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Controlled-release implant system formulated using biodegradable hemostatic gauze as scaffold

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Abstract

A unique polymer-based sustained-release implant was formulated using biodegradable hemostatic gauze as the scaffold. A piece of commercial gauze, Surgicel™ was coated with a poly(lactic-co-glycolic) acid (PLGA) solution in which drugs were loaded, followed by evaporating the solvent. Drug release kinetics from the PLGA coating was examined using phenol red (PhR, a hydrophilic dye) and carmustine (BCNU, a hydrophobic anti-tumor agent) as model drugs. With an additional drug-free PLGA over-layer coated on top of the drug-loaded PLGA coating, nearly zero order release was archived for both of the model drugs for a period of 12–14 days when the implants were incubated in PBS buffer at 37 °C. However, the drug release rate was independent of types of PLGA polymers such as lactide/glycolide ratio. A degradation study showed that the hydrophilic Surgicel™ scaffold itself degraded in 3 days of the release incubation regardless of the thickness of the polymer coating on top of it, suggesting that the loaded drug may be released through the diffusion channels created by the scaffold degradation. Characterization of this formulation using XRD and DSC indicated that the drug, BCNU, was distributed in the PLGA matrix in amorphous state. Images of scanning electron microscope showed that PLGA was coated on the outer and inner surfaces of the porous Surgicel™.

Keywords: PLGA; Surgicel™; Phenol red; BCNU; Drug delivery; Controlled release; Implant

1. Introduction

Biodegradable polymeric systems have been well studied as sustained-release drug implants in last decades (Brem and Gabikian, 2001; Yasukawa et al., 2005; Huynh et al., 2006). This type of sustained-release implants is especially attractive for post-surgical treatment of brain tumors because these formulations may offer a simple solution to overcome the drug transporting hurdles by the brain–blood barrier (BBB) (Sipos et al., 1997; Brem and Gabikian, 2001; Wang et al., 2002; Guerin et al., 2004). By taking the advantage of surgical operation, a drug-loaded sustained-release polymer implant may be administrated directly at the site of brain so that the systemic toxicity and side effects can be minimized.

While many injectable or implantable sustained-release dosage forms, such as microspheres (Painbeni et al., 1998; Woo et al., 2001; Hickey et al., 2002), nanoparticles (Brannon-Peppas and Blanchette, 2004), wafers (Dang et al., 1996; Seong et al., 2003; Chae et al., 2005; Kim et al., 2005; Lee et al., 2005), films (Witt and Kissel, 2001; Gómez et al., 2004; Wang et al., 2004) and fibers (Xu et al., 2006), are developed for local administration to brain tumors, there are still a number of technical aspects that need to be further improved. These technical issues include drug loading capacity (Dang et al., 1996), release kinetics (Lee et al., 2005; Xu et al., 2006), drug stability (Painbeni et al., 1998; Chae et al., 2005), and manufacture simplicity (Domb et al., 1999; Jain, 2000). For example, the sustained-release BCNU–PLGA wafer, 3.85% of drug loading, has to be prepared via a lengthy procedure involving spray-drying a PLGA solution of higher drug loading to microparticles and compressing the particles into a wafer (Dang et al., 1996). Direct compressing PLGA and drug powders into a wafer resulted in a formulation giving severe burst release (Chae et al., 2005; Lee et al., 2005). Since formulation complexity is undesired for large scale manufacture (Domb et al., 1999), a formulation strategy that offers effective yet simple solution for burst-free and complete sustained-release is highly demanded.

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The approach to address the above issues in the present study is to coat drugs with a sustained-release PLGA layer onto a clinically available biodegradable hemostatic material as the support (or scaffold). Surgicel™ is a biodegradable fibrous hemostat material that can be left at the surgical cavity to stop post-operation bleeding (Lagman et al., 2002; Sabel and Stummer, 2004). We found that Surgicel™ is an excellent scaffold material to support a sustained-release polymer coating. First, its biodegradability makes it possible for implant applications. Its fibrous and porous structure enables sufficient amount of drug-loaded-polymer to be coated.

Phenol red (PhR) and carmustine (BCNU) were used as model drugs to examine the Surgicel™-involved formulation approach in the present study. PhR is a water soluble dye and easily detectable, and can therefore serve as a model for hydrophilic drugs (Takahashi et al., 2004). BCNU is a poorly soluble compound and one of the most frequently used chemotherapeutic drug for cancer therapy whose efficacy in treatment of brain tumor can be greatly improved by sustained-release implant (Gliadel™) (Sipos et al., 1997; Wang et al., 1999; Brem and Gabikian, 2001). Its hydrophobic nature may serve as another type of model drug to examine the applicability of the gauze-coated sustained-release system.

The present study, the Surgicel™-supported polymer-based sustained-release formulations of PhR and BCNU will be examined and characterized by in vitro release kinetics, storage stability, X-ray powder Diffraction (XRD), Differential Scanning Calorimetry (DSC) and Scanning Electron Microscopy (SEM).

2. Materials and methods

2.1. Materials

Poly(DL-lactide-co-glycolic acid) (PLGA; L/G = 50/50, MW 47 kDa; L/G = 65/35, MW 45 kDa; L/G = 75/25, MW 45 kDa; L/G = 50/50, MW 20 kDa) was purchased from Lakeshore Bio-Materials, Inc. (OH, USA). Surgicel™ (oxidized regenerated cellulose) was supplied by Ethicon SARL (Neuchatel, Switzerland). Phenol red (PhR) was obtained from Sinopharm Chemical reagents Co., Ltd. (Shanghai, China). Carmustine (BCNU) was purchased from Dalian Hongfeng Pharmaceutical Co., Ltd. (Dalian, China).

2.2. Preparation of PLGA-based Surgicel™ implants

Drug-loaded PLGA-coated Surgicel™ implants were fabricated by painting a piece of Surgicel™ (2.5 cm × 1 cm) with a drug-containing PLGA solution dissolved in ethyl acetate, one of the most frequently used organic solvent in pharmaceutical industry. BCNU was loaded by co-dissolving it with the polymer in the solvent, while PhR was loaded by dispersing its fine powder in the PLGA solution due to its insolubility in ethyl acetate. After initial drying, the polymer-printed Surgicel™ was placed in a refrigerator set to −20°C for 10 h or longer, followed by further evaporation in vacuum below 0.1 mbar for 48 h to remove solvent residues. Some drug-loaded samples were printed with an additional drug-free PLGA over-layer for improving drug release kinetics. Table 1 summarizes all the formulations examined in this study.

2.3. Determination of drug loadings

The PhR loaded implants were washed with a co-solvent containing methanol and dichloromethane (1:1, v/v) (Takahashi et al., 2004). The resulted PhR solution was diluted and subjected to a UV–vis spectrophotometer (Unicon UV-2000) to measure absorbance at 425 nm.

The BCNU-loaded implants were washed with dichloromethane, followed by adding methanol (9 parts of dichloromethane) to precipitate the PLGA polymer. After centrifugation at 12,000 rpm for 5 min, the supernatant (20 μl) was injected to a Shimazu HPLC system (SPD-10AVP) equipped with a ODS column (Shim-pack VP-ODS, 150 mm × 4.6 mm) and a UV detector. A mobile phase containing methanol and water (7:3, v/v) were used at a flow rate of 1.0 ml/min. Chromatography was recorded by UV absorption at 237 nm (Seong et al., 2003; Lee et al., 2005).

The drug loads of the implants were determined by dividing the weight of recovered drug from an implant with the weight-gain after coating the Surgicel™ piece with the drug–PLGA layer. Standard deviation for the drug loading was calculated based on three repeated coating experiments.

2.4. In vitro release studies of PLGA-based Surgicel™ implant

Drug and PLGA-coated Surgicel™ (2.5 cm × 1 cm, 18 mg) implants were incubated in a 100 mM PBS buffer (pH 7.4) at 37 °C under shaking. For PhR, the release medium was replaced by fresh buffer at scheduled date and its drug contents were assayed using a UV–vis spectrophotometer as described above. For BCNU, since the compound is not stable in the release medium, drugs left in the implants were measured at scheduled dates. At the experimental procedure, an implant to be sampled was removed from the release medium and lyophilized. Then BCNU in the sample was recovered and assayed by the same procedure used for determining drug loading as above. For each sample, the drug release experiment was repeated three times and the release profiles were calculated based on the average of the three experiments.

2.5. Degradation kinetics

Degradation kinetics of plain Surgicel™ and coated Surgicel™ implants were investigated by incubating the samples to be measured in a PBS buffer under identical conditions as drug release experiments. In brief, accurately weighted samples were placed in a test tube containing 2 ml of the PBS (pH 7.4) and shacked at 37 °C. The incubation was terminated at scheduled date, and the remaining solid samples were lyophilized and weighted. The sample degradation rates were calculated

Table 1
Formulations of drug-loaded PLGA-based Surgicel™ implants

Sample	Drug	PLGA (L/G)	Targeted drug-loaded rate (%)	Drug-loaded polymer solution coated (μl)	Drug-free polymer solution coated layer (μl)	PLGA MW (g/mol)
P1	PhR	50/50	5.00	100	–	47 kDa
P2	PhR	50/50	10.00	100	–	47 kDa
P3	PhR	50/50	20.00	100	–	47 kDa
P4	PhR	50/50	5.00	100	–	20 kDa
P5	PhR	65/35	5.00	100	–	45 kDa
P6	PhR	75/25	5.00	100	–	45 kDa
P7	PhR	50/50	5.00	100	100	47 kDa
P8	PhR	50/50	5.00	100	200	47 kDa
P9	PhR	50/50	5.00	100	400	47 kDa
P10	PhR	50/50	10.00	100	100	47 kDa
P11	PhR	50/50	10.00	100	200	47 kDa
P12	PhR	50/50	10.00	100	400	47 kDa
P13	PhR	50/50	20.00	100	100	47 kDa
P14	PhR	50/50	20.00	100	200	47 kDa
P15	PhR	50/50	20.00	100	400	47 kDa
B1	BCNU	50/50	5.00	100	–	47 kDa
B2	BCNU	50/50	10.00	100	–	47 kDa
B3	BCNU	50/50	20.00	100	–	47 kDa
B4	BCNU	50/50	5.00	100	–	20 kDa
B5	BCNU	65/35	5.00	100	–	45 kDa
B6	BCNU	65/35	10.00	100	–	45 kDa
B7	BCNU	65/35	20.00	100	–	45 kDa
B8	BCNU	75/25	5.00	100	–	45 kDa
B9	BCNU	50/50	5.00	100	200	47 kDa
B10	BCNU	65/35	5.00	100	200	45 kDa
B11	BCNU	65/35	10.00	100	200	45 kDa
B12	BCNU	65/35	20.00	100	200	45 kDa

according to the following equation:

$$\text{System Degradation Rate} = \frac{W_i - W_r}{W_i} \times 100\%$$

Where W_i is the initial sample weight and W_r is the remaining sample weight after lyophilization.

2.6. Scanning electron microscopy (SEM)

Scanning electron microscopic (SEM) images of Surgicel™ and drug-loaded Surgicel™ implants were obtained using a Hitachi S-2150 SEM system. Prior to image scanning, the samples were coated with gold vapor under argon atmosphere.

2.7. X-ray powder diffractometry (XRD)

Crystalline structures of BCNU loaded with PLGA polymer on Surgicel™ implants were determined by X-ray diffraction recorded on a Rigaku D/MAX 2000 XRD system equipped with Cu Kα radiation source (40 kV, 20 mA). The BCNU-loaded Surgicel™ implants were cut into small pieces and grounded to powder. Then the powder was loaded on a quartz sample holder and subjected to the XRD machine for measurement. The 2θ scan-rate was $8^\circ\,\text{min}^{-1}$ from 5° to 80°. As controls, physical mixture of BCNU, PLGA and powdered Surgicel™ were subjected to the identical XDR measurement.

2.8. Differential scanning calorimetry (DSC)

The samples were characterized using a differential scanning calorimeter (DSC, TA Q10) for their thermal properties. A BCNU-loaded Surgicel™ implant, 8 mg in weight, was placed in an aluminium pan, sealed hermetically and heated in a nitrogen flow from 0 °C to 180 °C at the rate of 10 °C/min.

3. Results

3.1. Drug load and yield of drug loading

The projected and actual drug loads of some coated Surgicel™ implants and the yield of the drug–polymer coating are summarized in Table 2. For PhR, the drug loading yield was 94% for the samples projecting 5% drug loads (the drug reached 5% of the total mass of the coating material). For the samples with higher projected PhR loads, (10% and 20%), however, the drug-loading yield dropped to 72%. In the case of BCNU-coated Surgicel™ implants, high drug loading yields (99% and 98%) were observed for the samples of 5% and 10% projected drug loads. When the drug loads increased to 20%, the yield of drug loading dropped to 78%. For the Surgicel™ implants loaded with various amount of drugs, same amount of PLGA solution was used (see Table 1). The dependence of PhR loading yields on projected drug loads may partially be due to the small scale preparation for which PhR particles stuck on the surfaces of the container and the spraying equipment represent a considerable

Table 2
Drug loading capacity and yield of drug loading of the implants

Sample	Drug	PLGA (L/G)	Targeted drug-loaded rate (%)	Actual drug-loaded rate (%)	Yield (%)
P1	PhR	50/50	5.00	4.72 ± 0.21	94.34 ± 4.25
P2	PhR	50/50	10.00	7.22 ± 0.25	72.17 ± 2.50
P3	PhR	50/50	20.00	14.41 ± 0.10	72.06 ± 0.50
B5	BCNU	65/35	5.00	4.99 ± 0.29	99.76 ± 6.34
B6	BCNU	65/35	10.00	9.80 ± 0.23	98.03 ± 2.33
B7	BCNU	65/35	20.00	15.66 ± 1.13	78.28 ± 5.64

fraction of total PhR. For BCNU-loaded Surgicel™ implants, since the drug was evenly dissolved in the PLGA solution, the drug loading yield remained as high as 98% for the sample of 10% projected drug load. The result that further increase in projected drug load resulted in a decrease in actual drug loading yield may be attributed to that for high drug concentration, some BCNU may precipitated during the spraying process for which the solvent was evaporated by the spraying gas. While drug loading yield is, in general, an important issue for manufacture, the factors that cause low yield of drug loading are only seen in small scale preparation, and will not affect large scale manufacture.

3.2. Release kinetics of PhR loaded in PLGA-coated Surgicel™ implants

The cumulative release profiles of phenol red (PhR), the hydrophilic model drug, from all 15 formulations of PLGA-coated Surgicel™ implants were summarized in Fig. 1. Fig. 1A shows cumulative release profiles of PhR from the PLGA (average molecular weight = 47 kDa) coated Surgicel™ as a function of drug loads. The rate of cumulative release increased as the projected drug load was increased from 5%, 10% to 20%. For the samples loaded with 5% and 10% PhR, 40% of the loadings were released in the first day, followed by a linear release till day 10. After day 10, the drug release ceased and the release curve suggests 85% of the total loading was depleted (Fig. 1A). For the samples with 20% drug load (actual loading was 14%), the amount of first day release drastically increased to 82% of the total drug load, and the drug release ceased in only 3 days by which 85% of the loading was released.

Fig. 1B compares PhR release profiles from Surgicel™ implants loaded with the same amount of drug (5%), but with PLGA of different molecular weights and lactide/glycolide (L/G) ratio. The samples having L/G ratio of 50/50, 65/35 and 75/25 but same molecular weight (47 kDa) showed similar drug release profiles: a burst release of 45% of drug loading in the first day, followed by a linear release curve up to 85–90% of drug loading till day 10–15. For the sample coated with shorter PLGA (20 kDa, L/G = 50/50), however, the drug release was remarkably faster: the first day burst was doubled as compared with samples coated with the high molecular weight PLGA, and the loaded drugs were depleted within 4 days (Fig. 1B). The drastic increase in PhR release rate when the PLGA (L/G = 50/50) molecular weight decreased from 47 kDa to 20 kDa is interesting.

Fig. 1C–E display the PhR release profiles from the Surgicel™ implants coated with an additional layer of drug-free PLGA (L/G = 50/50, MW = 47 kDa) of different thickness on top of the drug-loaded PLGA (L/G = 50/50, MW = 47 kDa) coatings with various drug loads (5%, 10% and 20%), respectively. The thickness of the drug-free PLGA top-layer was adjusted by the volumes of the drug-free PLGA solution (100 μl, 200 μl and 400 μl) applied to coat a Surgicel™. For comparison, release profiles of the sample with corresponding drug load but without the additional drug-free PLGA top-layer (0 μl) are shown in each the figure, respectively. For all the drug loadings, coating of the additional drug-free PLGA top-layer substantially reduced in burst release. As the amount of the drug-free PLGA coating increased from 0 μl (no additional coating), to 400 μl, the cumulative drug release in day 1 decreased from 50% to 5% for the samples that the first PLGA coating contained 5% and 10% drug (Fig. 1C and D). For the sample loaded with 20% drug, the cumulative drug release in the first day dropped from 80% to 12% when the drug-free PLGA coating was increased from 0 μl to 400 μl (Fig. 1E). In addition, linear release profiles for the initial 12, 9 and 6 days were achieved for the drug loadings of 5%, 10% and 20%, respectively, by coating 400 °C l the drug-free PLGA solution to top of the drug loading PLGA layer (Fig. 1C–E). The cumulative release of PhR in day 1 for the three drug loads (5%, 10% and 20%) are plotted against the amount of drug-free PLGA coating (0 μl, 100 μl, 200 μl and 400 μl), respectively, in Fig. 1F. As the top-coating of the drug-free PLGA layer increased, drug release in day 1 decreased significantly for all the drug loads. These results strongly suggest that the top-coating of the drug-free PLGA layer effectively suppressed the burst release and involved in determining the drug release mechanism.

3.3. Release kinetics of BCNU loaded in PLGA-coated Surgicel™ implants

Fig. 2A and B summarize release profiles of BCNU from coated Surgicel™ of various drug loadings and PLGA types. Interestingly, as shown in Fig. 2A, the samples without the drug-free PLGA top-coating all showed a similar first order BCNU-release profile regardless of the differences in drug loadings and polymer types (L/G ratio and molecular weight).

Fig. 2B shows the BCNU-release profiles from drug-loaded Surgicel™ which had a drug-free PLGA layer coated on the top of the drug-loaded PLGA layer. For comparison, the same

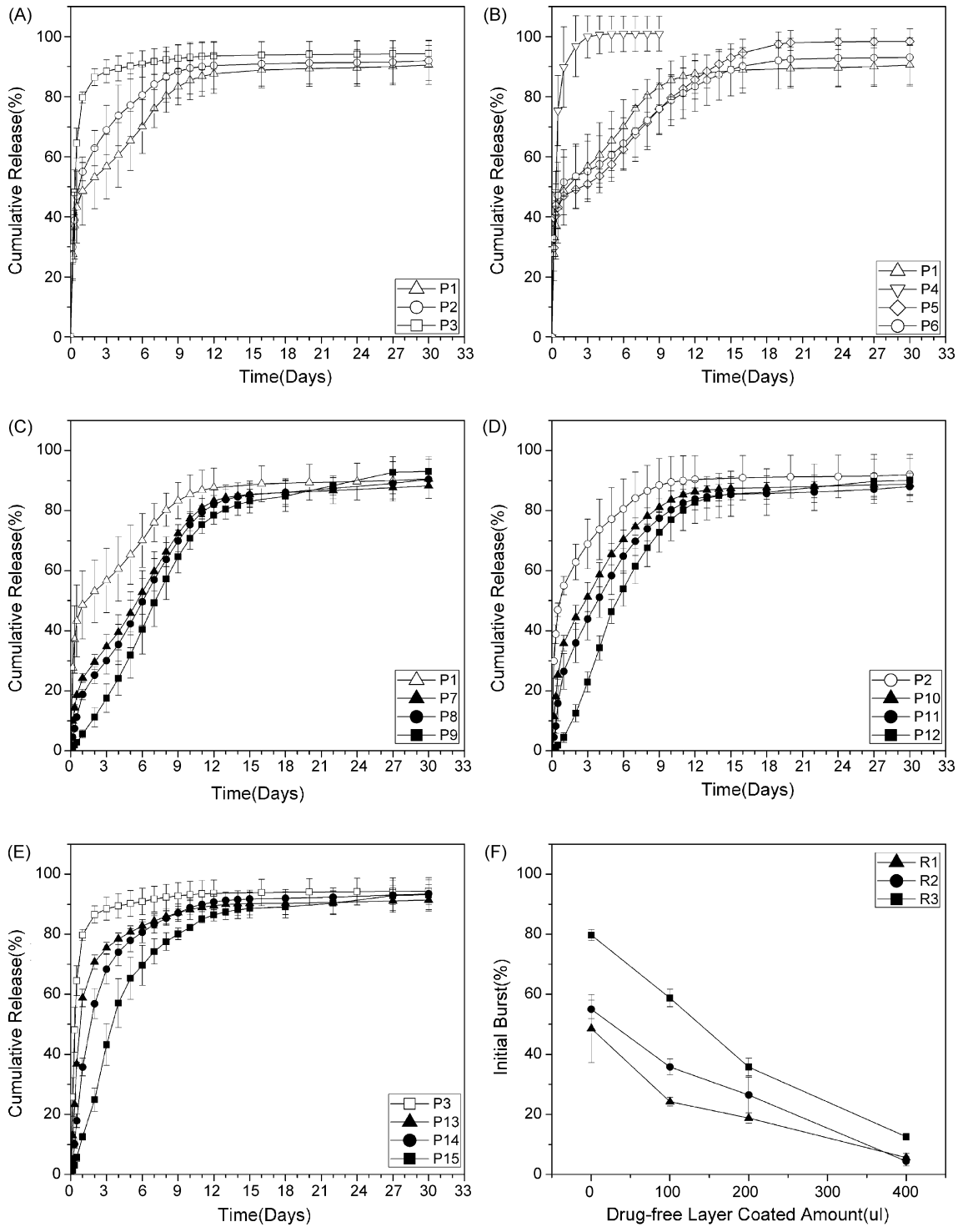

Fig. 1. Release profiles of PhR-loaded implants: (A) different drug loading rate and polymer MW (without layer): P1 (5%), P2 (10%), and P3 (20%) PLGA (50/50, 47 kDa); (B) different monomer ratio (5% without layer): P1, PLGA (50/50, 47 kDa); P5, PLGA (65/35, 45 kDa); P6, PLGA (75/25, 47 kDa); P4 PLGA (50/50, 20 kDa). Different drug-free PLGA (50/50, 47 kDa) coated amount: (C) P1, P7, P8 and P9 with 0 μl, 100 μl, 200 μl, and 400 μl layer at 5%. (D) P2, P10, P11 and P12 with 0 μl, 100 μl, 200 μl, and 400 μl layer at 10%. (E) P3, P13, P14 and P15 with 0 μl, 100 μl, 200 μl, and 400 μl layer at 20%. (F) Different coated amount on initial burst at different drug loading rate: R1 (5%), R2 (10%) and R3 (20%).

drug-loaded Surgicel™ but without the drug-free PLGA top-coating are re-displayed in Fig. 2B. As clear as shown in this figure, diffusion-limited drug release profiles were obtained for the samples of all the three drug loads with the drug-free PLGA top-layer.

3.4. *Degradability of Surgicel™ and coated Surgicel™ implants*

To elucidate the drug release mechanism, degradability of Surgicel™ and Surgicel™ coated with BCNU and PLGA poly-

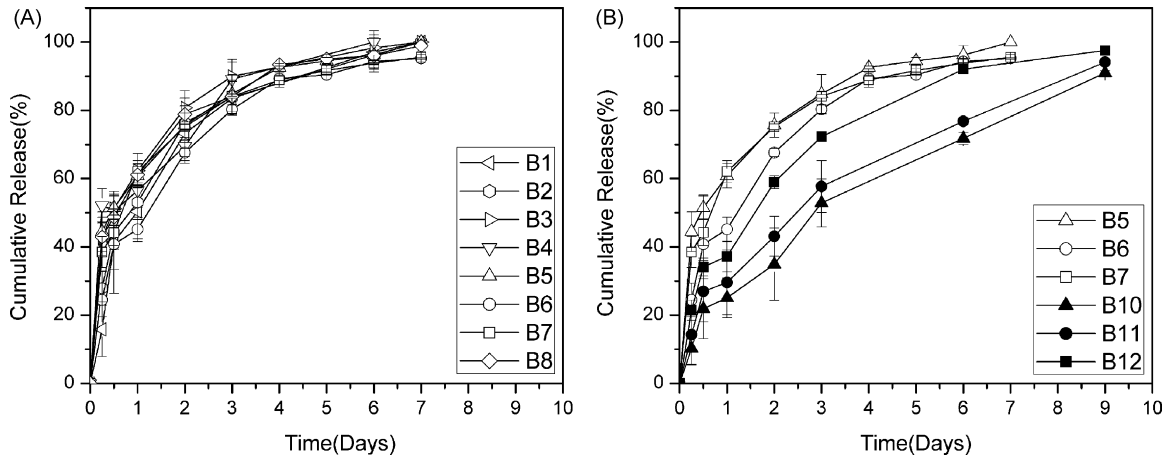

Fig. 2. Release profiles of BCNU-loaded implants: (A) B1, 5% PLGA (50/50, 47 kDa); B2, 10% PLGA (50/50, 47 kDa); B3, 20% PLGA (50/50, 47 kDa); B4, 5% PLGA (50/50, 20 kDa); B5, 5% PLGA (65/35, 45 kDa); B6, 10% PLGA (65/35, 45 kDa); B7, 20% PLGA (65/35, 45 kDa); 5% PLGA (75/25, 45 kDa) were implants without layer. (B) B10, 5% PLGA (65/35, 45 kDa); B11, 10% PLGA (65/35, 45 kDa); B12, 20% PLGA (65/35, 45 kDa) were implants with 200 μl blank polymer layer.

mer were examined in this study. Fig. 3 summarizes the result of the degradation study by plotting weight of each formulation against the time of release incubation at 37 °C. When a piece of un-coated Surgicel™ (2.5 cm × 1 cm, 18 mg) was incubated in 2 ml PBS at 37 °C, the total mass completely disappeared within 3 days (Fig. 3). As unique as shown in Fig. 3, the coated Surgicel™ scaffold with a drug-loaded PLGA layer and with additional drug-free PLGA layer all showed the same weight loss in the same time period, suggesting that the rapid weight loss was due to degradation of Surgicel™ was independent of the PLGA coatings.

3.5. Morphologic study of coated Surgicel™

To better understand the controlled-release patterns of drug from the PLGA layers coated on Surgicel™, morphology of the

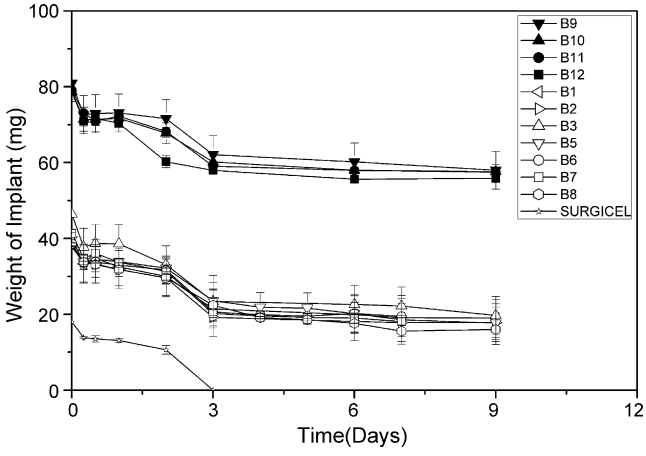

Fig. 3. System degradation diagram: B1, 5% PLGA (50/50, 47 kDa); B2, 10% PLGA (50/50, 47 kDa); B3, 20% PLGA (50/50, 47 kDa); B5, 5% PLGA (65/35, 45 kDa); B6, 10% PLGA (65/35, 45 kDa); B7, 20% PLGA (65/35, 45 kDa); B8, 5% PLGA (75/25, 47 kDa) were implants without layer. B9, 5% PLGA (50/50, 47 kDa); B10, 5% PLGA (65/35, 45 kDa); B11, 10% PLGA (65/35, 45 kDa); B12, 20% PLGA (65/35, 45 kDa) were implants with 200 μl blank polymer layer. Surgicel™ was also tested for comparison.

samples were characterized using scanning electron microscopy (SEM). Fig. 4 shows the SEM images of the samples treated by stepwise PLGA coatings. The left column are the images taken from top of the Surgicel™ after each coating step, while the right column are images taken from cutting edges of the Surgicel™ after each step. The Surgicel™ scaffold itself showed a fibrous structure in which a group of fine fibers piled into each larger bouquet fiber, roughly 10 μm in diameter (by which Surgicel was weaved, Fig. 4A). Fig. 4B shows the cross-section of the bouquet fibers. Coating Surgicel™ with a PLGA layer, with and without 5% BCNU, lead to complete coverage of the top of the bouquet fibers from which the fibrous structure became invisible (Fig. 4C and E). Images of the cross-section of the fibers showed that each of the bouquet fiber is surrounded by PLGA layer (Fig. 4D and F). For the sample with additional drug-free PLGA coating on top of drug-loaded coating (Fig. 4G and H), the Surgicel™ fibers (Fig. 4H) were covered by significantly thicker and more complete PLGA layers. Clearly, this thicker and complete PLGA coating was responsible for regulating the release rate of both PhR and BCNU (Figs. 1 and 2).

3.6. Crystalline structure of BCNU loaded on sustained-release implants

BCNU is a drug with polymorphism character. In order to elucidate effect of the in-polymer dispersion of BCNU on polymorphs of the drug, the drug loaded in PLGA coatings was characterized using X-ray diffraction (XRD). For crystalline BCNU, the XRD patterns featured with a series of peaks, of which the peak at 18.46° is the highest (Fig. 5A). PLGA without BCNU did not show sharp XRD peaks but a broad one from 15° to 25° (Fig. 5B). The sample composed of physically mixed PLGA and crystalline BCNU showed a combined XRD pattern in Fig. 5A–C. Surgice™, coated with drug-free PLGA, with PLGA containing 5% BCNU, and with both 5% BCNU-loaded PLGA and drug-free PLGA layer showed no XRD patterns but a broad bulge curve of the base line (Fig. 5D–G). The XRD

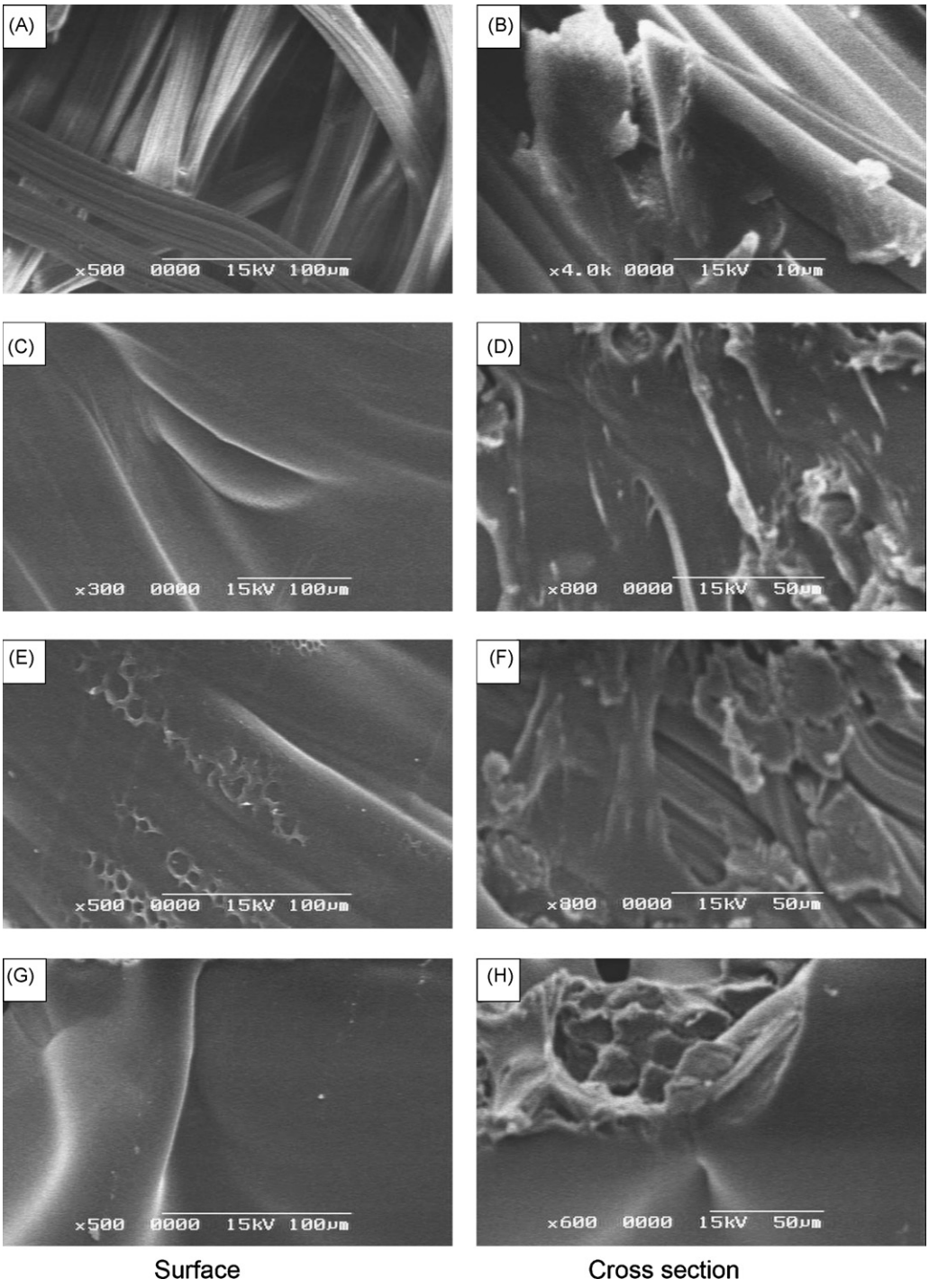

Fig. 4. SEM profile of surface and cross-section of Surgicel™ (A and B); blank Surgicel™ implant, (C and D); 5% BCNU-loaded implant without layer, (E and F); 5% BCNU-loaded implant with blank layer (G and H).

patterns suggest that BCNU loaded in the PLGA layer were in amorphous state.

3.7. Thermal properties of the implants

The Surgicel™ samples loaded with BCNU were characterized using differential scanning calorimetry (DSC) for possible interaction between the drug and the sustained-release polymer. As shown in Fig. 6. pure BCNU exhibited a sharp endothermic peak at 33.6 °C (its melting point), indicating its crystalline structure (Fig. 6A), while pure PLGA generated a curve with a point of inflection at 49.3 °C, indicating its phase transition temperature (Fig. 6B). Mixing BCNU and PLGA caused broadening of the peak for BCNU melting point and shifting of PLGA phase transition temperature, suggesting that there might be some interaction between BCNU and PLGA within the physically mixed powders (Fig. 6C). The Surgicel™ scaffold itself showed a broad endothermic curve centered at 77.8 °C (Fig. 6D).

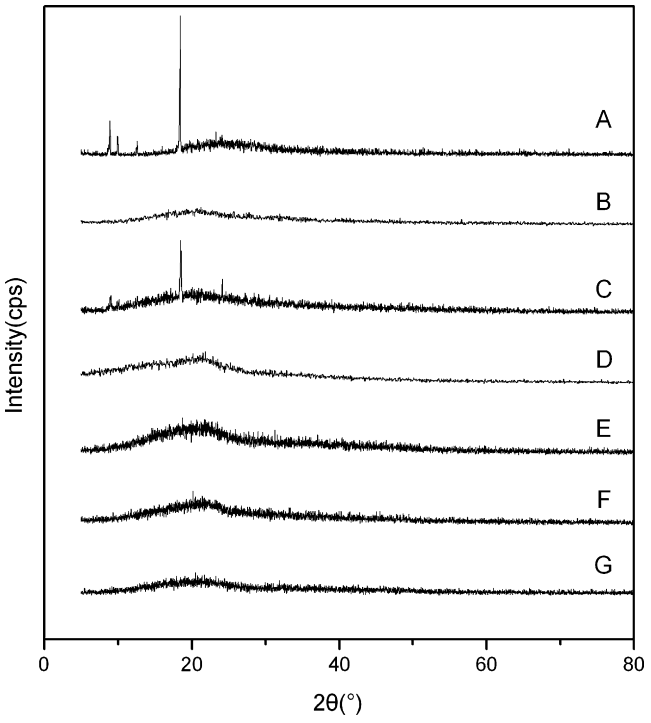

Fig. 5. XRD spectra of the following sample particles: (A) BCNU; (B) PLGA; (C) mixture of BCNU and PLGA; (D) Surgicel^{TM}; (E) blank Surgicel^{TM} implant; (F) B5, 5% BCNU-loaded implant without layer and (G) B10, 5% BCNU-loaded implant with 200 μl blank layer. PLGA (65/35, 45 kDa) were used in the tested samples.

This may be due to dehydration of this cellulose-based material. Coating Surgicel^{TM} with drug-free PLGA, drug-loaded PLGA and the both (with the drug-free layer at the top) lead to flatting of the dehydration curve (Fig. 6E–G), suggesting that dehydration occurred during the coating process. The likelihood may be that the water adsorbed on Surgicel^{TM} was desorbed during evaporation of ethyl acetate, the solvent used to dissolve PLGA. For all the samples coated with PLGA layers, the weak signal for PLGA phase transition (Fig. 6B) became invisible probably because of attenuation of PLGA as a thin layer on the Surgicel^{TM} scaffold (Fig. 6E–G). The peak for melting point of BCNU was not seen in the drug-loaded PLGA coatings (Fig. 6F and G).

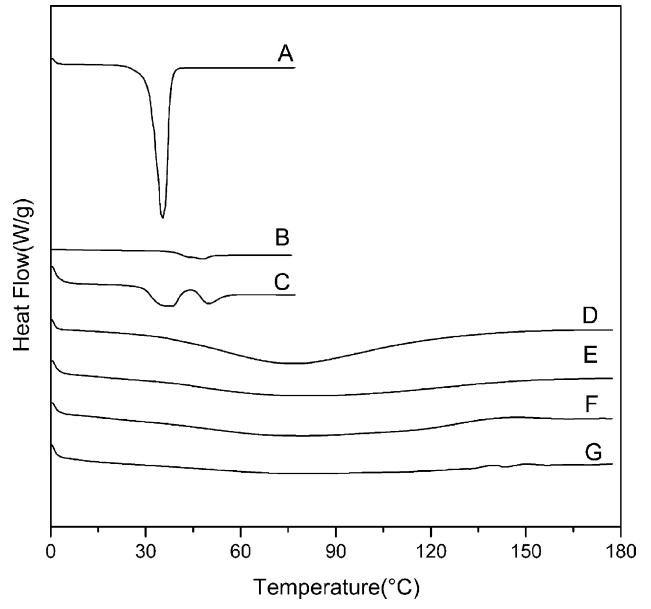

Fig. 6. DSC thermograms of sample particles tested: (A) BCNU, (B) PLGA (65/35, 45 kDa), (C) mixture of BCNU and PLGA (65/35, 45 kDa), (D) Surgicel^{TM}, (E) blank Surgicel^{TM} implant (65/35, 45 kDa), (F) sample B5: 5% BCNU-loaded implant and (G) sample B10: 5% BCNU-loaded implant with 200 μl blank layer.

4. Discussion

4.1. Release mechanism of PhR from PLGA-coated Surgicel^{TM}

While for many PLGA-based controlled-release systems, degradation of the polymer is often the rate-limiting step for release of therapeutics, it is not the case for the system developed in the present study. Because the time taken for complete release of PhR and BCNU (<12 days, Figs. 1 and 2) is substantially less than that taken for PLGA degradation (normally from 2 weeks to months), the rate-limiting step for PhR release is unlikely to be PLGA degradation, but some other factors. The main difference of the gauze-supported PLGA sustained-release drug delivery system is that the hydrophilic gauze scaffold degrades much faster than the PLGA coatings (Fig. 3). This degradation may create diffusion channels for the drugs to release from the system. Since degradation of Surgicel to molecular pieces takes 1–2 weeks [http://store.k12webstore.com/mkgz24.html], the complete weight loss due to Surgicel degradation at day 4 of the release incubation (Fig. 3) suggests that degradated pieces might be fragments larger than single molecules. By taking degradation of the gauze scaffold into account, the conceivable mechanisms for drug release from the gauze-supported PLGA-based system include: (1) diffusion through the channels created by Sugicel degradation; (2) diffusion through the channels created by dissolution of hydrophilic drug itself; (3) diffusion of drugs through hydrated PLGA matrix.

For the PhR loaded Surgicel^{TM} implants, the highly soluble drug may be dissolved by water absorbed into the implants and create diffusion channels. For the implants loaded with 5% and 10%, PhR showed similar sustained-release profiles (Table 1 and Fig. 1A), while that loaded with 20% PhR showed a remarkably increased release (Table 1 and Fig. 1A), suggesting that dissolution of PhR played an important role in for the implant of high PhR load. Probably for implants of low PhR loading (5% and 10%), the drug was mainly released through the diffusion channels created by hydration and degradation of the gauze support (scaffold), and the diffusion channels created by PhR dissolution can be negligible. For the implant with high PhR loading (20%), however, diffusion channels created by increased PhR loading probably became comparable to these created by degradation of the Surgicel support. Drugs diffused through the channels created by dissolved PhR became considerable. This speculation is supported by comparison between PhR release profiles from Surgicel^{TM} implants coated with PLGA of different L/G ratio (Table 1 and Fig. 1B). In general, PLGA microspheres made of PLGA of various L/G ratio (such as 50/50,

65/35 and 75/25) release their loadings at significantly decreased rate with increase in L/G ratio due to decreased hydration and degradation rate of the polymer (Feng et al., 2006). The almost identical release profiles of PhR from the implants coated with PLGA of different L/G ratio and 45 kDa in molecular weight strongly suggest that cross-PLGA diffusion was not an important contributing mechanism for PhR release for the samples coated with PLGA, 45 kDa in molecular weight (Table 1 and Fig. 1B). For the implant coated with low molecular PLGA (MW = 12 kDa, L/G = 50/50), however, the PhR (5% loading) release was drastically accelerated (Fig. 1B). Low molecular weight PLGA, especially those with the L/G ratio of 50/50 and with the end carboxylic groups un-blocked, are extensively hydrated and swollen in an aqueous environment so that cross-PLGA diffusion of PhR became comparable to other diffusion channels.

4.2. Release mechanism of BCNU from PLGA-coated Surgicel™

Release of BCNU from *PLGA-coated Surgicel*™ underwent different profiles from those of PhR (Fig. 2A and B). A first order profile of BCNU-release was observed from the *PLGA-coated Surgicel*™ regardless drug loading, L/G ratio of PLGA and molecular weight of PLGA, suggesting that the rate limit step for BCNU-release was dissolution of this less soluble drug. Rapid degradation of the Surgicel™ scaffold creates sufficient water-filled diffusion channels for BCNU-release for all the implants coated with different types of PLGA. With these diffusion channels, diffusion rate of BCNU became significantly higher than that of BCNU dissolution, thus the overall release rate was limited by dissolution of BCNU. For the implants coated a drug-free with PLGA top-layer, diffusion-limited release profiles of BCNU were observed (Fig. 2B). Clearly the drug-free PLGA top-layer created a diffusion barrier for BCNU by which the cross-PLGA diffusion of BCNU became the rate-limiting step.

For the effect of BCNU morphology on release kinetics, while the XRD study showed that the drug dispersed in the PLGA coating layer was amorphous (Fig. 5), it is not clear why the amorphous BCNU loaded in PLGA of different L/G ratio and molecular weight (which reflect degree of hydrophilicity and water penetration rate) showed similar release profiles (Fig. 2A).

4.3. Advantages and potential applications of Surgicel™-supported PLGA sustained-release implants

Compared with previously reported systems, the primary advantages of this new system is its simplicity in formulation process and its flexibility in adjusting release kinetics. While formulating BCNU into PLGA microspheres prior to wafer fabrication lead to relatively satisfied drug release kinetics (Painbeni et al., 1998), the microencapsulation process involved lengthy and laborious steps. Direct compressing a BCNU and PLGA polymer mixture to sustained-release a wafer or tablet may simplify the formulation process, it results in severe burst release (Lee et al., 2005). By coating drug-loaded PLGA solution on a gauze scaffold, satisfied sustained-release delivery of the drug can easily be achieved without complicated microencapsulation process. In addition, desired release kinetics such as level of initial burst release can easily be achieved for both hydrophilic and hydrophobic drugs and regardless drug loading simply by additional coating of a drug-free PLGA top-layer of different thickness (Figs. 1 and 2). By right thickness, the drug-free top-layer of PLGA may provide an additional control of drug release overwhelming other release mechanisms. This approach of using additional PLGA coating to adjust drug release profiles cannot be easily applied to fibers, wafers and microspheres (Dang et al., 1996; Seong et al., 2003; Chae et al., 2005; Kim et al., 2005; Lee et al., 2005; Witt and Kissel, 2001; Gómez et al., 2004; Wang et al., 2004; Xu et al., 2006). For these micrometer-sized systems, drug release kinetics is strongly affected by drug loading. For example, the PLGA-based implant tablet prepared through direct compressing by Takahashi et al., 70% of PhR load was released in the initial 5 h when the drug loading was over 20% (Takahashi et al., 2004).

In addition to formulation simplicity for sustained-release delivery of BCNU, the system is flexible and applicable to other applications. For example, the PLGA-coated gauze (although lost the capability to absorb blood) can easily be attached with an un-coated Sugicel™ and applied on the wound surface during surgical operation to slop post-surgical bleeding or to prevent scar formation together with sustained-release drug therapy.

The system can also be used to load protein drugs on implants for sustained-release delivery without protein denaturing. Development of sustained-release delivery systems for protein drugs encountered a series of formidable difficulties due to conformational instability of these macromolecules (Sven and Daniel, 2005). Water-soluble proteins are easily denatured during formulation processes involving water–organic solvent interfaces used to form sustained-release microspheres (Ugo et al., 2005). While delicate proteins can be loaded in solvent-resistant polysaccharide particles without exposing them to water–oil interfaces, known to denature proteins, using a stabilized aqueous–aqueous emulsion (Jin et al., 2006), further microencapsulation process involves a "solid-in-oil-in-water" emulsification during which the protein-containing polysaccharide particles may be hydrated, leading an aqueous–oil phase interface. For the present gauze-coating system, the protein–polysaccharide particles can be suspended in the PLGA solution and coated onto gauze scaffold without contacting with water.

5. Conclusion

Using a clinically available hemostat as a scaffold, we demonstrated a unique PLGA-based formulation to achieve controlled-release delivery of both highly soluble and less soluble drugs. Drugs were released from this system through a unique mechanism due to rapid degradation of the Surgicel™ scaffold inside of the PLGA coatings that created diffusion channels independent of drug-loaded PLGA polymer. Such diffusion channels can be rationally blocked by an additional coating of drug-free polymer to reach desired drug release rate. In addi-

tion to nearly zero order and burst-free release kinetics, this formulation approach is simple and does not need complicated microencapsulation process, thus can easily be scaled up.

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References

Brannon-Peppas, L., Blanchette, J.O., 2004. Nanoparticle and targeted systems for cancer therapy. Adv. Drug. Deliv. Rev. 56, 649–1659.

Brem, H., Gabikian, P., 2001. Biodegradable polymer implants to treat brain tumors. J. Control Rel. 74, 63–67.

Chae, G.S., Lee, J.S., Kim, S.H., Seo, K.Su., Kim, M., Lee, H.B., Khang, G., 2005. Enhancement of the stability of BCNU using self-emulsifying drug delivery systems (SEDDS) and in vitro antitumor activity of self-emulsified BCNU-loaded PLGA water. Int. J. Pharm. 301, 6–14.

Dang, W.B., Daviau, T., Ying, P., Zhao, Y., Nowotnik, D., Clow, C.S., Tyler, B., Brem, H., 1996. Effects of GLIADEL(R) 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., Ben-Tolila, A., 1999. Preparation and characterization of carmustine loaded polyanhydride wafers for treating brain tumors. Pharm. Res. 16, 762–765.

Feng, L., Qi, X.R., Zhou, X.J., Maitani, Y., Wang, S.C., Jiang, Y., Nagai, T., 2006. Pharmaceutical and immunological evaluation of a single-dose hepatitis B vaccine using PLGA microspheres. J. Control Rel. 112, 35–42.

Gómez, C., Blanco, M.D., Bernardo, M.V., Olmo, R., Muñiz, E., Teijón, J.M., 2004. Cytarabine release from comatrices of albumin microspheres in a poly(lactide-co-glycolide) film: in vitro and in vivo studies. Eur. J. Pharm. Biopharm. 57, 225–233.

Guerin, C., Olivi, A., Weingart, J.D., Lawson, H.C., Brem, H., 2004. Recent advances in brain tumor therapy: local intracerebral drug delivery by polymers. Invest. New Drugs 22, 27–37.

Hickey, T., Kreutzer, D., Burgess, D.J., Moussy, F., 2002. Dexamethasone/PLGA microspheres for continuous delivery of an anti-inflammatory drug for implantable medical devices. Biomaterials 23, 1649–1656.

Huynh, G.H., Deen, D.F., Szoka, F.C., 2006. arriers to carrier mediated drug and gene delivery to brain tumors. J. Control Rel. 110, 236–259.

Jain, R.A., 2000. The manufacturing techniques of various drug loaded biodegradable poly(lactide-co-glycolide) (PLGA) devices. Biomaterials 21, 2475–2490.

Jin, T., Zhu, H., Zhu, J., 2006. Aquespheres, their preparation and uses thereof, US Patent, 6,998,393 (14 February).

Kim, M.S., Seo, S.K., Hyun, H., Kim, S.K., Khang, G., Lee, H.B., 2005. Sustained release of bovine serum albumin using implantable wafers prepared by MPEG-PLGA diblock copolymers. Int. J. Pharm. 304, 165–177.

Lagman, R., Walsh, D., Day, K., 2002. Oxidized cellulose dressings for persistent bleeding from a superficial malignant tumor. Am. J. Hosp. Palliat. Care 19, 417–418.

Lee, J.S., An, T.K., Chae, G.S., Jeong, J.K., Cho, S.H., Lee, H.B., Khang, G., 2005. Evaluation of in vitro and in vivo antitumor activity of BCNU-loaded PLGA wafer against 9L gliosarcoma. Eur. J. Pharm. Biopharm. 59, 169–175.

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. Biopharm. 45, 31–39.

Sabel, M., Stummer, W., 2004. The use of local agents: surgicel and Surgifoam. Euro. Spine J. 13, S97–S101.

Seong, H., An, T.K., Khang, G., Choi, S., Chong, O.L., Lee, H.B., 2003. BCNU-loaded poly(D,L-lactide-co-glycolide) wafer and antitumor activity against XF-498 human CNS tumor cells in vitro. Int. J. Pharm. 251, 1–12.

Sipos, E.P., Tyler, B., Piantadosi, S., Burger, P.C., Brem, H., 2008. Optimizing interstitial delivery of BCNU from controlled release polymers for the treatment of brain tumors. Cancer Chemoth. Pharm. 39, 383–389.

Sven, F., Daniel, E.O., 2005. Protein drugs stability: a formulation challenge. Nat. Rev. Drug. Discov. 4, 298–306.

Takahashi, M., Onishi, H., Machida, Y., 2004. Development of implant tablet for a week-long sustained release. J. Control Rel. 100, 63–74.

Ugo, B., Eric, A., Eric, D., 2005. Strategic approaches for overcoming peptide and protein instability within biodegradable nano- and microparticles. Eur. J. Pharm. Biopharm. 59, 375–388.

Wang, C.H., Li, J., Teo, C.S., Lee, T., 1999. The delivery of BCNU to brain tumors. J. Control Rel. 61, 21–41.

Wang, P.P., Frazier, J., Brem, H., 2002. Local drug delivery to the brain. Adv. Drug. Deliv. Rev. 54, 987–1013.

Wang, Y., Challa, P., Epstein, D.L., Yuan, F., 2004. Controlled release of ethacrynic acid from poly(lactide-co-glycolide) films for glaucoma treatment. Biomaterials 25, 4279–4285.

Witt, C., Kissel, T., 2001. Morphological characterization of microspheres, films and implants prepared from poly(lactide-co-glycolide) and ABA triblock copolymers: is the erosion controlled by degradation, swelling or diffusion. Eur. J. Pharm. Biopharm. 51, 171–181.

Woo, B.H., Fink, B.F., Page, R., Schrier, J.A., Jo, Y.W., Jiang, G., DeLuca, M., Vasconez, H.C., Deluca, P.P., 2001. Enhancement of bone growth by sustained delivery of recombinant human bone morphogenetic protein-2 in a polymeric matrix. Pharm. Res. 18, 1747–1753.

Xu, X., Chen, X., Xu, X., Lu, T., Wang, X., Yang, L., Jing, X., 2006. BCNU-loaded PEG-PLLA ultrafine fibers and their in vitro antitumor activity against Glioma C6 cells. J. Control Rel. 114, 16–307.

Yasukawa, T., Ogura, Y., Sakurai, E., Tabata, Y., Kimura, H., 2005. Intraocular sustained drug delivery using implantable polymeric devices. Adv. Drug. Deliv. Rev. 57, 2033–2046, http://store.k12webstore.com/mkgz24.html.