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Characterization of perivascular poly(lactic-co-glycolic acid) films containing paclitaxel

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Abstract

The objectives of this study were to investigate the use of poly(lactic-co-glycolic acid) (PLGA) for the formulation of paclitaxel loaded films and to characterize these films for potential application as perivascular "wraps" to prevent restenosis. Films were manufactured from PLGA blended with either methoxypolyethylene glycol (MePEG) or a diblock copolymer composed of poly(D,L-lactic acid)-block-methoxypolyethylene glycol, PDLLA-MePEG (diblock) by solvent evaporation on teflon discs. Elasticity was determined by gravimetric stress/strain analysis. Thermal analysis was determined using differential scanning calorimetry (DSC). Changes in film composition and degradation in aqueous media were determined using gel permeation chromatography (GPC). Paclitaxel release from films was measured by incubation of the films in phosphate buffered saline (PBS) with drug analysis by HPLC methods. The addition of MePEG or diblock to PLGA caused a concentration dependent increase in the elasticity of films, due to plasticizing effects. DSC analysis showed that MePEG and diblock caused a concentration dependent decrease in the glass transition temperature (T_g) of PLGA indicating miscibility of the polymers. When placed in aqueous media, more than 75% of MePEG dissolved out of the PLGA films within 2 days, whereas diblock partitioned slowly and in a controlled manner out of the films. Paclitaxel release from PLGA/MePEG films was very slow with less than 5% of the encapsulated drug being released over 2 weeks. The addition of 30% diblock to paclitaxel loaded PLGA films caused a substantial increase (five- to eight-fold) in the release rate of paclitaxel. PLGA films containing 30% diblock and either 1% or 5% paclitaxel were partially or completely degraded following perivascular implantation in rats.
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Keywords: Perivascular drug delivery; Biodegradable polymers; Paclitaxel; Poly(lactic-co-glycolic acid); Films

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1. Introduction

Restenosis is a vascular disease characterized by intimal hyperplasia, tissue remodeling and lumen narrowing (Kimura et al., 1997; Lafont et al., 1995; Mintz et al., 1996). The disease is a frequent complication arising from vascular interventional procedures such as balloon angioplasty, stent placement or graft insertion (Kumpe, 1997; Serruys et al., 1993; Veith et al., 1995). Following vascular trauma, the process of restenosis involves smooth muscle cell activation, proliferation and migration from the arterial media layer to the intimal layer. Smooth muscle cell proliferation and secretion of an extracellular matrix leads to the formation of neointima and a narrowing of the arterial lumen (for a review, see Chorny et al., 2000).

Localized drug delivery to the vessel wall is believed to be the optimal approach for preventing restenosis and three general strategies have been described: perivascular, intraluminal and endoluminal drug delivery (Chorny et al., 2000). Endoluminal delivery includes the use of drug coated stents. Antiproliferative drugs such as rapamycin and paclitaxel coated onto coronary stents have been shown to effectively inhibit restenosis in clinical trials (Tanabe et al., 2003). Intraluminal delivery involves the deposition of drug solution or drug loaded nanoparticles into the vessel wall via penetration of the endothelium using, for example, microporous angioplasty balloons (Chorny et al., 2000; Axel et al., 1997). In perivascular drug delivery, polymer-based carriers loaded with drug are applied to the outer adventitial surface of the blood vessel. Drug uptake into the vessel wall occurs by passive diffusion and is facilitated by the extensive adventitial vasa vasorum that supplies the vessel wall (Chorny et al., 2000). Edelman and coworkers first demonstrated the use of films of the non-degradable polymer ethylene-vinyl acetate (EVA) for perivascular delivery of heparin (Edelman et al., 1990) and subsequently EVA films have been used to deliver a number of different agents, including antisense oligonucleotides, growth factors and colchicine (Mishaly et al., 1997; Lopez et al., 1996; Chorny et al., 2000). We have described the complete inhibition of intimal hyperplasia in balloon injured rat carotid arteries by the perivascular delivery of paclitaxel loaded in EVA films (Signore et al., 2001). In this study paclitaxel was loaded into EVA films, wrapped around the vessel and sutured in place. Paclitaxel was also loaded into a paste of poly(ε-caprolactone) with 20% MePEG. The films were elastic, easy to manipulate for implantation around the vessel wall and provided excellent controlled drug release profiles. The paste formulation was applied either as a molten paste at 50 °C around the vessel or as solidified pre-cast molds. Although these delivery systems showed efficacy, they were not considered to be optimal formulations for further development. The poly(ε-caprolactone)-based paste and mold were brittle and inflexible, difficult to manipulate and might cause mechanical irritation of the blood vessel in the long term. In addition, this polymer possesses a very long degradation lifetime of well over one year (Pitt et al., 1981). Although the EVA films had excellent handling characteristics, these films were non-degradable and would ultimately require surgical removal.

Film formulations based on the biocompatible and biodegradable polymer PLGA have been prepared for the controlled release of a number of different drugs (Dorta et al., 2002; Gumusderelioglu and Deniz, 2000; Kranz et al., 2000; Webber et al., 1998). Biodegradable polymers clearly possess advantages over non-degradable polymers and films and gels of drug loaded polyanhydride polymers and Pluronic F-127 have been used for perivascular delivery (Teomim et al., 1999; Edelman et al., 2000). The biodegradable copolymer poly(lactic-co-glycolic acid) (PLGA) may be cast into thin films which may be rendered flexible or elastic by the addition of biocompatible plasticizers (Kranz et al., 2000; Webber et al., 1998). The objectives of this study were to investigate the use of PLGA for the formulation of paclitaxel loaded films and to characterize these films for potential application as perivascular "wraps" to prevent restenosis. We found two additives that were miscible with PLGA and produced thin, elastic and flexible film formulations. These plasticizers were methoxypolyethylene glycol (MePEG) (molecular weight 350) and a diblock copolymer, previously synthesized by our group (Zhang et al., 1996; Burt et al., 1999), composed of poly(D,L- lactic acid)-block-methoxypolyethylene glycol (PDLLA-MePEG), termed "diblock" (calculated molecular weight, 3333). Following perivascular implantation in rats, control and paclitaxel loaded films were partially or completely degraded after 28 days in the rats.

2. Materials and methods

2.1. Materials

PLGA with weight percentages of lactic acid to glycolic acid of 85:15 (IV = 0.61 dl/g) and 50:50 (IV = 0.66 dl/g) were obtained from Birmingham Polymers (Birmingham, AL). Methoxypolyethylene glycol (MePEG), molecular weight 350 g/mol, was obtained from Union Carbide (Danbury, CT). The PDLLA-MePEG diblock copolymer was obtained from Angiotech Pharmaceuticals Inc. (Vancouver, BC) and was synthesized using MePEG molecular weight 2000 g/mol and weight percentages of D,L-lactic acid and MePEG of 40: 60. Paclitaxel was obtained from Hauser chemical company (Boulder, CO). All solvents were HPLC grade and were obtained from Fisher Scientific.

2.2. Film preparation

Film casting solutions were made by dissolving the appropriate amount of polymers (PLGA containing MePEG or diblock copolymer) and paclitaxel in 2 ml of dichloromethane (DCM) at a final, total concentration of 10% w/v. For example, films composed of 80% PLGA, 20% MePEG and 5% paclitaxel were cast from a solution containing 5 mg of paclitaxel, 19 mg of MePEG and 76 mg of PLGA per ml of DCM. The solutions were allowed to stand with occasional swirling for approximately 1 h until all components had completely dissolved and the solutions were visually clear. For stress–strain determinations, 1 cm × 2.5 cm Teflon® strips were cut and attached to glass microscope slides to provide a surface for the films to form. Two hundred micro-litres of the 10% w/v polymer solutions were pipetted onto each strip and the DCM was allowed to evaporate in a fume hood for three days (20 mg films). For drug release and weight loss studies the same method as above was used except that 80 μl of polymer/ drug solution was deposited onto 0.8 cm × 0.8 cm Teflon® strips (8 mg films).

2.3. Differential scanning calorimetry (DSC)

DSC was performed using a Perkin Elmer Pyris 1 calorimeter. Approximately 10 mg of film was placed in a crimped aluminum DSC pan. The sample was heated to 80 °C, then rapidly cooled to −80 °C at 200 °C per minute, followed by heating at a rate of 40 °C per minute.

2.4. Stress–strain determinations

Stress–strain determinations were performed as previously described (Jackson et al., 2002). Briefly, rectangular films measuring 1 cm × 2.5 cm × 0.1 mm (20 mg) were placed in a device that clamped the films at the 1 cm ends. The thickness of the film was measured using a digital micrometer (Mitutoyo, Japan) and the length of the film between the clamps was measured using calipers. This clamp device was fixed at one end and suspended in the optical path of a microscope (clamped and oriented in the horizontal direction) with a calibrated eyepiece micrometer. The microscope was focused on a mark on the film. Increasing weights were then applied to the lower end of the film and the extension of the film was measured using the eyepiece micrometer. Films always returned to their original length when weights were removed.

Stress was determined as the force applied per unit area [9.81 × weight applied (kg)/width × thickness (m^2)] (N/m^2).

Strain was determined as the change in film length (extension)/original length (m).

2.5. Gel permeation chromatography (GPC)

Quantitative GPC was performed on the film samples from various time points in the weight loss experiments and also included freshly manufactured non-incubated films at ambient temperature. The system comprised a Shimadzu LC-10 AD HPLC pump, a Shimadzu RID-6A refractive index detector coupled to a 50 Å Hewlett Packard Plgel column. The mobile phase was chloroform with a flow rate of 1 ml/min. The injection volume of the polymer sample was 50 μl at a polymer concentration of approximately 0.25% (w/v) i.e. a 2.5 mg piece of film dissolved in 1 ml of chloroform.

For weight loss determinations, quantitative calibration graphs were made using PLGA, diblock or MePEG solutions containing known concentrations of each polymer in chloroform.

For PLGA degradation studies a calibration graph of log molecular weight versus retention time was es-

tablished for the 50 Å Plgel column using polystyrene standards with molecular weights of 300, 600.1, 4K, 9K, 20K and 30K g/mol. (Polymer Laboratories, Church Stretton, Salop, England).

2.6. Drug release experiments

Drug release experiments were performed as follows: 5 mg films were placed in 16 ml test tubes and 15 ml of 10 mM phosphate buffered saline (pH 7.4) (PBS) were pipetted on top. The tubes were capped and incubated at 37 °C with end-over-end rotation at 8 rpm. At appropriate times, all the 15 ml of buffer was removed to a separate tube and replaced with fresh buffer. One millilitre of DCM was added to the collected sample of buffer and the tubes were capped and shaken for 1 min and then centrifuged at 200 × *g* for 2 min. The supernatant was then discarded (approximately 15 ml) and the lower, paclitaxel-rich DCM phase was evaporated to dryness under gentle heat (40 °C) and nitrogen gas. The dried paclitaxel was then reconstituted in 1 ml of 60:40 acetonitrile:water (v/v) and analyzed by HPLC. HPLC analysis of paclitaxel was performed using a Waters HPLC system (mobile phase 58:37:5 acetonitrile:water:methanol 1 ml/min, 20 μl injection, C18 Novapak Waters column with detection at 232 nm). This method allowed for the recovery of greater than 97% of the drug. All the released drug eluted on the HPLC with a retention time of 2.6 min and a minor component (less than 20% of total drug) at 3.8 min. These retention times confirmed that the released drug was intact (non-degraded) paclitaxel (2.6 min) or 7-epi-paclitaxel (3.8 min) (a pharmacologically active, epimerised paclitaxel).

2.7. Weight loss determinations

Sample films (0.8 cm × 0.8 cm × 0.1 mm) (approximately 8 mg) formulated with either 66.5% w/w 50:50 PLGA, 28.5% w/w diblock and 5% w/w paclitaxel (70:30 ratio of PLGA:diblock) or 80% w/w 50:50 PLGA with 20% w/w MePEG (no paclitaxel) were placed in 14 ml PBS in a culture tube and oscillated at 150 rpm in a 37 °C incubator. At regular intervals, the supernatant was removed and replaced with fresh PBS at 37 °C to maintain sink conditions. At various time points the supernatant was completely removed and the film was dried down at 30 °C under a stream of nitrogen gas. Once completely dry, the film was dissolved in 1 ml of chloroform and the amount of diblock or MePEG remaining in the film was quantitated by GPC. The samples were assayed at ambient temperature by GPC with an injection volume of 50 μl and a mobile phase of chloroform flowing at a rate of 1 ml/min. Separation was achieved through a 50 Å Plgel column (Hewlett Packard). The film components were detected by refractive index detection and the peak areas were used to determine the amount of diblock or MePEG remaining in the films at the appropriate time point of the study. Stock solutions containing PLGA, diblock or MEPEG in the 0–5 mg/ml concentration range were analyzed by GPC and peak areas were used to create separate calibration curves for each polymer. The average concentrations of the diblock or MePEG film components from the 0 day films were assigned the values of 30% w/w or 20% w/w, respectively. The decreases in the diblock or MePEG peak areas for films on subsequent days of the experiment were expressed as weight percentages relative to the 0 day film.

2.8. In vivo film degradation studies

Thirty Wistar rats weighing 400–500 g were purchased from the Animal Care Center of the University of British Columbia. All procedures involving animals were approved by the Animal Care Committee of the University of British Columbia. The animals were anesthetized with 1.5% halothane in oxygen and a 1 cm long segment of the left external carotid artery was exposed. The arteries were injured using an inflated balloon embolectomy catheter procedure according to the method of Signore et al. (2001). Films composed of 50:50 PLGA containing 30% w/w diblock and loaded with 0% (control), 1% or 5% w/w paclitaxel were wrapped around the injured carotid artery and sutured in place ($n = 10$ in each group). The wound was then closed and, in each group, five animals were kept for 28 days and five animals for 12 weeks. At the time of sacrifice, the animals were euthanized with carbon dioxide and pressure perfused at 100 mmHg with 10% phosphate buffered formaldehyde for 15 min. The surgical areas were examined for evidence of degree of intactness and residual pieces of the films.

3. Results

The DCM solvent casting method allowed for the preparation of films with a thickness of approximately 100 μm. Based on ease of handling for perivascular placement, this film thickness was assessed to be appropriate. Thin films (50 μm) tended to fold and self-adhere and 200 or 300 μm films did not bend as readily as the 100 μm films. Therefore, films with dimensions of 0.8 cm × 0.8 cm × 0.1 mm were used in drug release and weight loss studies or 1 cm × 2.5 cm × 0.1 mm for stress–strain determinations. Sample films which were dissolved in DCM and analysed by HPLC were found to contain the expected amount of paclitaxel, confirming 100% encapsulation of the drug in the films. Quantitative GPC confirmed 100% encapsulation of MePEG or diblock in the PLGA films.

3.1. Differential scanning calorimetry

The addition of MePEG to both 50:50 and 85:15 PLGA copolymers caused a concentration dependent decrease in the T_g value of the blended mixture (Fig. 1A and B). DSC scans showed the presence of a single T_g

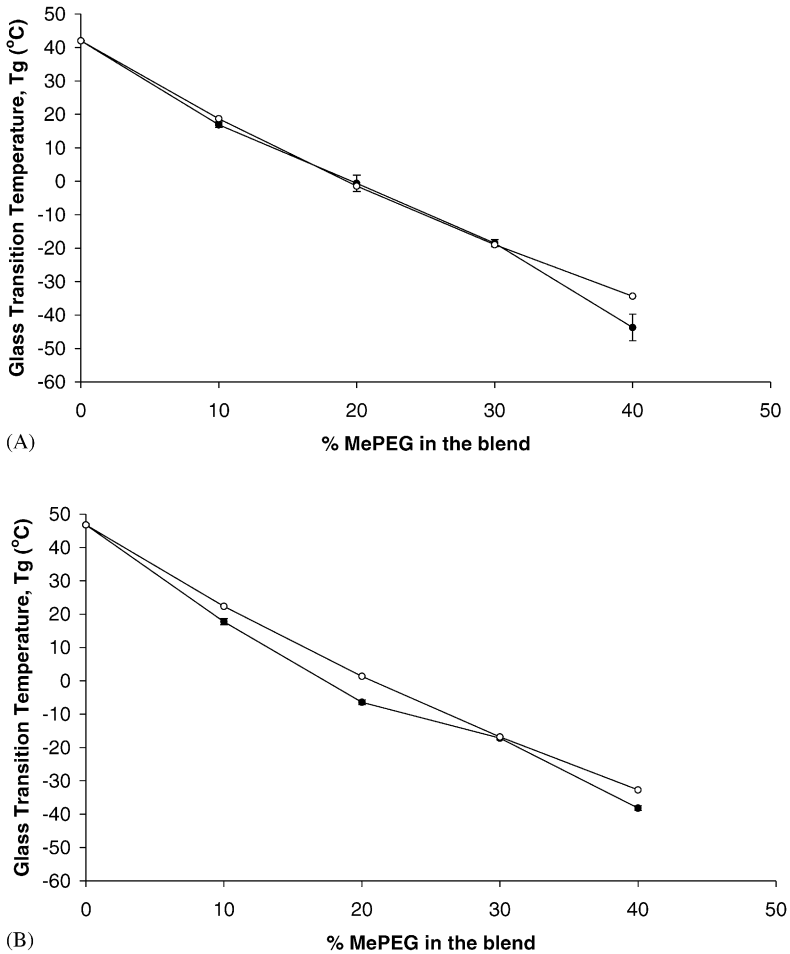

Fig. 1. Effect of blending increasing amounts of MePEG into PLGA films on the glass transition temperature (T_g) of the blend. Theoretical values are based on the Fox equation (◯) and observed values are obtained by DSC (●) (scans obtained at heating rates of 40 °C/min). (A) PLGA composition of 50:50 and (B) PLGA composition of 85:15. Each data point represents the mean of $n = 3$ scans.

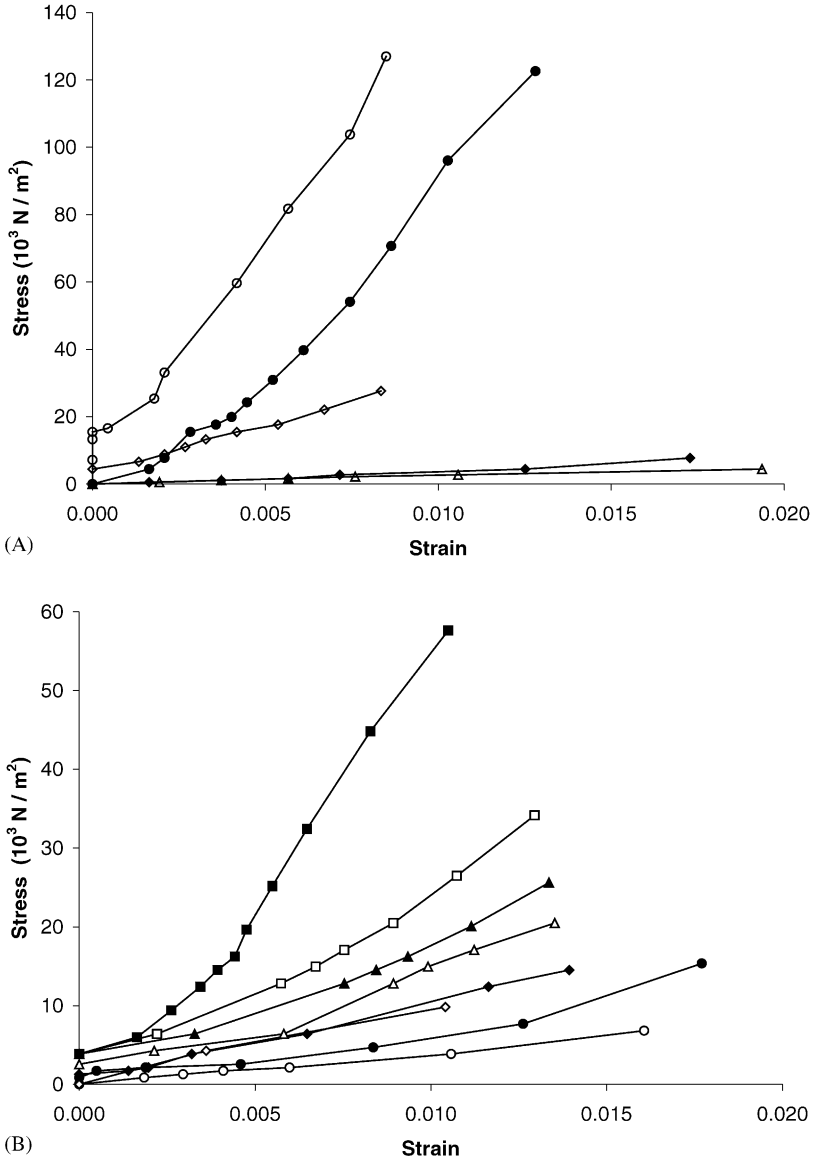

Fig. 2. (A) Effect of blending increasing amounts of MePEG into 85:15 PLGA on the stress/strain properties of the films. Films contained: (◯), no MePEG; (●), 5%w/w MePEG; (◊), 10%w/w MePEG; (♦), 15%w/w MePEG; and (▲), 20%w/w MePEG. (B) Effect of blending increasing amounts of paclitaxel into 85:15 PLGA films containing 15%w/w MePEG on the stress/strain properties of the films. Films contained the following amounts of paclitaxel: (◯), no paclitaxel; (●), 2.5%w/w; (◊), 5%w/w; (♦), 7.5%w/w; (△), 10%w/w; (▲) 15%w/w; (□) 20%w/w; (■), 30%w/w.

for each blend composition. The T_g values for the pure components PLGA 50:50, 85:15 and MePEG were 42, 47 and −98 °C, respectively. Also shown in Fig. 1A and B are the theoretical T_g values for the blended components based on the Fox equation for complete miscibility of the components:

$$\frac{1}{T_g(\text{blend})} = \frac{A}{T_g(\text{MePEG})} + \frac{B}{T_g(\text{PLGA})}$$

where A is the weight fraction of MePEG and B the weight fraction of PLGA.

DSC scans for diblock added to PLGA 50:50 or 85:15 also showed a single T_g for each blend composition. The T_g for the diblock was $-35\,^{\circ}C$ and the addition of increasing amounts of diblock to either 50:50 or 85:15 PLGA up to 40% diblock by weight produced a concentration dependent decrease in T_g of the blend.

3.2. Stress–strain determinations

Films made from PLGA alone were brittle and could not be analyzed by this method of measuring film extension with increasing stress. The effect of adding increasing amounts of either MePEG or diblock to the PLGA films was to increase the elasticity and flexibility of the films. The addition of increasing amounts of MePEG or diblock to the 85:15 PLGA copolymer reduced the gradient of the stress:strain curves (as shown in Figs. 2A and 3A, respectively) and increased the elastic properties of the films. A similar effect for the addition of diblock to 50:50 PLGA copolymer was observed (Fig. 3B). PLGA films prepared from the 85:15 copolymer blended with 15%w/w MePEG were very elastic as indicated by the low gradient of the stress–strain curve in Fig. 2A. However the addition of paclitaxel in the 2.5 to 30% (w/w) range caused a concentration dependent decrease in the elasticity of these films as shown by the increase in the gradient of the stress–strain curves in Fig. 2B.

3.3. Release of MePEG or diblock copolymer from films by GPC

When PLGA films blended with either MePEG or diblock were dissolved in chloroform and analyzed by GPC, there were two distinct peaks in the chromatograms. The earlier peak arose from the higher molecular weight PLGA copolymer and the later peak from the low molecular weight MePEG or from the diblock copolymer. A set of standards containing various weight ratios of PLGA and MePEG or of PLGA and diblock gave quantitative calibration curves for each polymer with correlation coefficients (R^2) greater than 0.98. Following incubation of PLGA films blended with MePEG or diblock in PBS, these calibration graphs were used to quantitate the residual amounts of each component in the incubation tubes at times up to 72 h. The release profile for MePEG from films of PLGA blended with 20%w/w MePEG is shown in Fig. 4. MePEG released rapidly from the PLGA films in the first 8 h (approximately 50% of total content released). This was followed by a slower phase of release so that by 72 h, only about 15% of the MePEG remained in the film. The release profile for diblock from films of PLGA blended with 30%w/w diblock and loaded with 5% paclitaxel is shown in Fig. 5. There was a small burst phase of diblock release in the first day of film incubation in buffer but this represented less than 15% of the total diblock content of the films. This was followed by an almost linear release of diblock from the films at a rate of approximately 3% of the loaded diblock per day.

3.4. Drug release from films

At all paclitaxel loadings, paclitaxel was released via a short burst phase from 50:50 PLGA films blended with 10%w/w MePEG (Fig. 6). Release profiles after the burst phase followed approximately zero order kinetics for all films (R^2 values greater than 0.94). The rate constants were calculated to be 0.21, 0.99, 2.78 and 3.53 μg/day for the 2.5, 7.5 15 and 30%w/w paclitaxel loaded films, respectively. Likewise, the 50:50 PLGA films loaded with 1%w/w paclitaxel and varying amounts of diblock also displayed approximately zero order kinetics after the burst phase with rate constants of 0.0132, 0.0336, 0.0327, 0.094 and 0.2595 μg/day for the 0, 5, 10, 20 and 30%w/w diblock loaded films, respectively (Fig. 7). The addition of diblock up to 20% loading caused a small, concentration dependent increase in the release rate of paclitaxel from the films. However, the addition of 30% diblock copolymer caused between a five- to eight-fold increase in the release rate of the drug over the first 10 days After 10 days, less than 2% of the loaded paclitaxel was released from films containing diblock concentrations of 5, 10 and 20%w/w. However, paclitaxel release increased about six-fold to 13% after 10 days, for films containing 30%w/w diblock copolymer. Similar release profiles were obtained for 50:50 PLGA films loaded with 5%w/w paclitaxel and containing increasing amounts of diblock copolymer so that, for example, by 30 days less than 5% of the drug had released from films containing 5%w/w diblock and approximately 20% of

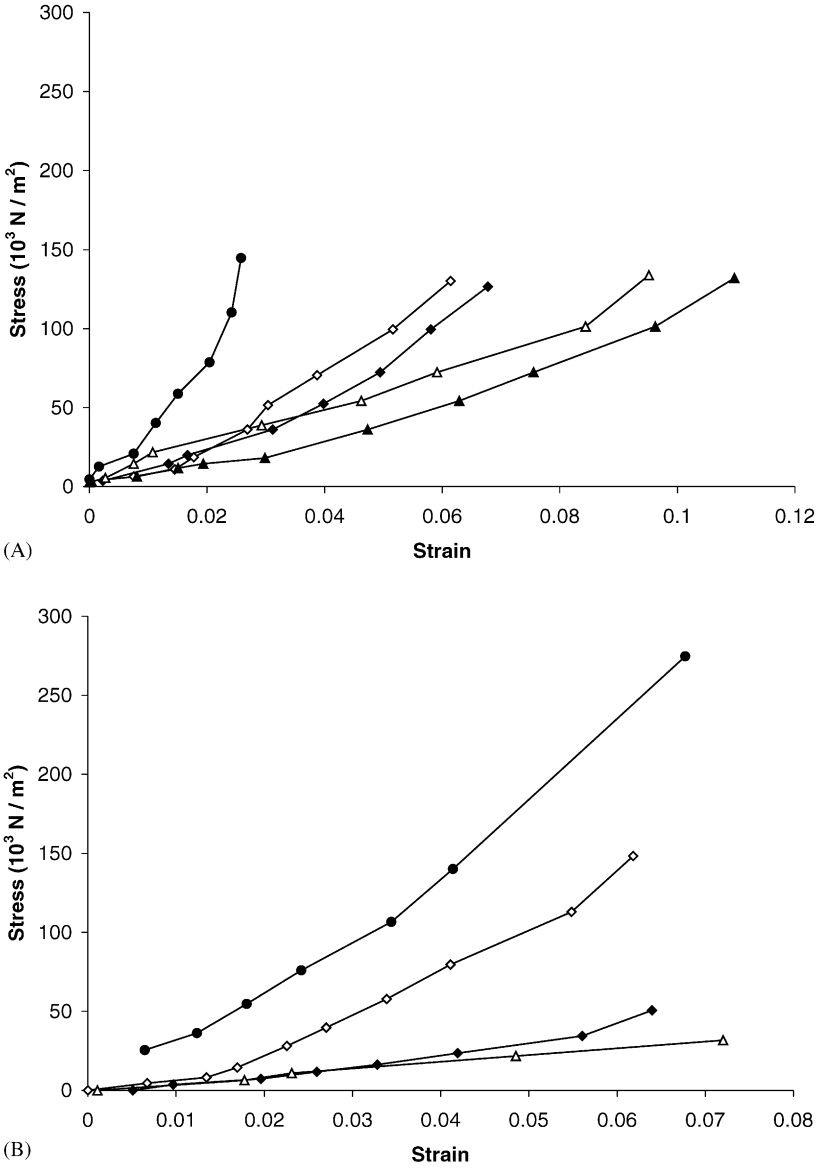

Fig. 3. Effect of blending increasing amounts of diblock copolymer into PLGA films on the stress/strain properties of the films. Films contained: (●): 5%w/w diblock, (◇): 10%w/w diblock, (◆): 20%w/w diblock, (△): 30%w/w diblock and (▲): 40%w/w diblock. (A) PLGA composition of 85:15 and (B) PLGA composition of 50:50.

the drug had released from films containing 30%w/w diblock.

3.5. *In vivo degradation studies*

One animal died in the 1%w/w paclitaxel loaded film (28 day group) so the sample size in this group was $n = 4$. All other groups had a sample size of $n = 5$. Gross observation at the time of sacrifice showed no trace of the control (no paclitaxel) 50:50 PLGA films (blended with 30%w/w diblock) in animals at 28 days. The films had partially degraded, and broken pieces of the films were visible in the 1 and 5%w/w paclitaxel loaded film groups at 28 days. At

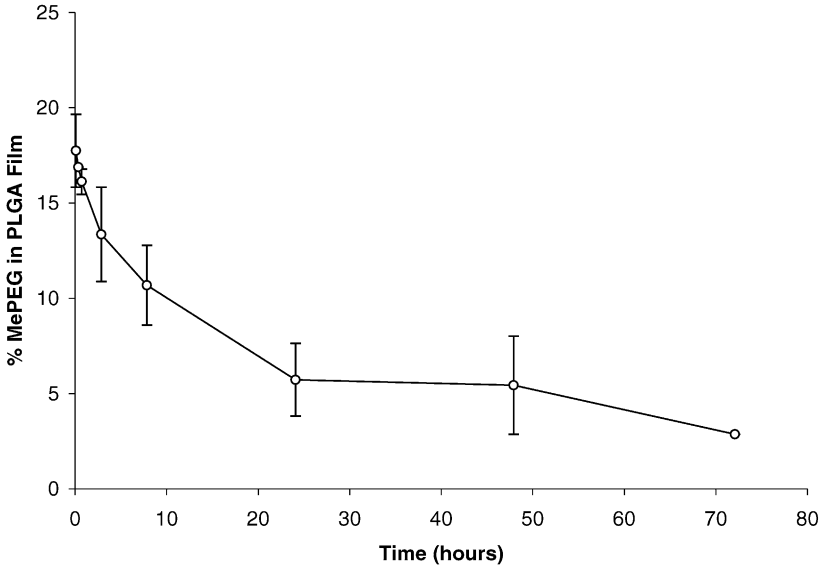

Fig. 4. Time course of MePEG loss from films of 50:50 PLGA blended with 20%w/w MePEG, incubated in PBS, as determined by quantitative gel permeation chromatography.

12 weeks, no film was visible in the control and 1%w/w paclitaxel loaded film groups but traces of polymer were visible in the 5%w/w paclitaxel loaded film group.

4. Discussion

PLGA copolymers with 50:50 and 85:15 weight percentages of lactic acid to glycolic acid content showed

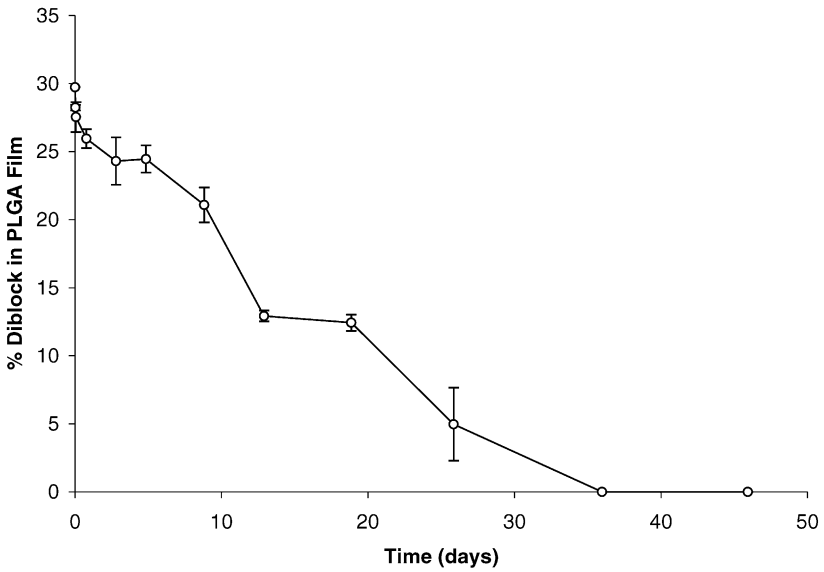

Fig. 5. Time course of diblock loss from films of 50:50 PLGA blended with 30% diblock and 5% paclitaxel, incubated in PBS, as determined by quantitative gel permeation chromatography.

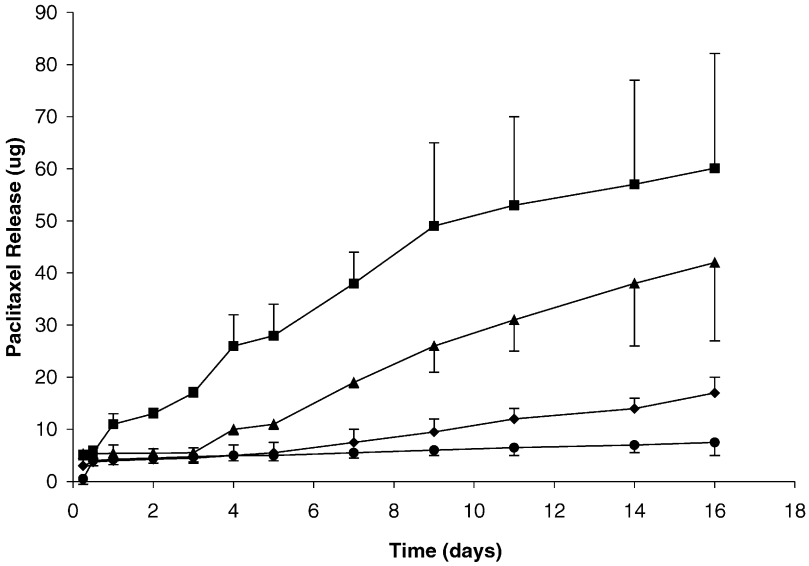

Fig. 6. Time course of paclitaxel release (μg) from 50:50 PLGA films (5 mg) containing 10%w/w MePEG in PBS, pH 7.4 at 37 °C. Films contained the following paclitaxel loadings: (●), 2.5%w/w; (♦), 7.5%w/w; (▲), 15%w/w; (■), 30%w/w (n = 4).

T_g values well above 37 °C (42 and 47 °C) and were brittle at room temperature. The addition of 10%w/w MePEG to either the 50:50 or 85:15 PLGA copolymer caused more than a 25 °C depression of T_g (see Fig. 1A and B), sufficient to allow for the formation of flexible films at room temperature using either polymer. The concentration dependent depression of T_g by the addition of MePEG to PLGA showed good agree-

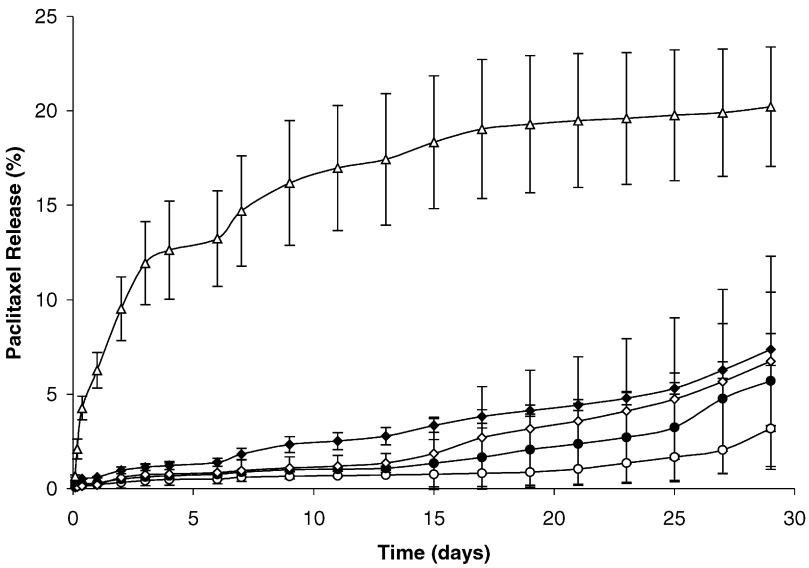

Fig. 7. Effect of blending increasing amounts of diblock copolymer in 50:50 PLGA films containing 1%w/w paclitaxel on the time course of drug release (%) from the films (5 mg). Films contained: (◯), no diblock; (●), 5%w/w diblock; (◊), 10%w/w diblock; (♦), 20%w/w diblock; (△), 30%w/w diblock. (n = 4).

ment with the Fox equation. The single T_g value for the polymer blends, intermediate between the T_g values of the pure components, is indicative of the miscibility of the components in the films (Rosen, 1993). The addition of 30%w/w diblock to either 50:50 or 85:15 PLGA also produced flexible films at room temperature and showed a very large suppression of T_g of more than 40 °C indicating miscibility of the diblock with the PLGA. All films containing MePEG or diblock were clear and one phase.

The elasticity of PLGA films increased with the addition of either MePEG or diblock as determined by stress–strain measurements (Figs. 2A and 3A,B), demonstrating the plasticizing effects of MePEG and diblock copolymer. For example, the addition of 10%w/w MePEG or diblock caused a four- to five-fold increase in elasticity, respectively. The structure of the diblock copolymer was a polyether-polyester, PDLLA-MePEG, and possessed a relatively low calculated molecular weight of around 3333. The short chains of the diblock were miscible with the PLGA, lowered the T_g and functioned effectively as a plasticizer for the PLGA matrix. There are reports of the blending of low and high molecular weight polyesters with each other to modify the properties of the blended matrix (Bodmeier et al., 1989; Bain et al., 1999; Asano et al., 1991). Although the addition of paclitaxel into 85:15 PLGA film formulations, containing 15%w/w MePEG partially reversed these increases in elasticity (Fig. 2B), this effect was minor at drug loadings up to 10%, with no observable deleterious effects on film flexibility or ease of handling. The decrease in elasticity measurements caused by the addition of increasing amounts of paclitaxel to the PLGA/MePEG matrix may have been due to a stiffening effect due to enhanced interactions between paclitaxel and PLGA chains via hydrogen bonding. Increased interactions between poly(D,L-lactic acid) and an added drug were proposed by Yamakawa et al. (1992) to explain a significant increase in the T_g observed for the polymer. The addition of paclitaxel to poly(L-lactic acid) blend microspheres has been shown by our group to increase the T_g (Liggins and Burt, 2004).

MePEG was shown to partition out of the PLGA films over a period of 72 h. Almost 75% of the total MePEG content in a 20%w/w MePEG loaded 50:50 PLGA film was released in 24 h as shown in Fig. 4. The films became stiffer (less elastic) as the MePEG dissolved out but the films remained intact with no evidence of fragmentation. The rapid release of MePEG was not unexpected since, although it is miscible with PLGA, this low molecular weight additive is freely soluble in water. In contrast, the diblock released rapidly from the PLGA films for about one day and then showed controlled release over one month (Fig. 5). Given that the diblock is soluble in water (similar to MePEG), this release profile indicates that there was likely an affinity of the PDLLA block of the diblock copolymer for the PLGA polymer chains that slowed down the release of the diblock from the films. It is anticipated that PLGA films containing diblock might be expected to remain flexible in vivo for extended periods since the plasticizing effect of this additive would be retained for long periods.

Paclitaxel loaded into all PLGA films was observed by optical microscopy to be a dispersion of particulate drug throughout the film. In vitro, paclitaxel released from PLGA/MePEG films very slowly with less than 5% of the loaded drug being released within 16 days (Fig. 6). However, paclitaxel has been reported to release very slowly from hydrophobic polymers such as poly(caprolactone) and the inclusion of MePEG into poly(caprolactone) matrices was reported to further inhibit the release rate of paclitaxel (Winternitz et al., 1996). The inclusion of the diblock copolymer in paclitaxel loaded PLGA films had little effect on drug release rates at diblock loadings up to 20%w/w. However, there was a dramatic increase in release rates at 30%w/w diblock content for both 1%w/w (Fig. 6) and 5%w/w paclitaxel loaded films. It is possible that the addition of 30%w/w diblock represented a critical loading of water soluble additive in the PLGA/diblock/paclitaxel matrix that allowed for a significantly enhanced hydrophilicity of the matrix, with increased water uptake, the formation of water-filled channels throughout the matrix and greatly increased paclitaxel release rates.

Films composed of 50:50 PLGA, blended with 30%w/w diblock and loaded with paclitaxel (1%w/w and 5%w/w) or without paclitaxel (controls) were selected for in vivo evaluation of degradation and erosion. These films possessed sufficient strength to be handled and sutured in place around the vessel, but were also elastic and flexible to allow for perivascular implantation. Loss of the diblock out of the film via partitioning and diffusion in the presence of aqueous fluids, was a

slow and controlled process and it was anticipated that the films would retain their flexibility and not revert to being brittle in vivo. The PDLLA-MePEG diblock copolymer has been shown to be biocompatible and non-toxic in a range of in vitro and in vivo evaluations (Burt et al., 1999). All control films were degraded and resorbed within 4 weeks and all but traces of matrix were visible after 12 weeks of implantation for 5%w/w paclitaxel loaded films. Measurements of the molecular weight (by GPC) of the 50:50 PLGA copolymer incubated in phosphate buffered saline, pH 7.4, showed the molecular weight drop from approximately 35000 to 5000 g/mol over one month with the copolymer almost completely degraded by 2 months (data not shown).

In conclusion, we believe the PLGA films blended with 30%w/w diblock and loaded with paclitaxel represent suitable biodegradable perivascular drug delivery systems for the prevention of restenosis and efficacy studies with these films are underway.

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