initial molecular weight on the erosion of wafer and release of BCNU. J. Control. Rel. 42, 83–92.

Domb, A.J., Israel, Z.H., Elmalak, O., Teomim, D., Bentolila, A., 1999. Preparation and characterization of carmustine loaded polyanhydride wafers for treating brain tumors, Pharm. Res 16, pp. 762–765.

Emerich, D.F., Winn, S.R., Hu, Y., Marsh, J., Snodgrass, P., LaFreniere, D., Wiens, T., Hasler, B.P., Bartus, R.T., 2000. Injectable chemotherapeutic microspheres and glioma I: enhanced survival following implantation into the cavity wall of debulked tumors. Pharm. Res. 17, 767–775.

Engelhard, H.H., 2000. The role of interstitial BCNU chemotherapy in the treatment of malignant glioma. Surg. Neurol. 53, 458–464.

Göpferich, A., Karydas, D., Langer, R., 1995. Predicting drug release from cylindric polyanhydride matrix discs. Eur. J. Pharm. Biopharm. 42, 81–87.

Göpferich, A., Langer, R., 1993. Modeling polymer erosion. Macromolecules 42, 81–87.

Hochberg, F.H., Pruitt, A.A., Beck, D.O., DeBrun, G., Davis, K., 1985. The rationale and methodology for intra-arterial chemotherapy with BCNU as treatment for glioblastoma. J. Neurosurg. 63, 876–880.

Hutchison, F.G., Furr, A.B.J., 1990. Biodegradable polymer systems for the sustained release of polypeptides. J. Control. Rel. 13, 279–294.

Kornblith, P.L., Walker, M., 1998. Chemotherapy for malignant gliomas. J. Neurosurg. 68, 1–17.

Langer, R., 1990. New methods of drug delivery. Science 249, 1527–1533.

Leong, K.W., Brott, B.C., Langer, R., 1985. Bioerodible polyanhydrides as drug-carrier matrices. I: characterization, degradation, and release characteristics. J. Biomed. Mater. Res. 19, 941–955.

Loo, T.L., Dion, R.L., Dixon, R.L., Rall, D.P., 1966. The antitumor agent, 1,3-bis(2-chloroethyl)-1-nitrosourea. J. Pharm. Sci. 55, 492–497.

Menei, P., Daniel, V., Montero-Menei, C., Brouillard, M., Pouplard-Barthelaix, A., Benoit, J.P., 1993. Biodegradation and brain tissue reaction to poly(D, L-lactide-co-glycolide) microspheres. Biomaterials 14, 470–478.

Painbeni, T., Venier-Julienne, M.C., Benoit, J.P., 1998. Internal morphology of poly(D, L-lactide-co-glycolide) BCNU-loaded microspheres. Influence on drug stability. Eur. J. Pharm. 45, 31–39.

Paoletti, P., 1984. Therapeutic strategy for central nervous system tumors: present status, criticism and potential. J. Neurosurg. Sci. 28, 51–60.

Park, T.G., 1995. Degradation of poly(lactic-co-glycolic acid) microspheres: effect of copolymer composition. Biomaterials 16, 1123–1130.

Reinhard, C.S., Radomsky, M.L., Saltzman, W.M., Hilton, J., Brem, H., 1991. Polymeric controlled release of dexamethasone in normal rat brain. J. Control. Rel. 16, 331–340.

Sampath, P., Brem, H., 1998. Implantable slow-release chemotherapeutic polymers for the treatment of malignant brain tumors. Cancer Control 5, 130–137.

Sipos, E.P., Tyler, B., Piantadosi, S., Burger, P.C., Brem, H., 1997. Optimizing interstitial delivery of BCNU from controlled release polymers for the treatment of brain tumors. Cancer Chemother. Pharmacol. 39, 383–389.

Skehan, P., Sterng, R., Scudiero, D., Monks, A., McMahon, J., Vistica, D., Warren, J.T., Bikesch, H., Denney, S., Boyd, M.R., 1990. New colorimetric cytotoxicity assay for anticancer-drug screening. J. Natl. Cancer Inst. 82, 1107–1112.

Tamada, J., Langer, R., 1993. Erosion mechanism of hydrolytically degradable polymers. Proc. Natl. Acad. Sci. USA 90, 552–556.

Wu, X.S., 1995. Synthesis and properties of biodegradable lactic/glycolic acid polymers. In: Wise, et al. (Eds.), Encyclopedic Handbook of Biomaterials and Bioengineering. Mercel Dekker, New York, pp. 1015–1054.

Yang, M.B., Tarmargo, R.J., Brem, H., 1989. Controlled delivery of 1,3-bis(2-chloroethyl)-1-nitrosourea from ethylene–vinyl acetate polymer. Cancer Res. 49, 5103–5107.