Available online at www.sciencedirect.com
ScienceDirect
International Journal of Pharmaceutics 342 (2007) 6–17
INTERNATIONAL JOURNAL OF PHARMACEUTICS
www.elsevier.com/locate/ijpharm

The characterization of paclitaxel-loaded microspheres manufactured from blends of poly(lactic-*co*-glycolic acid) (PLGA) and low molecular weight diblock copolymers

John K. Jackson, Tawny Hung, Kevin Letchford, Helen M. Burt *

Faculty of Pharmaceutical Sciences, 2146 East Mall, University of British Columbia, Vancouver, BC, Canada V6T 1Z3

Received 3 January 2007; received in revised form 20 April 2007; accepted 20 April 2007
Available online 3 May 2007

Abstract

Paclitaxel-loaded biodegradable drug delivery systems manufactured from poly(lactic-*co*-glycolic acid) (PLGA) are known to release the drug at extremely slow rates. The objective of this study was to characterize paclitaxel-loaded microspheres composed of blends of PLGA with low molecular weight ampipathic diblock copolymers. The encapsulation and release of a series of poly(ε-caprolactone) (PCL)- or poly(D,L-lactic acid) (PDLLA)-*co*-methoxypolyethylene glycol (MePEG) diblock copolymers was measured using quantitative gel permeation chromatography. Polymeric miscibility was determined by glass transition temperature measurements using differential scanning calorimetry and paclitaxel release was measured using HPLC methods. The PCL- and PDLLA-based diblock copolymers encapsulated at high efficiency and were miscible in PLGA microspheres (30–120 μm size range). The burst phase of paclitaxel release was increased up to 20-fold by the inclusion of diblock copolymers in PLGA microspheres. Approximately 10% of the more hydrophobic PCL-based copolymers released from the microspheres in a short burst over 3 days followed by very slow release over the following 10 weeks. Only the PDLLA-based copolymer released from the PLGA microspheres in a controlled manner over 10 weeks. All microspheres containing PEG were found to have more hydrophilic surfaces (as measured by contact angle) with improved biocompatibility (reduced neutrophil activation) compared to PLGA only microspheres. These results indicate that low molecular weight polyester-based diblock copolymers may be effectively encapsulated in PLGA microspheres to increase paclitaxel release (probably through a micellization process) and improve biocompatibility.

Keywords: PLGA; Paclitaxel; Diblock copolymers; Microspheres

1. Introduction

Paclitaxel, a naturally occurring taxane isolated from the Pacific yew tree, *Taxus brevifolia*, is an antiproliferative drug indicated for the treatment of ovarian and breast cancer (Huizing et al., 1995). However, the Cremophor® El/ethanol formulation of this drug, (Taxol™), designed for systemic administration, is associated with serious toxicities (Onetto et al., 1993). This has led to the development of novel polymeric drug delivery systems for both systemic and local delivery of paclitaxel, such as micelles, injectable pastes and microsphere formulations (Zhang et al., 1996; Liggins et al., 2004; Demetrick et al., 1997; Lapidus et al., 2004; Winternitz et al., 1996; Jackson et al., 2000b).

Recently, paclitaxel has been proposed for the localized or site-directed treatment of other proliferative diseases, such as restenosis and rheumatoid arthritis (Liggins et al., 2004; Signore et al., 2001; Burt and Hunter, 2006) and has been formulated in polymer-based controlled release films, device coatings, pastes or microspheres.

Microsphere formulations of paclitaxel are particularly well suited to the local treatment of diseases as they are injectable, the drug encapsulates at high efficiency and is released slowly over a period of months (Liggins et al., 2000; Dordunoo et al., 1995; Burt et al., 1995; Liggins and Burt, 2001; Gupte and Ciftci, 2004; Ruan and Feng, 2003). However, modulation of paclitaxel release from the commonly used biodegradable polyesters such as poly(lactic acid) (PLA) or poly(lactic-*co*-glycolic acid) (PLGA) has proved difficult due to the strong hydrophobic interactions between paclitaxel and these hydrophobic polymers. Typically, slow paclitaxel release profiles of between 5% and

* Corresponding author. Tel.: +1 604 822 6354; fax: +1 604 822 3035.
E-mail address: burt@interchange.ubc.ca (H.M. Burt).

0378-5173/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijpharm.2007.04.022

35% of loaded drug released in 1 month (Liggins et al., 2000; Dordunoo et al., 1995; Burt et al., 1995; Liggins and Burt, 2001; Mu and Feng, 2001) may be produced and may not match the more rapid release requirements for a particular disease.

For localized release at tumor sites, it would be optimal to release the drug at a rate that maintains a localized therapeutic concentration for extended periods. Generally, the IC_{50} for paclitaxel against cancer cells lines is of the order of 10–100 nM (approximately 10–100 ng/mL) (Jackson et al., 2000b). We believe that a burst phase of release followed by consistent and controlled release of paclitaxel over several weeks may be optimal to eliminate tumor cells.

The use of very low molecular weight poly(L-lactic acid) (PLLA) polymers, or the inclusion of excipients along with the drug, have been proposed as methods of increasing the paclitaxel release rates from microspheres (Liggins and Burt, 2001; Mu and Feng, 2001). Very low molecular weight (2000 g/mol) PLLA formed well-rounded microspheres with high paclitaxel loadings primarily due to the semi-crystalline nature of PLLA providing sufficient structural integrity to form microspheres (Liggins and Burt, 2001). These microspheres produced relatively rapid paclitaxel release profiles. The inclusion of additional excipients in PLGA microspheres has been shown to have little effect (Wang et al., 2003) or an inhibitory effect on paclitaxel release (Mu and Feng, 2001). This inhibitory effect on paclitaxel release caused by the addition of water-soluble excipients such as methoxypoly(ethylene glycol) (MePEG) to hydrophobic polymers has been previously reported by Winternitz et al. (1996). Wang et al. (2003) noted a PEG-induced increase in paclitaxel release rates from PLGA microparticles manufactured by spray drying but found that the inclusion of PEG caused agglomeration of the microparticles, limiting the effectiveness of this strategy. Another strategy to increase the release rate of paclitaxel has been to avoid the use of PLGA and manufacture microspheres from a triblock copolymer composed of poly(lactic acid) copolymerized with short PEG chains (Ruan and Feng, 2003). This method produced porous microspheres, with the suggested advantage of a hydrophilic PEG corona to improve biocompatibility (Ruan and Feng, 2003). A similar strategy to improve biocompatibility has been used in nanoparticulate technology whereby amphipathic diblock copolymers containing PEG may encapsulate hydrophobic drugs such as paclitaxel in a hydrophobic core of the nanoparticle, leaving PEG on the outer surface with resulting longer circulation times (Stolnik et al., 2001; Torchilin, 2007; Fonseca et al., 2002).

Recently, we have reported that amphipathic diblock copolymers were miscible with PLGA and that paclitaxel release from PLGA films was enhanced by the inclusion of diblock copolymers (Jackson et al., 2004a). Specifically, a diblock copolymer composed methoxypoly(ethylene glycol)-block-poly(D,L-lactic acid) (MePEG-b-PDLLA) (molecular weight of about 3500) blended into paclitaxel-loaded PLGA films was found to induce a five-fold increase in paclitaxel release during the first week.

The objective of this study was to encapsulate diblock copolymers of MePEG-b-PDLLA or methoxypoly(ethylene glycol)-block-poly(caprolactone) (MePEG-b-PCL) in paclitaxel loaded PLGA microspheres and to determine the miscibility and the effect of the diblock copolymers on drug release rates in vitro. Diblock copolymers manufactured from MePEG-b-PCL of different block lengths and MePEG-b-PDLLA were found to encapsulate well in PLGA microspheres and modulated paclitaxel release from these microspheres. Surface characterization of the diblock copolymer/PLGA blend microspheres showed evidence of surface localization of the MePEG blocks of the ampipathic copolymers at the microsphere surface.

2. Materials and methods

2.1. Materials

Paclitaxel was obtained from Hauser Chemicals, Inc. (Boulder, CO). PLGA 85/15 (IV = 0.61 dL/g) was obtained from Birmingham Polymers (Birmingham, AL). Methoxypoly(ethylene glycol) 750 (MePEG) (Fluka), ε-caprolactone (Fluka), stannous octoate (Sigma) and pyrene (Aldrich) were used as supplied without further purification. The solvents dichloromethane (DCM), chloroform and acetone were obtained from Fisher Scientific. D,L-lactide and poly vinyl alcohol (PVA) (98% hydrolyzed, MW = 13,000–23,000) were obtained from Aldrich Chemical Co., Milwaukee, WI. Poly(ethylene glycol) and polystyrene standards were obtained from Polymer Laboratories, Inc., Amherst, MA.

2.2. Diblock copolymer synthesis and characterization

MePEG-b-PDLLA was synthesized by a ring opening polymerization using MePEG (MW = 2000) and D,L-lactide to give a final weight ratio of MePEG to D,L-lactide of 60:40 as previously described (Zhang et al., 1996). MePEG-b-PCL diblock copolymers were synthesized as previously described (Letchford et al., 2004) using MePEG 750 conjugated to 2, 5, or 10 units of caprolactone to give diblock copolymers with molecular weights of approximately 1000, 1250, and 1875. These diblock copolymers are termed $MePEG_{17}$-b-PCL_2, $MePEG_{17}$-b-PCL_5 and $MePEG_{17}$-b-PCL_{10}. All copolymers were characterized by NMR (Bruker, AC-200E) at 200 MHz and gel permeation chromatography (GPC).

2.3. Preparation of microspheres

Microspheres (size range 30–120 μm) were prepared using a solvent evaporation method as previously described (Liggins et al., 2000). Polymer solutions containing PLGA and paclitaxel, with or without diblock copolymer, were made up in DCM to a final concentration of 10% (w/v). For example, to manufacture microspheres containing 30% diblock and 5% paclitaxel, 50 mg of paclitaxel, 285 mg of diblock copolymer, and 665 mg of PLGA were dissolved in 10 mL of DCM. Ten milliliters of polymer solution were then pipetted into 100 mL of 2.5% PVA stirring at 600 rpm. After 2 h, the microspheres were collected by centrifugation, washed three times with distilled water, and dried under vacuum at room temperature.

2.4. Microsphere size and morphology

The PLGA microspheres (20 mg) were homogeneously dispersed in distilled water (70 mL) with a few drops of 1% polysorbate 80 solution. Particle size distribution of microspheres was determined using a Malvern Mastersizer 2000 laser diffraction particle size analyzer.

2.5. Gel permeation chromatography

PLGA and diblock copolymer molecular weights were determined by gel permeation chromatography (GPC) against polyethylene glycol standards (Polymer Laboratories Inc.) in the range of 670–11,800 g/mol. Samples were injected using a Waters (Milford MA) model 717 plus autosampler. Chloroform with a flow rate of 1 mL/min was used as the mobile phase and separation was achieved through two Waters Styragel columns (HR 3 and HR 0.5) connected in series. Detection was by a Waters refractive index detector. The relative concentration of diblock copolymer in microsphere samples was determined using quantitative GPC. Calibration curves of both PLGA and diblock copolymers were obtained using standards in the 0–50 mg/mL range.

2.6. Paclitaxel analysis by HPLC

Paclitaxel was analyzed using reverse phase HPLC methods as previously described (Jackson et al., 2004a). Briefly, the system combined a Waters HPLC system with Millennium software control, a C18 Novapak column, and a mobile phase of 58:37:5 acetonitrile:water:methanol flowing at 1 mL/min with UV detection at 232 nm.

2.7. Encapsulation efficiency

The encapsulation efficiency for the diblock copolymer was determined by dissolving 10 mg of microspheres in 1 mL of chloroform and analyzing by quantitative GPC as previously described (Jackson et al., 2004a). The encapsulation efficiency of paclitaxel was determined by dissolving 7 mg of microspheres in 1 mL of DCM in a 16 mL screw cap (Teflon) tube. Fifteen milliliters of acetonitrile:water (60:40, v/v) were added and the tubes were shaken vigorously for 30 s. The contents were then allowed to settle for 30 min resulting in a phase separation of approximately 8 mL (upper phase, paclitaxel-rich) and 8 mL (lower phase). Both phases were analyzed by HPLC methods to determine the amount of paclitaxel encapsulated in the microsphere sample. This method allows for total dissolution of microsphere components and has been shown to results in complete drug partitioning into the upper phase.

2.8. Thermal analysis

Differential scanning calorimetry (DSC) of microspheres was studied using a TA Instruments Q100 DSC, with refrigerated cooling. The purge gas was prepurified nitrogen at a pressure of 20 psi. Microspheres weighing 3–5 mg were placed in crimped, but not hermetically sealed, aluminum pans using an empty pan as a reference. The samples were heated from −80 to 80 °C at 10 °C/min.

2.9. In vitro release of paclitaxel from microspheres

Ten milligrams of microspheres were placed in 16 mL screw cap (Teflon) tubes and 15 mL of phosphate buffered saline (10 mM PBS, pH 7.4) were added. The tubes were rotated end-over-end at 8 rpm at 37 °C. At appropriate times, the tubes were centrifuged at 1000 × *g* for 5 min, the PBS was removed and saved, and fresh PBS was placed in the tube. One milliliter of DCM was added to the PBS samples and the contents were shaken in a capped tube for 30 s. The contents were then allowed to phase separate for 30 min and the aqueous phase (drug-free) was aspirated from the 1 mL DCM phase (drug-rich). The DCM was then evaporated to dryness at 40 °C under nitrogen gas and the contents were re-dissolved in 1 mL of acetonitrile:water (60:40, v/v) and analyzed by HPLC methods as previously described.

2.10. Diblock copolymer release experiments

Ten milligrams of 30% diblock containing microspheres (paclitaxel-free) were weighed into 2 mL plastic Eppendorf tubes and 2 mL of PBS were added. The tubes were capped and incubated at 37 °C. In all tubes, the PBS was replaced weekly. At appropriate times, the tubes were centrifuged at 14,000 × *g* and the supernatant was discarded. The contents were then dried under vacuum for 2 days and dissolved in 1 mL of chloroform for quantitative GPC analysis. The determination of the percentage of diblock remaining in the microspheres allowed for the calculation of the amount of diblock copolymer released in that time period.

2.11. Microsphere degradation

Ten milligrams of 5% paclitaxel-loaded microspheres were incubated in 2 mL of PBS as previously described for the diblock release studies. Following drying under vacuum, a small sample was taken for examination by scanning electron microscopy and the remainder was dissolved in 1 mL of chloroform for analysis of PLGA molecular weight by GPC. Scanning electron microscopy was performed by mounting microspheres on aluminum disks with a double sided adhesive tape impregnated with carbon. The mounted samples were coated with 100 Å of gold-palladium using a Hummer sputter coater and analyzed by scanning electron microscopy (Hitachi).

2.12. Microsphere-induced neutrophil chemiluminescence studies

Neutrophils were separated from fresh human blood by dextran sedimentation and Ficol-paque centrifugation techniques. Blood was collected from healthy volunteers according to protocols approved by the UBC Clinical Research Ethics Board. The cells were suspended at 2.5 million cells per mL in Hanks

buffered salt solution, stored on ice and used within 2 h of preparation. Eppendorf tubes (1.5 mL) containing 25 mg of microspheres were prewarmed to 37 °C and 0.5 mL of the neutrophil suspension (also prewarmed) were added with mild shaking. Luminol was added to a final concentration of 5 μM. The tubes was placed in the holder of an LKB chemiluminometer (LKB instruments) at 37 °C and chemiluminescence was measured at 20 s intervals with shaking of the tubes between readings.

2.13. Contact angle measurements

Contact angle determinations were made on PLGA films containing various concentrations of each diblock cast on Teflon squares and on microspheres layered on glass slides using a computerized contact angle instrument (Image capture retiga camera, (Qimaging, Burnaby, BC Canada), with Northern Eclipse 6 software (Empix Imaging: www.empix.com)). Films were cast on 1 cm × 1 cm Teflon squares adhered to glass microscope slides. A 4 μL drop of water was pipetted onto the surface of the polymer, focused by optical microscopy and the video image captured for angle analysis using the software. Microspheres were suspended in water and slowly dried on glass slides at 37 °C so that a continuous layer of microspheres, approximately 200 μm deep, were layered on the slide. The dry microsphere slide was gently warmed to 50 °C for 5 min. This temperature was above the glass transition temperature of PLGA and caused a slight softening of the microspheres. This softening caused the microspheres to partially fuse together and effectively eliminated any gaps or openings between the microspheres on the slide. Water was found to bead well on the surface of these microsphere layers and the contact angle could be measured using this method.

3. Results

3.1. Gel permeation chromatography and NMR of polymers

There was good separation between the PLGA and diblock copolymer peaks of microsphere samples dissolved in chloroform. A representative chromatogram for a mixture of PLGA, MePEG-b-PDLLA diblock copolymer and paclitaxel is shown in Fig. 1. PLGA eluted at 10.6 min whereas MePEG-b-PDLLA, $MePEG_{17}$-b-PCL_{10}, $MePEG_{17}$-b-PCL_5 and $MePEG_{17}$-b-PCL_2 eluted between 11.7 and 14.1 min as shown in Table 1. A series of dilution standards for each polymer yielded linear calibration curves in the 0–20 mg/mL range with similar calibration coefficients and R^2 values close to 1 (Table 1). When present, paclitaxel was also detected at 14.8 min and standards gave a linear calibration curve for this drug.

NMR data for all copolymers established the appropriate molecular composition of the MePEG and PDLLA or PCL constituents. The NMR data for the MePEG-b-PDLLA copolymer has been previously described (Zhang et al., 1996) and a representative NMR for the $MePEG_{17}$-b-PCL_5 copolymer is shown in Fig. 2. The allocation of constituent peaks (A–G) is shown in the legend to this figure.

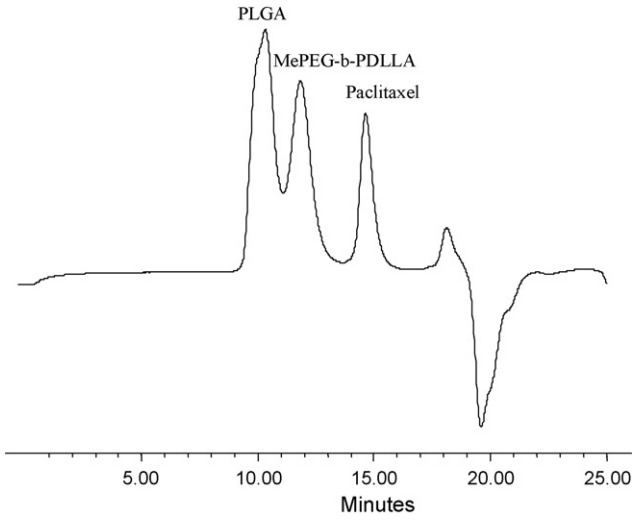

Fig. 1. GPC chromatogram of a mixture of PLGA (85:15) (57%), MePEG-b-PDLLA diblock copolymer (38%) and paclitaxel (5%).

3.2. Microsphere characterization

Paclitaxel encapsulated in all microsphere samples at over 90% efficiency levels (Table 2). The more hydrophilic $MePEG_{17}$-b-PCL_2 diblock copolymer encapsulated very poorly in PLGA microspheres as shown in Table 2. However, MePEG-b-PDLLA, $MePEG_{17}$-b-PCL_{10}, $MePEG_{17}$-b-PCL_5 all encapsulated well at loading concentrations as high as 30% diblock (70% PLGA) and gave reasonable yields of microspheres. There were distinct differences in microsphere morphology especially at higher diblock loadings. Microspheres containing MePEG-b-PDLLA were spherical whereas $MePEG_{17}$-b-PCL_5 or $MePEG_{17}$-b-PCL_{10}-containing microspheres were dimpled and became distorted at 30% loadings (Table 2 and Fig. 3). This distortion effect was most noticeable for microspheres containing $MePEG_{17}$-b-PCL_{10} at 30% loading. However particle size analysis showed that all microspheres fell within the 30–120 μm size range irrespective of morphology (data not shown).

3.3. Thermal analysis

Microsphere samples were cooled from room temperature to minus 80 °C and then heated at 10 °C/min up to

Table 1
GPC calibration data for standard curves of paclitaxel, PLGA (85:15) and diblock copolymers of MePEG-b-PDLLA and MePEG-b-PCL

Component	Retention time (min)	Calibration coefficient	R^2
Paclitaxel	14.9	37,522	0.999
PLGA	10.7	4913.5	0.994
$MePEG_{17}$-b-PCL_2	14.1	4935.5	0.999
$MePEG_{17}$-b-PCL_5	13.7	4536.2	0.991
$MePEG_{17}$-b-PCL_{10}	13.1	4905.7	0.980
$MePEG_{44}$-b-$PDLLA_{15}$	11.7	5058.2	0.984

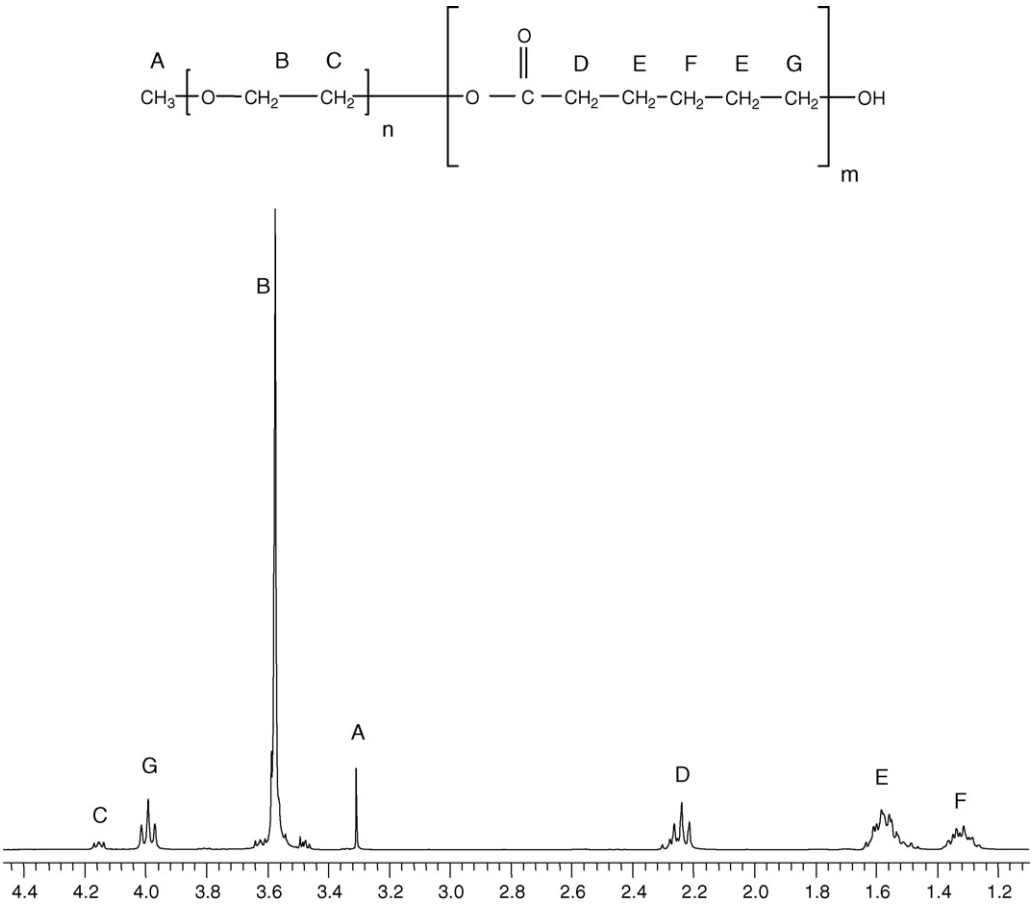

Fig. 2. NMR data for $MePEG_{17}$-b-PCL_5 diblock copolymer. Structure of methoxypoly(ethylene glycol) block poly(caprolactone) (MePEG-b-PCL) where *n* is the number of ethylene oxide repeat units and *m* is the number of caprolactone repeat units and ^{1}H NMR of $MePEG_{17}$-b-PCL_5 displaying peak assignments, labelled A–G.

+80 °C. Representative thermograms for PLGA microspheres, the MePEG-b-PDLLA diblock copolymer and 30% diblock-loaded PLGA microspheres are shown in Fig. 4. PLGA microspheres gave a glass transition (T_g) at 48 °C. The MePEG-b-PDLLA diblock gave a T_g at −48 °C, a crystallization peak at −25 °C and a melting endotherm at 42 °C. The blending of 30% MePEG-b-PDLLA in PLGA microspheres resulted in a single T_g at 22 °C intermediate between the two pure PLGA and MePEG-b-PDLLA components and a small melting peak at 56 °C. Thermal analysis of $MePEG_{17}$-b-PCL_5- and $MePEG_{17}$-b-PCL_5-containing microspheres resulted in a similar intermediate position of the T_g. The Fox equation (as shown in Table 3) was used to determine theoretical T_g values for the blends. These were in good agreement with experimental values, indicating miscibility of the diblock copolymer and PLGA polymer.

3.4. Paclitaxel release

Paclitaxel was released from PLGA (no diblock added) microspheres with a small burst phase (approximately 1% of the total encapsulated drug released after 3 days) followed by a very slow release over the following 2 months (see Fig. 5A). The release rate of paclitaxel from PLGA microspheres containing the MePEG-b-PDLLA diblock copolymer was greater than the release of the drug from PLGA microspheres that did not contain the diblock copolymer (Fig. 5A) For 20% and 22% loaded MePEG-b-PDLLA diblock copolymer-loaded microspheres, the release profiles were characterized by a short burst phase of release followed by a slower, more controlled release over 2 months.

Both 10% and 20% $MePEG_{17}$-b-PCL_5 diblock copolymer blended with PLGA microspheres released paclitaxel faster than PLGA only microspheres, reaching 10% of total drug released after 70 days (Fig. 5B) as compared to less than 2% released from PLGA microspheres (Fig. 5A). However, microspheres with 27% $MePEG_{17}$-b-PCL_5 resulted in a very large increase in both the burst phase of paclitaxel release (approximately 20% of encapsulated drug after 5 days) and the slower controlled release phase over the following 65 days (Fig. 5B). The addition of 10% $MePEG_{17}$-b-PCL_{10} diblock copolymer to PLGA microspheres caused only minor increases in drug release rates compared to PLGA microspheres (Fig. 5C). However the 20% and 30% diblock copolymer blended with PLGA microspheres were associated with large increases in both the initial burst of drug release during the first 5 days and in the slower release phase over the next 2 months Fig. 5C. PBS pH 7.4 is used routinely for paclitaxel release studies but we repeated the drug release

Table 2
The effect of blending MePEG-b-PDLLA and MePEG-b-PCL diblock copolymers with PLGA (85:15) on encapsulation, yields, size and morphology of microspheres loaded with 20% paclitaxel

Theoretical composition[a,b] (%)	Diblock encapsulation efficiency[c] (%)	Yield[d] (%)	Morphology[e]
PLGA:$MePEG_{44}$-b-$PDLLA_{15}$			
100:0	–	–	Round
90:10	100	96	Round
80:20	100	77	Round
70:30	74	93	Round
60:40	42	44	–
PLGA:$MePEG_{17}$-b-PCL_2			
90:10	–	74	Round
80:20	9	67	Round
70:30	21	74	Round
60:40	15	52	–
PLGA:$MePEG_{17}$-b-PCL_5			
90:10	95	91	Round
80:20	100	74	Dimpled
70:30	95	92	Dimpled
60:40	97	61	Dimpled
PLGA:$MePEG_{17}$-b-PCL_{10}			
90:10	98	87	Round
80:20	98	82	Dimpled
70:30	106	88	Dimpled/distorted
60:40	–	–	–

[a] Theoretical encapsulation based on weight percents.
[b] Paclitaxel encapsulated at more than 90% efficiency in all formulations determined by HPLC assay.
[c] Diblock copolymer encapsulation efficiency determined by GPC assay.
[d] Microsphere yield determined by gravimetric analysis.
[e] Microsphere morphology determined by SEM.

experiments for the microspheres containing different amounts of the $MePEG_{17}$-b-PCL_5 diblock copolymer using 50% RPMI (cell culture media) and there was no difference in the release rate of paclitaxel compared to those performed using PBS over a 28-day period.

3.5. Diblock copolymer release

The MePEG-b-PDLLA diblock copolymer released from the diblock/PLGA microspheres in a controlled manner over 10 weeks as shown in Fig. 6. The release profile was characterized by a large burst of diblock copolymer release (more than 30%) in the first few days followed by sustained release over 10 weeks. A burst phase (approximately 20%) was also observed for both $MePEG_{17}$-b-PCL_5 and $MePEG_{17}$-b-PCL_{10} release from PLGA microspheres. After that time $MePEG_{17}$-b-PCL_5 released very slowly over the next 12 weeks but there was negligible release of $MePEG_{17}$-b-PCL_{10} which was largely retained within the PLGA microspheres. The data points in Fig. 5 represent between $n = 1$ to 3 at different time points. Because the buffer was changed weekly in all tubes (to remove released diblock copolymer and maintain sink conditions) there was a loss of microspheres from many samples due to handling. Therefore some tubes did not have enough microspheres remaining for GPC determinations to be made and they were eliminated from the study. For this reason error bars are not shown in Fig. 6.

Table 3
Glass transition temperature obtained using DSC for diblock copolymers, PLGA (85:15) and microspheres manufactured from PLGA (approximately 70%) and $MePEG_{44}$-b-$PDLLA_{15}$, $MePEG_{17}$-b-PCL_5 and $MePEG_{17}$-b-PCL_{10} (30%)

Sample	Observed T_g (°C)	Theoretical T_g (°C)[a]
$MePEG_{17}$-b-PCL_5	−70	–
$MePEG_{17}$-b-PCL_{10}	−72	–
$MePEG_{44}$-b-PLA_{15}	−48	–
PLGA (85:15)	48	–
PLGA (73%) + $MePEG_{17}$-b-PCL_5 (27%, w/w)	6.6	4.5
PLGA (68%) + $MePEG_{17}$-b-PCL_{10} (32%, w/w)	0	−3.5
PLGA (78%) + $MePEG_{44}$-b-$PDLLA_{15}$ (22%, w/w)	22	20.5

[a] Theoretical T_g of microspheres containing diblock as determined by the Fox equation: $1/T_{g\,theoretical} = wt_{PLGA}/T_{g\,PLGA} + wt_{diblock}/T_{g\,diblock}$.

3.6. Microsphere degradation

Following incubation in PBS buffer at 37 °C for 10 weeks, there was some evidence of a loss in microsphere integrity and changes in morphology for all samples as shown by SEM images (Fig. 7A–D). All microspheres showed signs of degradation such as fragmentation and loss of surface smoothness. These effects were minimal for PLGA only microspheres (Fig. 7A–D) and extensive for microsphere containing the $MePEG_{17}$-b-PCL diblock copolymers.

3.7. Neutrophil chemiluminescence studies

PLGA microspheres caused the rapid activation of neutrophils as described by the chemiluminescence arising from the production of reactive oxygen species as shown in Fig. 8. After 5 min incubation, chemiluminescence values had reached approximately 190 mV and these values slowly reduced to approximately 75 mV after 15 min incubation. Microspheres containing diblock copolymers at concentrations of approximately 30% diblock ($MePEG_{17}$-b-PCL_5 or $MePEG_{17}$-b-PCL_{10} copolymer) (w/w to PLGA) or 22% MePEG-b-PDLLA diblock copolymer also induced rapid neutrophil chemiluminescence as shown in Fig. 7. However, at all time points these values were less than those induced by PLGA microspheres. Peak chemiluminescence values at 5 min were reduced to 84 mV (MePEG-b-PDLLA), 29 mV ($MePEG_{17}$-b-PCL_5) and 17 mV ($MePEG_{17}$-b-PCL_{10}) for diblock containing microspheres.

3.8. Contact angle determinations

PLGA films that were solvent cast from dichloromethane solutions caused water to bead into drops with high contact angles (approximately $83 \pm 2°$). However, for films containing diblock copolymers, the contact angle reduced in a concentration dependent manner as shown in Fig. 9. Similar results were found for PLGA films containing $MePEG_{17}$-b-PCL_5

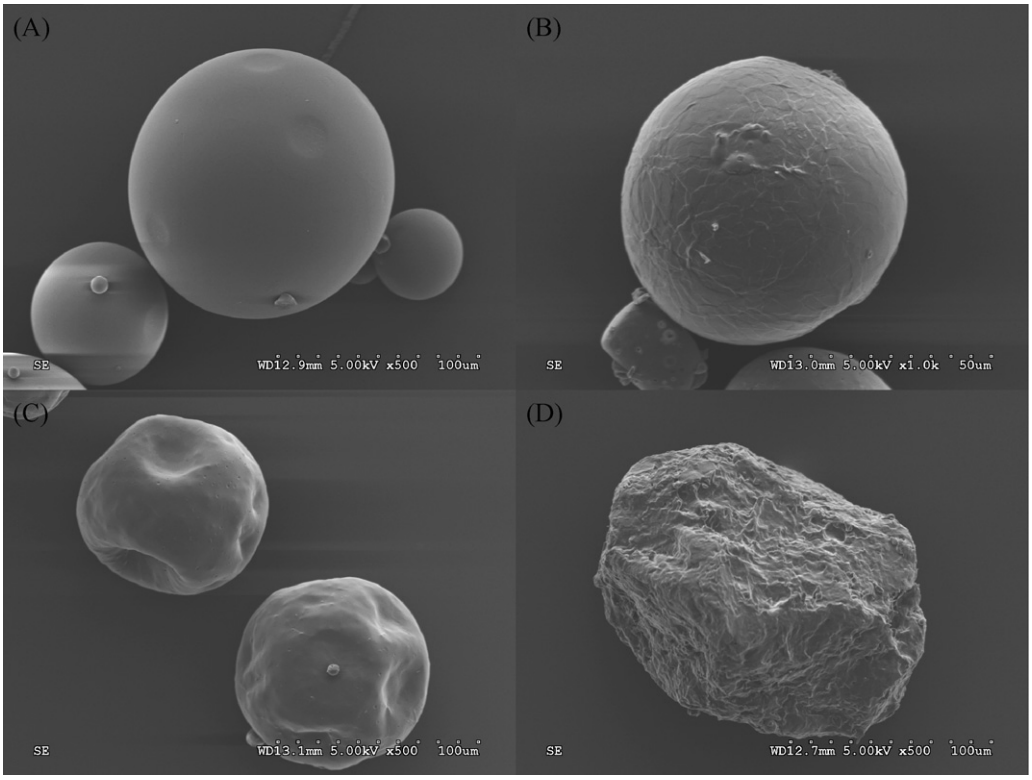

Fig. 3. Scanning electron micrographs of 5% paclitaxel-loaded PLGA (85:15) microspheres blended with, A: No diblock, B: 22% MePEG-b-PDLLA, C: 27% $MePEG_{17}$-b-PCL_5 and D: 32% $MePEG_{17}$-b-PCL_{10}.

and $MePEG_{17}$-b-PCL_{10} diblock copolymers (data not shown). Microspheres of PLGA formed into a fused, but uneven film gave contact angles similar to PLGA films ($83 \pm 3^\circ$). Similar treated blended PLGA microspheres containing the MePEG-b-PDLLA diblock copolymer at 22% loading gave contact angles of $60 \pm 3^\circ$ and microspheres containing $MePEG_{17}$-b-PCL_5 or $MePEG_{17}$-b-PCL_{10} at 27% or 32% (respectively) loadings gave contact angles of $46.7 \pm 2^\circ$ or $46.6 \pm 2^\circ$, respectively.

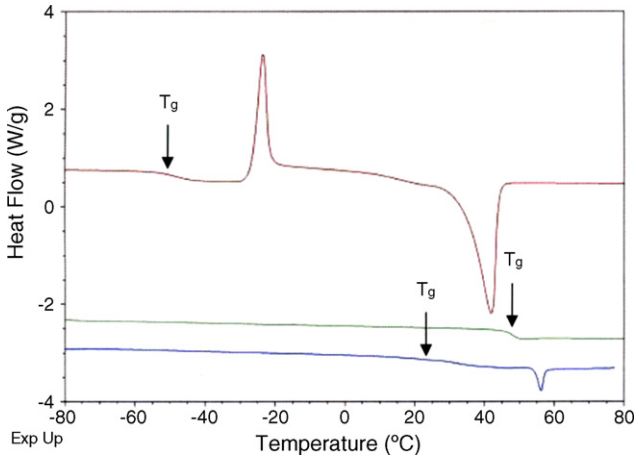

Fig. 4. Thermal analysis: representative DSC heating curves (10 °C/min) for MePEG-b-PDLLA diblock copolymer loaded PLGA microspheres. Top curve: PDLLA diblock copolymer alone, middle curve: PLGA alone and bottom curve: MePEG-b-PDLLA diblock copolymer (22%) loaded PLGA microspheres.

4. Discussion

The inclusion of ampipathic diblock copolymers in PLGA solutions did not disrupt the solvent evaporation-microsphere manufacture process and paclitaxel encapsulation remained over 90% for all microsphere preparations. The size range (30–120 μm) of microspheres used in these studies have been previously shown to be suitable for injection and localized delivery of drugs into intratumoral, intraperitoneal, or intraarticular sites (Liggins et al., 2000, 2004). However the methods for diblock copolymer encapsulation would be equally suitable for the manufacture of smaller or larger microspheres. The 85/15 (LA:GA ratio) with an IV of 0.61 degrades in vivo over a 2–6-month period which is considered suitable for in vivo applications. Although particle size analysis demonstrated that all microspheres were in the desired 30–120 μm size range, there were noticeable differences in microsphere morphology that depended on both the type and the loading concentration of the diblock copolymer. At higher loadings (27%) of $MePEG_{17}$-b-PCL_5 there was significant dimpling of the spherical morphology and for $MePEG_{17}$-b-PCL_{10} (32%) the microspheres became distorted (Table 2 and Fig. 3). However, these morphological changes did not affect drug encapsulation efficiency or microsphere yields. Other groups have noted that the addition of additives into paclitaxel-loaded PLGA microspheres or higher loadings of paclitaxel in PLLA microspheres, resulted in significant changes in microsphere surface morphology (Liggins et al., 2000; Mu and Feng, 2001; Wang et al., 2003).

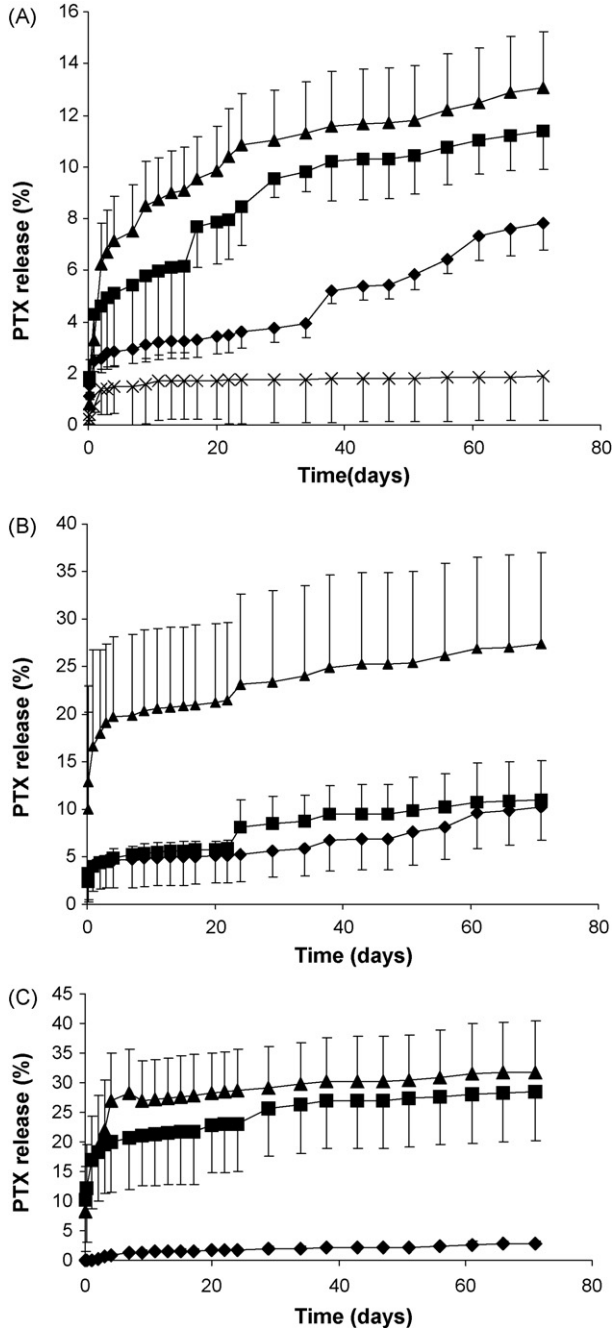

Fig. 5. (A) Paclitaxel release from PLGA (×) or PLGA microspheres blended with 10% (♦), 20% (■) or 22% (▲) MePEG-b-PDLLA diblock copolymer. Microspheres loaded with 5% PTX. (B) Paclitaxel release from PLGA microspheres blended with 10% (♦), 20% (■) or 27% (▲) $MePEG_{17}$-b-PCL_5 diblock copolymer. Microspheres loaded with 5% PTX. (C) Paclitaxel release from PLGA microspheres blended with 10% (♦), 20% (■) or 32% (▲) $MePEG_{17}$-b-PCL_{10} diblock copolymer. Microspheres loaded with 5% PTX.

The use of GPC for the quantitative analysis of blended polymers has been previously described (Jackson et al., 2004a). In this study the different copolymers eluted at slightly longer time points as the molecular weight decreased (MePEG-b-PDLLA: 11.7 min, $MePEG_{17}$-b-PCL_{10}: 13.1 min, $MePEG_{17}$-b-PCL_5: 13.7 min and $MePEG_{17}$-b-PCL_2: 14.1 min) as expected. These differences allowed for good peak separation from PLGA peaks

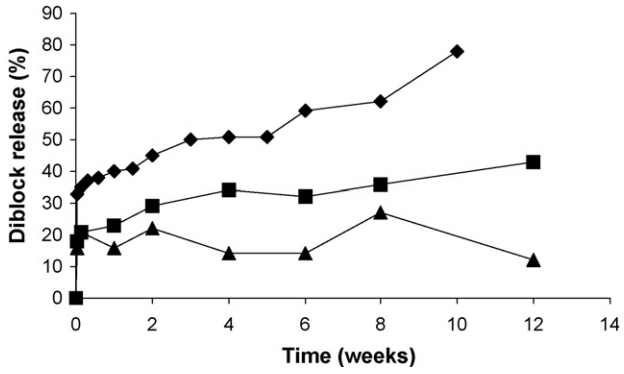

Fig. 6. Release of diblock copolymers from PLGA microspheres loaded with 22% (w/w) MePEG-b-PDLLA (♦), 27% (w/w) $MePEG_{17}$-b-PCL_5 (■) and 32% (w/w) $MePEG_{17}$-b-PCL_{10} (▲) as determined by quantitative GPC.

for blended microspheres which could be analyzed down to concentrations as low as 5% of all diblock copolymers in 95% PGLA. A representative chromatogram for MePEG-b-PDLLA loaded PLGA microspheres is shown in Fig. 1, demonstrating good peak separation for the least well separated diblock copolymer. Interestingly, paclitaxel also gave a distinct peak (14.8 min) when included in the blended polymer samples. In this case GPC was not used to quantitate all three microsphere components as HPLC methods are more sensitive for drug analysis.

The more hydrophobic copolymers (lower HLB) $MePEG_{17}$-b-PCL_{10} and $MePEG_{17}$-b-PCL_5, were retained in the microspheres with little loss to the external phase during preparation. Using GPC, it was determined that the diblock copolymers with longer hydrophobic blocks (MePEG-b-PDLLA, $MePEG_{17}$-b-PCL_5 or $MePEG_{17}$-b-PCL_{10}) encapsulated well during the microsphere manufacture process. However, the $MePEG_{17}$-b-PCL_2 diblock copolymer with the short hydrophobic chain was only poorly encapsulated. This probably resulted from the increased water solubility of this polymer (Letchford et al., 2004) and dissolution into the PVA solution during manufacture. The miscibility of MePEG-b-PDLLA, $MePEG_{17}$-b-PCL_5 and $MEPEG_{17}$-b-PCL_{10} diblock copolymers with PLGA in microsphere preparations was confirmed by DSC. Only one T_g, intermediate in value between the T_g of the pure blend components (diblock and PLGA) was evident in all samples and the T_g values for blended microspheres were in close agreement with theoretical values determined from the Fox equation values assuming 100% miscibility (Table 3). The MePEG-b-PDLLA diblock copolymer released from the PLGA microspheres in a controlled manner characterized by a burst phase of release during the first few days and followed by a slower release over 10 weeks (Fig. 6). The release studies were terminated at this time because the diblock copolymer remaining in the PLGA had dropped below the detection limit (5% diblock in 95% PLGA) of the GPC method. We have previously shown that MePEG released from PLGA films over a period of a few days and the MePEG-b-PDLLA diblock copolymer released slowly over a period of more than 1 month (Jackson et al., 2004a) in general agreement with the release rate observed in this study. This particular diblock copolymer is soluble in water up to 200 mg/mL (Letchford et al., 2004) so that retention within the

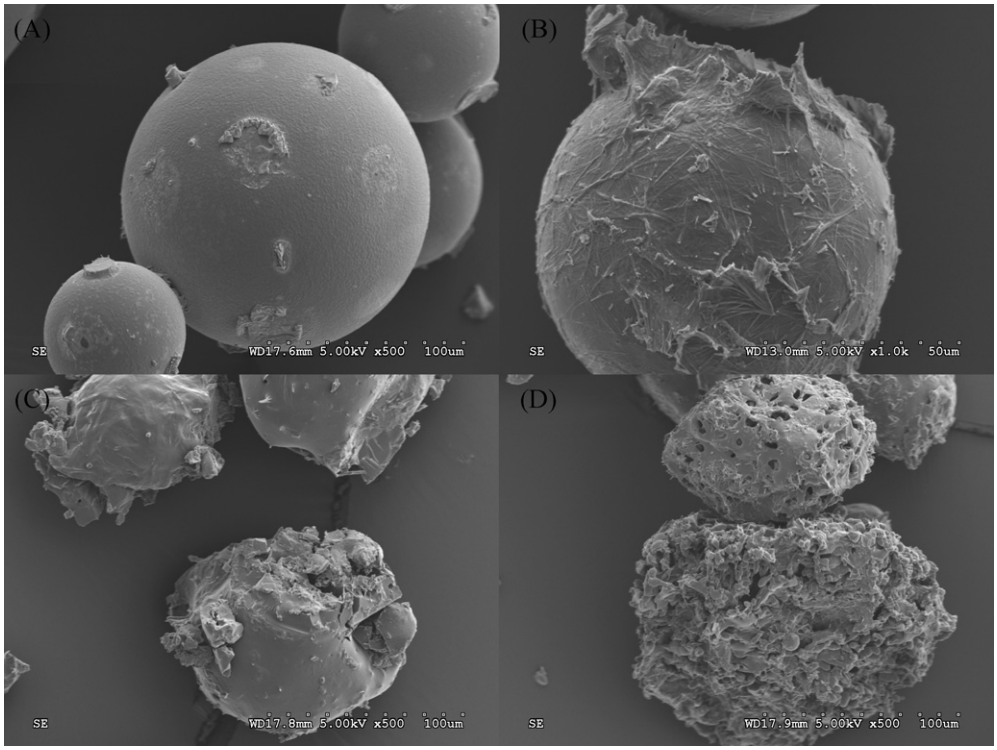

Fig. 7. Scanning electron micrographs of 5% paclitaxel-loaded PLGA microspheres blended with, A: No diblock copolymer, B: 22% MePEG-b-PDLLA, C: 27% $MePEG_{17}$-b-PCL_5 and D: 32% $MePEG_{17}$-b-PCL_{10} following 10 weeks incubation in PBS at 37 °C.

PLGA matrix probably arises from the hydrophobic interactions between the PDLLA blocks of the diblock copolymer and the PLGA polymer chains. However, the release profiles of the $MePEG_{17}$-b-PCL_5 and $MePEG_{17}$-b-PCL_{10} diblock copolymers from the microspheres were quite different and characterized by a smaller burst of release at the start of the study with very slow ($MePEG_{17}$-b-PCL_5) or negligible ($MePEG_{17}$-b-PCL_{10}) release after that time. The retention of the $MePEG_{17}$-b-PCL_{10} diblock copolymer in the microspheres might be explained by the low aqueous solubility of this copolymer (2 mg/mL at 37 °C (Letchford et al., 2004)) and a strong hydrophobic interaction between the polycaprolactone blocks and the PLGA chains. The $MePEG_{17}$-b-PCL_5 diblock copolymer has a water solubility of approximately 100 mg/mL (Letchford et al., 2004) so that the retention of the diblock in the microsphere is not likely explained by its low water solubility. Both the $MePEG_{17}$-b-PCL_5 and MePEG-b-PDLLA diblock copolymers have similar ratios of hydrophilic (MePEG) to hydrophobic (PCL or PLA) chains (approximately 60:40). Therefore, it seems likely that the retention of the polycaprolactone-based copolymers in PLGA microspheres derives from the higher hydrophobicity of poly(caprolactone) compared to poly(lactic acid), allowing

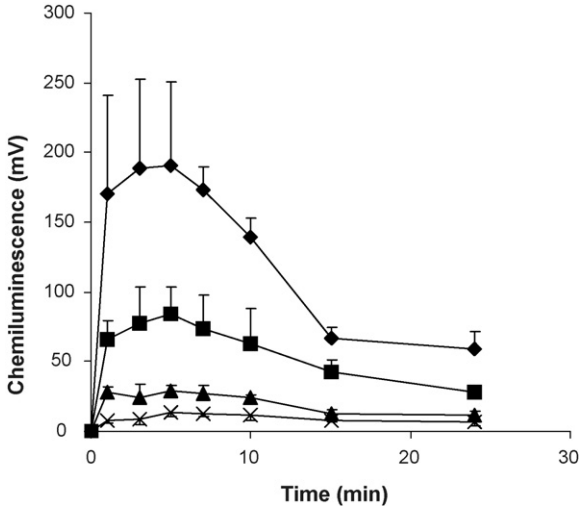

Fig. 8. Chemiluminescence response of neutrophils to PLGA microspheres (♦) or PLGA microspheres containing MePEG-b-PDLLA (■), $MePEG_{17}$-b-PCL_5 (▲) or $MePEG_{17}$-b-PCL_{10} (×) diblock copolymers at 22%, 27% or 32% (w/w).

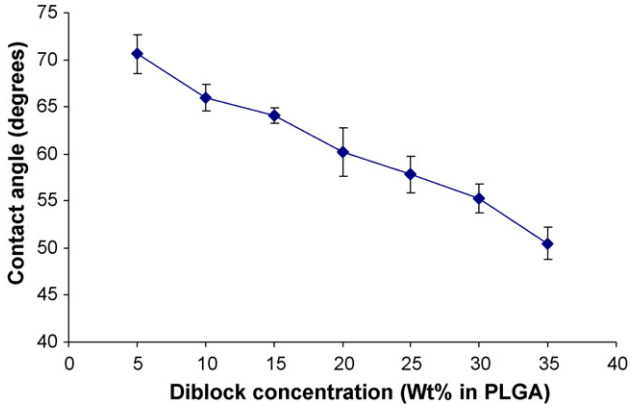

Fig. 9. Effect of increased concentrations of PLA diblock in PLGA films on the contact angle of water with the films. Films were cast by evaporation from dichloromethane solvent.

for a strong binding interaction with the PLGA chains. The biocompatibility of all polymers used in these studies is well established in the literature. At high concentrations, (approximately 1 mg/mL), diblock copolymers may cause membrane perturbation and cell lysis of normal cells after extended incubation times (unpublished data). Considering the slow rate of copolymer release from the microspheres, observed in these studies, together with continuous physiological fluid dilution effects, it is unlikely that the local concentrations of diblock copolymers would even approach these toxic levels.

In this study, we have shown that amphipathic diblock copolymers, originally designed to solubilize (micellize) hydrophobic drugs (Zhang et al., 1996; Letchford et al., 2004), may be encapsulated and form miscible blends in PLGA microspheres. Since the MePEG-b-PDLLA and $MePEG_{17}$-b-PCL_5 diblock copolymers released in a controlled manner from PLGA microspheres, we suggest that PTX release was increased via the solubilization of PTX within diblock copolymer micelles formed in water-filled pores within the microsphere matrix. These diblock copolymers form micelles at low concentrations and the diblocks with hydrophobic PCL core forming blocks solubilize large amounts of PTX (Letchford et al., 2004), particularly as the PCL block length increases. To determine whether the increased PTX release rate from diblock/PLGA microspheres was due to a drug micellization effect following PTX release into the PBS medium, PTX loaded PLGA microspheres (no diblock) were incubated in solutions of the different diblock copolymers (above the critical micelle concentration). There were no observed increases in the release rate of PTX from the microspheres (control data not shown). Therefore, it was likely that the PTX micellization process occurred within aqueous pores within the bulk matrix of the microspheres. Interestingly, many groups have incorporated PEG into polyester microspheres for the purpose of stabilizing encapsulated proteins (Castellanos et al., 2005; Zhou et al., 2003; Liu and Deng, 2002; Deng et al., 2001; Jiang and Schwendeman, 2001). Generally, these methods improve the encapsulation and stability of proteins and may affect protein release profiles. However, we have demonstrated that MePEG releases rapidly from PLGA films and injectable pastes and results in slower paclitaxel release from polyester matrices (Winternitz et al., 1996; Stolnik et al., 2001; Jackson et al., 2004a,b). The increased surface roughness of PLGA microspheres containing higher loadings of certain diblock copolymers (Table 2 and Fig. 3) might be expected to increase the rate of release of paclitaxel from these microspheres due to increased surface area effects. However, we have previously shown that only small changes in release rates occur between 10% loaded PLLA microspheres with 1000-fold differences in surface area to volume ratios (Liggins and Burt, 2004). Therefore, it is unlikely that the differences in surface roughness caused by the inclusion of diblock copolymers would significantly affect drug release rates in these microspheres.

When microspheres are injected into the body, they are usually recognized as foreign material and attempts are made by the host to eliminate or exclude these materials. Cells, such as macrophages or neutrophils may recognize IgG or complement opsonized on the surface of the microspheres. This may result in an unwanted inflammatory response. Attempts to inhibit the adsorption of plasma proteins and recognition by phagocytic cells usually involve reducing the hydrophobicity of the surface with PEG containing molecules. Both Faraasen et al. (2003) and Muller et al. (2003) successfully reduced plasma protein adsorption and the phagocytosis of PLGA microspheres by surface adsorbing poly(L-lysine)-block-poly(ethylene glycol) to the surface. Alternative methods include surface conjugation of PEG directly to the microsphere surface (Gorbet and Sefton, 2005; Byun et al., 2004) or the manufacture of microspheres directly from insoluble copolymers of poly(lactic acid)-block-poly(ethylene glycol) (Liu and Deng, 2002; Deng et al., 2001). The use of PEGylated surfaces on nanodelivery systems (liposomes or nanoparticles) has been shown to produce "stealthy" systems that allow for long circulation times of these drug delivery systems (Crosasso et al., 2000). Brigger et al. (2002), demonstrated that nanospheres manufactured from insoluble diblock copolymers containing PEG allowed for over three-fold increases in passive tumor targeting due to increased circulation times. For microspheres, surface coating with PEG containing Pluronics has also been shown to significantly reduce plasma protein binding and to inhibit neutrophil chemiluminescence responses to microspheres manufactured from different types of hydrophobic polymers (Jackson et al., 2000a). Although all these methods represent viable techniques that provide PEG surface coatings on microspheres, they do not influence drug release or degradation processes.

In this study, MePEG containing diblock copolymers were encapsulated within the matrix of PLGA microspheres. Since these copolymers are amphipathic and the manufacturing method creates a polymer solution interface with water, it seems likely that some MePEG blocks of the diblock copolymers may orientate at the polymer/water interface and thus form part of the outer microsphere surface. Contact angle measurements are frequently employed to investigate changes in the surface hydrophilicity and wetting properties of hydrophobic polymers (Zielhuis et al., 2005; Norris et al., 1999; Meese et al., 2002; Balakrishnan et al., 2005). In this study, the surface activity of the MePEG blocks of the diblock copolymers was confirmed by the reduced contact angle measurements (increased hydrophilicity) for PLGA films containing increased concentrations of three diblock copolymers (Fig. 9). Determinations of contact angles for PLGA microspheres were complicated by water percolation through packed layers of microspheres. This problem was overcome to some degree by gentle heat fusion of the microspheres to reduce the inter-microsphere void size. However, the method is still limited by the undulations and roughness of the microsphere layer. Using this method, we determined that microspheres containing MePEG-b-PDLLA, $MePEG_{17}$-b-PCL_5 or $MePEG_{17}$-b-PCL_{10} had significantly reduced contact angle measurements as compared to PLGA (no diblock) microspheres, demonstrating that some of the MePEG blocks in the diblock copolymers were orientated away from the microsphere surface. Other groups have demonstrated similar reductions in contact angles for PLGA or PLLA microspheres or films modified with surface coatings of hydrophilic agents such as PEG,

PVA or poloxamer (Zielhuis et al., 2005; Norris et al., 1999; Meese et al., 2002; Balakrishnan et al., 2005).

These reductions in contact angle due to surface associated MePEG blocks resulted in increased biocompatibility as measured by neutrophil chemiluminescence (Fig. 8). Neutrophils and macrophages represent the first line of phagocytic defense against invasive material in vivo and neutrophil activation (as measured by chemiluminescence) may be used as an in vitro assay of the biocompatibility of microparticulates (Faraasen et al., 2003; Muller et al., 2003; Jackson et al., 2000a). The reduced chemiluminescence response of neutrophils to PLGA microspheres containing MePEG-b-PDLLA, $MePEG_{17}$-b-PCL_5 or $MePEG_{17}$-b-PCL_{10} diblock copolymers supports our suggestion that there is reduced opsonization by plasma components and that diblock/PLGA blended microspheres have increased biocompatibility compared to PLGA only microspheres. Interestingly, the $MePEG_{17}$-b-PCL_5 and $MePEG_{17}$-b-PCL_{10} diblock copolymers appeared to be the most effective agents in reducing neutrophil activation compared to the MePEG-b-PDLLA diblock copolymer (Fig. 8). This effect may arise from the increased encapsulation and loadings of these copolymers (approximately 30%, w/w) in PLGA as compared to the PDLLA diblock copolymer (22%) and to the increased rate of loss of MePEG-b-PDLLA from the PLGA microspheres in the first hour of incubation (Fig. 6).

In this study, we have shown that low molecular weight diblock copolymers may be encapsulated in PLGA microspheres where they form miscible blends and that the copolymer/PLGA blends increase the rate of paclitaxel release. The inclusion of these MePEG containing diblock copolymers in paclitaxel loaded PLGA microspheres also offers a simple method of sterically localizing MePEG blocks on the microsphere surface to improve the biocompatibility of the microspheres.

References

Balakrishnan, B., Kumar, D.S., Yoshida, Y., Jayakrishnan, A., 2005. Chemical modification of poly(vinyl chloride) resin using poly(ethylene glycol) to improve blood compatibility. Biomaterials 26, 3941–3951.

Brigger, I., Morizet, J., Aubert, G., Chacun, H., Terrier-Lacombe, M.J., Couvreur, P., Vassal, G., 2002. Poly(ethylene glycol)-coated hexadecylcyanoacrylate nanospheres display a combined effect for brain tumor targeting. J. Pharmacol. Exp. Ther. 303, 928–936.

Burt, H.M., Jackson, J.K., Bains, S.K., Liggins, R.T., Oktaba, A.M.C., Arsenault, A.C., Hunter, W.C., 1995. Controlled delivery of taxol from microspheres composed of a blend of ethylene-vinyl acetate copolymer and poly (D,L-lactic acid). Cancer Lett. 88, 73–79.

Burt, H.M., Hunter, W.L., 2006. Drug eluting stends: a multidisciplinary success story. Adv. Drug Rev. 58, 350–357.

Byun, J.W., Kim, J.U., Chung, W.J., Lee, Y.S., 2004. Surface-grafted polystyrene beads with comb-like poly (ethylene glycol) chains: preparation and biological application. Macromol. Biosci. 4, 512–519.

Castellanos, I.J., Flores, G., Griebenow, K., 2005. Effect of the molecular weight of poly(ethylene glycol) used as emulsifier on alpha-chymotrypsin stability upon encapsulation in PLGA microspheres. J. Pharm. Pharmacol. 57, 1261–1269.

Crosasso, P., Ceruti, M., Brusa, P., Arpicco, S., Dosio, F., Cattel, L., 2000. Preparation, characterization and properties of sterically stabilized paclitaxel-containing liposomes. J. Control. Release 63, 19–30.

Demetrick, J.S., Liggins, R.T., Machan, L., Davis, N.L., Burt, H.M., Hunter, W.L., 1997. The development of a novel intraperitoneal tumor seeding prophylactic. Am. J. Surg. 173, 403–406.

Deng, X., Zhou, S., Li, X., Zhao, J., Yuan, M., 2001. In vitro degradation and release profiles for poly-DL-lactide-poly(ethylene glycol) microspheres containing human serum albumin. J. Control. Release 71, 165–173.

Dordunoo, S.K., Jackson, J.K., Arsenault, L.A., Oktaba, A.M.C., Hunter, W.C., Burt, H.M., 1995. Taxol encapsulation in poly(caprolactone) microspheres. Cancer Chemo. Pharmacol. 36, 279–282.

Faraasen, S., Voros, J., Csucs, G., Textor, M., Merkle, H.P., Walter, E., 2003. Ligand specific targeting of microspheres to phagocytes by surface modification with poly(L-lysine)-grafted poly(ethylene glycol) conjugate. Pharm. Res. 20, 237–246.

Fonseca, C., Simoes, S., Gaspar, R., 2002. Paclitaxel-loaded PLGA nanoparticles: preparation physicochemical characterization and in vitro anti-tumoral activity. J. Control. Release 83, 273–286.

Gorbet, M.B., Sefton, M.V., 2005. Complement inhibition reduces material-induced leukocyte activation with PEG modified polystyrene beads (Tentagel) but not polystyrene beads. J. Biomed. Mater. Res. 74A, 511–522.

Gupte, A., Ciftci, K., 2004. Formulation and characterization of Paclitaxel, 5-FU and Paclitaxel + 5-FU microspheres. Int. J. Pharm. 276, 93–106.

Huizing, M.T., Misser, V.H.S., Pieters, R.C., Huinink, W.W.T., Vencor, C.H.N., Vermorken, J.B., Pinedo, H.M., Beijnen, J.H., 1995. Taxanes—a new class of anticancer agents. Cancer Invest. 4, 381–404.

Jackson, J.K., Springate, C.M.K., Hunter, W.L., Burt, H.M., 2000a. Neutrophil activation by plasma opsonized polymeric microspheres: inhibitory effect of Pluronic F127. Biomaterials 21, 1483–1491.

Jackson, J.K., Gleave, M.E., Yago, V., Beraldi, E., Hunter, W.L., Burt, H.M., 2000b. The suppression of human prostate tumor growth in mice by the intratumoral injection of a slow release polymeric paste formulation of paclitaxel. Cancer Res. 60, 4146–4151.

Jackson, J.K., Letchford, K., Babiuk, K.A., Machan, L., Signore, P., Hunter, W.L., Wang, K., Burt, H.M., 2004a. Characterization of perivascular poly(lactic-*co*-glycolic acid) films containing paclitaxel. Int. J. Pharm. 283, 97–109.

Jackson, J.K., Zhang, X., Llewellen, S., Hunter, W.L., Burt, H.M., 2004b. The characterization of novel polymeric paste formulations for intratumoral delivery. Int. J. Pharm. 270, 185–198.

Jiang, W., Schwendeman, S.P., 2001. Stabilization and controlled release of bovine serum albumin encapsulated in poly (D,L-lactide) and poly(ethylene glycol) microsphere blends. Pharm. Res. 18, 878–885.

Lapidus, R.G., Dang, W., Rosen, D.M., Gady, A.M., Zabelinka, Y., O'Meally, R., DeWeese, T.L., Denmeade, S.R., 2004. Anti-tumor effect of combination therapy with intratumoral controlled-release paclitaxel (PACLIMER microspheres) and radiation. Prostate 58, 291–298.

Letchford, K., Zastre, J., Liggins, R., Burt, H.M., 2004. Synthesis and micellar characterization of short block length methoxy poly(ethylene glycol)-block-poly(caprolactone) diblock copolymers. Colloids Surf. B: Biointerfaces 35, 81–91.

Liggins, R.T., D'Amours, S., Demetrick, J.S., Machan, L.S., Burt, H.M., 2000. Paclitaxel loaded poly(L-lactic acid) microspheres for the prevention of intraperitoneal carcinomatosis after a surgical repair and tumor cell spill. Biomaterials 21, 1959–1969.

Liggins, R.T., Burt, H.M., 2001. Paclitaxel loaded poly(L-lactic acid) microspheres: properties of microspheres made with low molecular weight polymers. Int. J. Pharm. 222, 19–33.

Liggins, R.T., Cruz, T., Min, W., Liang, L., Hunter, W.L., Burt, H.M., 2004. Intra-articular treatment of arthritis with microsphere formulations of paclitaxel: biocompatibility and efficacy determinations in rabbits. Inflamm. Res. 53, 363–372.

Liggins, R.T., Burt, H.M., 2004. Paclitaxel-loaded poly (L-lactic acid) microspheres 3: blending low and high molecular weight polymers to control morphology and drug release. Int. J. Pharm. 282, 61–71.

Liu, Y., Deng, X., 2002. Influences of preparation conditions on particle size and DNA-loading efficiency for poly(DL-lactic acid-polyethylene glycol) microspheres entrapping free DNA. J. Control. Release 83, 147–155.

Meese, T.M., Hu, Y., Nowak, R.W., Marra, K.G., 2002. Surface studies of coated polymer microspheres and protein release from tissue-engineered scaffolds. J. Biomater. Sci. Polym. Ed. 13, 141–151.

Mu, L., Feng, S.S., 2001. Fabrication, characterization and in vitro release of paclitaxel (Taxol) loaded poly (lactic-*co*-glycolic acid) microspheres prepared by spray drying technique with lipid/cholesterol emulsifiers. J. Control. Release 76, 239–254.

Muller, M., Voros, J., Csucs, G., Walter, E., Danuser, G., Merkle, H.P., Spencer, N.D., Textor, M., 2003. Surface modification of PLGA microspheres. J. Biomed. Mater. Res. 66A, 55–61.

Norris, D.A., Puri, N., Labib, M.E., Sinko, P.J., 1999. Determining the absolute surface hydrophobicity of microparticulates using thin layer wiking. J. Control. Release 59, 173–185.

Onetto, N., Canetta, R., Winograd, B., Catane, R., Dougan, M., Grechko, J., Burroughs, J., Rozencweig, M., 1993. Overview of taxol safety. J. Natl. Cancer Inst. Monogr. 15, 131–139.

Ruan, G., Feng, S.S., 2003. Preparation and characterization of poly(lactic acid)-poly(ethylene glycol)-poly(lactic acid) (PLA-PEG-PLA) microspheres for controlled release of paclitaxel. Biomaterials 24, 5037–5044.

Signore, P.E., Machan, L.S., Jackson, J.K., Burt, H.M., Bromley, P., Wilson, J.E., McManus, B.M., 2001. Complete inhibition of intimal hyperplasia by perivascular delivery of paclitaxel in balloon-injured rat carotid arteries. J. Vasc. Interv. Radiol. 12, 70–88.

Stolnik, S., Daudali, B., Arien, A., Whetstone, J., Heald, C.R., Garnett, M.C., Davis, S.S., Ilium, L., 2001. The effect of surface coverage and conformation of poly(ethylene oxide) (PEO) chains of poloxamer 407 on the biological fate of model colloidal drug carriers. Biochim. Biophys. Acta 1514, 261–279.

Torchilin, V.P., 2007. Micellar nanocarriers: pharmaceutical perspectives. Pharm. Res. 24, 1–16.

Wang, J., Ng, C.W., Win, K.Y., Shoemakers, P., Lee, T.K., Feng, S.S., Wang, C.H., 2003. Release of paclitaxel from polylactide-*co*-glycolide (PLGA) microparticles and discs under irradiation. J. Microencapsul. 20, 317–327.

Winternitz, C.I., Jackson, J.K., Oktaba, A.M., Burt, H.M., 1996. Development of a polymeric surgical paste formulation for taxol. Pharm. Res. 13, 368–375.

Zhang, X., Jackson, J.K., Burt, H.M., 1996. Development of amphiphilic diblock copolymers as micellar carriers of taxol. Int. J. Pharm. 132, 195–206.

Zhou, S., Deng, X., Yang, H., 2003. Biodegradable poly(ε-caprolactone)-poly(ethylene glycol) block copolymers: characterization and their use as drug carriers for a controlled delivery system. Biomaterials 24, 3563–3670.

Zielhuis, S.W., Nijsen, J.F.W., Figueredo, R., Feddes, B., Vredenberg, A.M., van het Schip, A.D., Hennink, W.E., 2005. Surface characteristics of holmium-loaded poly(L-lactic acid) microspheres. Biomaterials 26, 925–932.