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The effect of polyethylene glycol structure on paclitaxel drug release and mechanical properties of PLGA thin films

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ABSTRACT (232 WORDS)

Thin films of poly-lactic-co-glycolic acid (PLGA) incorporating paclitaxel have typically had slow release rates of paclitaxel on the order of $1 \mu\text{g/d}\cdot\text{cm}^2$. For implementation on medical devices, a range of zero-order release rates (i.e. $1\text{-}15 \mu\text{g/d}\cdot\text{cm}^2$) is desirable for different tissues and pathologies. Polyethylene glycol (PEG) of 8k and 35k molecular weight was incorporated at 15, 25, and 50% weight ratios in PLGA containing 10% w/w paclitaxel. The mechanical properties were assessed for potential use on medical implants and the rates of release of paclitaxel were quantified in % release and the more clinically useful $\mu\text{g/d}\cdot\text{cm}^2$. Paclitaxel quantitation was correlated to the release of PEG from PLGA, to further understand its role in paclitaxel/PLGA release modulation. PEG release was found to correlate with paclitaxel release and the level of crystallinity of the PEG in the PLGA film, as measured by Raman spectra. This supports the concept of using a phase separating, partitioning compound to increase the release rates of hydrophobic drugs such as paclitaxel from PLGA films, where paclitaxel is normally homogenously distributed/dissolved. Two formulations are promising for medical device thin films, when optimized for tensile strength, elongation, and drug release. For slow rates of paclitaxel release, an average of $3.8 \mu\text{g/d}\cdot\text{cm}^2$ using 15% 35k PEG for >30 d was achieved, while a high rate of drug release of $12 \mu\text{g/d}\cdot\text{cm}^2$ was maintained using 25% 8k PEG for up to 12 days.

1.0 Introduction

Localized drug or protein release from drug-eluting implants has now become a powerful tool to treat many pathologies. These bio-engineered implants and medical devices allow controlled dosages of potent drugs to be administered where they are most likely to have the strongest effect. This limits the amount of drug needed while reducing or eliminating systemic side effects and first-pass metabolism.

Some diseases, (i.e. prostate tumors and coronary atherosclerotic plaques) are also untreatable by systemic therapy, and would benefit from drug eluting implants devices. Syringe injectable poly lactic-co-glycolide (PLGA)/doxorubicin cylinders are a type of implant that has shown positive in vivo results for non-surgical treatment of prostate-confined cancer [1].

If the drug under consideration is potent, it can be implanted as a thin film (neat or encapsulated within a polymer matrix) or coated onto an existing medical device. Bare metal stents have been coated with numerous polymers encapsulating paclitaxel or sirolimus, offering improved angiographic results [2]. Recent clinical findings have supported that the stent may be able to be replaced with focally delivered paclitaxel by angioplasty balloons in certain cases [3]. In these cases, paclitaxel would benefit from a biocompatible, dissolvable carrier film that would extend the release for optimal reduction in scar-tissue (caused by angioplasty balloon inflation). Scar tissue, caused by neo-intimal growth of vascular smooth muscle cells, has been shown to be arrested by paclitaxel [4, 5].

When designing these carrier films, material scientists have a wide range of polymers to choose from. Non-biodegradable polymeric matrices are characterized by their durability, tissue compatibility, and mechanical strength that endure in vivo conditions without erosion or considerable degradation. Polyurethanes, poly (ethylene vinyl acetate), and polydimethylsiloxane are examples of polymer films [6-8] that follow predictable Fickian diffusion [9] or can be modified for linear or near-zero order release [10, 11]. Drawbacks of these non-biodegradable polymer devices are their occasional need for a second surgical procedure to remove the device, which leads to increased cost and associated discomfort/inconvenience for the patient.

Biodegradable matrices offer enhanced patient compliance and reduced side effects, as these drug-impregnated polymers offer extended dosing for intraocular, intravaginal, and cardiovascular pathologies [12-14]. The most commonly employed class of biodegradable polymers are the polyesters, which consist mainly of poly(caprolactone), poly(lactic acid), and the frequently exploited poly-lactide-co-glycolide (PLGA). Like most other biodegradable polymers, PLGA matrices undergo a more complicated release profile than that of their non-degradable counterparts. These release profiles are typically triphasic. The three phases can be summarized by initial burst release from the matrix surface, followed by a phase where the encapsulated drug diffuses more slowly out of the inner bulk matrix (similar to non-degradable matrices), and then finally after a period of incubation, a final drug release phase activated by the bulk degradation of the polymer [15-17].

One of the aims in this manuscript was to modify the release profile of thin film PLGA matrices such that most of the drug is released via the more predictable diffusion pathway before the more unpredictable bulk degradation phase commences, thus combining the advantages of a degradable carrier with avoidance of a late-stage burst. Our approach to accelerate the pre-degradation phase release was to use leachants as pore-formers.

When encapsulating highly hydrophobic drugs such as rapamycin (octanol-water partition coefficient, logP, of 5.77 [18]) or paclitaxel (logP of 4.0-4.4 [19, 20]) burst release is minimized (< 10%) in thin films as the drug is homogeneously distributed throughout the hydrophobic PLGA polyester. For example, in stent coatings of rapamycin/PLGA, a 50% mixture of rapamycin had to be formulated before any burst release was seen (vs. 5% and 25% rapamycin-containing films), and this was likely due to formulation homogeneity had been lost at this 50% ratio, and phase separation of the drug and PLGA was apparent by confocal Raman microscopy [21].

To increase the diffusion phase release of polyester/hydrophobic drug formulations, a number of techniques are available. Increasing the surface area by forming PLGA nanoparticles and microparticles offers improvement in drug release at the cost of unfettered, freely diffusible particles. PLGA/paclitaxel nanoparticles (~300-500 nm) were able to release ~15 µg/d paclitaxel for 30 days (after burst release) using 10 mg of dried nanoparticles [22]. Microparticle formulations of 1:4 w/w PLLA/PLGA yielded paclitaxel release of ~13 µg/d with

20 mg of material [23]. Due to their large surface area, nano- and microparticle burst release of 10-30% of encapsulated paclitaxel was seen in the cited formulations.

For thin films, increasing surface area requires methods to make matrices more porous. Porous matrices can be achieved by particulate leaching [24, 25], gaseous foaming of the matrix [26], and mixing with more hydrophilic polymers, such as polyethylene glycol (PEG).

Diffusion modulation by low molecular weight (MW) PEG incorporation has been used in a number of drug releasing formulations i.e. etanidazole pressed discs-PEG [27], sirolimus stent coatings [28], and spray dried films [29]. Low-MW PEG (2-4kDa) has also been revealed as a versatile plasticizer for PLGA [28, 30], but phase separation can undermine the film integrity if the concentration exceeds a limit. Incorporation of PEG into similar block copolymer polyesters can also affect mechanical properties [31].

To our knowledge, no systematic investigation has been undertaken to assess the properties of PEG incorporated into PLGA at various concentrations and MWs. While drug release from these matrices is of utmost importance in thin films for medical devices, an important aspect often overlooked is the parallel release of the additives or modifiers, in this case of PEG. In this work, we have correlated the rate of PEG release and its direct effect on the release of paclitaxel.

Yield strength and percent elongation have also been correlated to paclitaxel content, % PEG, and the PEG MW in PLGA thin films. It was our hypothesis that low-MW PEG would be more beneficial for increasing the rate of diffusion based drug release, while high-MW PEG would be more favorable for the mechanical properties due to the presence of intermolecular entanglements.

2.0 Materials and Methods

2.1 Materials

Poly (DL-lactide-co-glycolide) 53/47 (PLGA) with intrinsic viscosity of 1.03 was purchased from Purac, Netherlands. Paclitaxel was purchased from Yunnan Hande Bio-Tech, China. HPLC-grade dichloromethane (DCM) and acetonitrile was purchased from Tedia, USA. Deuterated chloroform (CDCl_3 + 0.03 % v/v TMS D99.8% + silver foil) was purchased from Cambridge Isotope Laboratories, Andover, USA. Polyethylene glycol (PEG) of molecular weight of 8000 (8k) and 35000 (35k), and polysorbate 80 (Tween 80) were purchased from Sigma-Aldrich, Singapore. All other polar solvents used were of high performance liquid chromatography (HPLC) grade and purchased from Sigma-Aldrich, Singapore. All chemicals and materials were used as received.

2.2 Film Formulation

The polymer solutions of 20 % w/v were prepared with 10 % w/w paclitaxel in DCM. A typical film formulation consisted of 60 mg of PCTX and 600 mg of polymer (PLGA + 0-50 % PEG) in 3 mL of DCM. For example, a 25 % 8k PEG/PLGA 53/47 solution was dissolved in 3 mL DCM overnight with 60 mg paclitaxel, 450 mg of PLGA 53/47 and 150 mg of 8k PEG. Film applicator height was set at 300 μm and the viscous solution was casted onto PET sheets (or TeflonTM if needed) at 50 mm/s, room temperature (RT), in a fume hood. DCM was evaporated at RT for 24 h followed by vacuum oven at 55 °C for 5 d. Punch-outs of 6 and 15 mm dia. were made for release and degradation studies respectively. The film applicator was adjusted to 500 μm to obtain 40 μm films required for evaluation of mechanical properties. A schematic of the film formulation can be seen in Supplementary Figure 1.

2.3 Film Wettability

Thin films (15-20 μm thick) were sliced into rectangular strips (3 x 1 cm) and their surface properties analyzed by contact angle (degree) and wetting tension (dyne/cm) employing a contact angle goniometer using a static sessile drop technique. The static measurements were carried out at RT using distilled H_2O with syringe pump rate of 5 $\mu\text{L/s}$, in triplicate. An image was captured after allowing the droplet to relax (15-20 s) and analyzed with FTA32 software, version 2.0 build 276.2.

161

162 **2.4 Film Mechanical Properties**

163 The dried 40 μm thin films were sliced into rectangular strips (8 x 1 cm) according to ASTM
164 D882. Each rectangular film were fixed to Instron Model 5567 grips with a load cell capacity of
165 10 N, pulled at rate of 5 mm/min (10 %/min) and analyzed with Bluehill software version 3.00.
166 The yield strength and elongation at break were recorded in the perpendicular direction (n=5).
167 No isotropic effects on the mechanical properties were investigated. Significance between the
168 mean values (n=5) was calculated using unpaired Student's t-tests. Probability values $P < 0.05$
169 were considered significant.

170

171 **2.5 Raman Spectroscopy**

172 The thin films were placed under the microscope objective and laser power up to circa 10 – 50
173 mW was shone onto the surface of the sample. Raman point-by-point mapping measurements
174 were performed on the area of 60 μm $\times$ 60 μm or 100 μm $\times$ 100 μm with a step size of 5 μm in
175 both the x and y directions. The measurements were performed using a Raman microscope
176 (InVia Reflex, Renishaw) equipped with a near infrared enhanced deep-depleted
177 thermoelectrically Peltier cooled CCD array detector (576 $\times$ 384 pixels) and a high grade Leica
178 microscope. The sample was irradiated with a 785 nm near infrared diode laser and a 50 $\times$ or
179 100 $\times$ objective lens was used to collect the backscattered light. Measurement scans were
180 collected using a static 1800 groove per mm dispersive grating in a spectral window from 300 to
181 1800 cm^{-1} and the acquisition time for each spectrum was around 35 seconds. Spectral
182 preprocessing that includes spike removal and baseline correction were carried out first before
183 the data was further analyzed using the band-target entropy minimization (BTEM) algorithm.
184 The BTEM algorithm [32] was used to reconstruct the pure component spectra of underlying
185 constituents from a set of mixture spectra without recourse to any a priori known spectral
186 libraries and has been proven well to reconstruct the pure component spectra of minor
187 components [33, 34]. When all normalized pure component spectra of all underlying
188 constituents have been reconstructed, the relative contributions of each constituent can be
189 calculated by projecting them back onto the baseline-corrected and normalized data set. The
190 spatial distribution of each underlying constituents can then be generated.

2.6 PEG Quantification by ¹H NMR

One cm square films were immersed in PBS Buffer, in triplicate. At predetermined time points, films were transferred to another tube containing fresh medium. After lyophilization, the powder was dissolved in 1050 ± 10 µg (700 µL) of CDCl₃, vortexed, and centrifuged at 10,000 rpm for 5 min prior to transferring the supernatant into NMR tubes. ¹H NMR spectra were recorded on Bruker Advance Spectrometer at 400 MHz using the signal of tetramethylsilane (TMS) present in deuterated chloroform at 0.03 % as an internal standard and can be seen in Supplementary Figure 2. ¹H NMR (400 MHz, CDCl₃, δ) 1.5-1.7 (bs, PLGA 3H, -C(=O)-CH(CH₃)-O-C(=O)-CH₂-O-), 3.45-3.85 (bs, PEG 4H, -O-CH₂-CH₂-O-), 4.6-5.0 (bs, PLGA 2H, -C(=O)-CH(CH₃)-O-C(=O)-CH₂-O-), 5.0-5.3 (bs, PLGA 1H, -C(=O)-CH(CH₃)-O-C(=O)-CH₂-O-).

2.7 In-Vitro Paclitaxel Release

The in-vitro release of paclitaxel was conducted in 2 mL of PBS with 2% Tween 80 release buffer (pH 7.4) at 37 °C, using 6 mm punch-outs (1 punch-out/well) in triplicate. At predetermined time-points 2 mL of buffer was removed and another 2 mL replaced with release buffer, maintaining sink conditions throughout the release. Withdrawn aliquots (or standards/dissolutions samples) were filtered through a 0.2 µm PTFE syringe filter directly into HPLC vials and immediately capped. Paclitaxel was quantified with an Agilent Series 1100 HPLC (Santa Clara, CA, USA) equipped with UV/Vis detector, autosampler, and column heater set to 35°C. A ZORBAX Eclipse XDB-C18 (5 µm) column of acetonitrile/water 60/40 (v/v) served as the mobile buffer, eluting the paclitaxel peak at ~ 5.4 minutes with a flow rate 1.0 mL/min and the UV/Vis detector recording at 227 nm. A total dissolution study of the 6 mm discs in triplicate was conducted by dissolving the films in acetone and diluting in release buffer to determine the surface concentration of paclitaxel (µg/mm²). The solubility limit of paclitaxel in PBS with 2% Tween-80 release buffer was determined to be 20 µg/mL.

2.8 Film Degradation Studies

PLGA films were incubated as described above in PBS with 2% Tween-80 release buffer and then removed at predetermined time points to be thoroughly dried in a 55°C vacuum for 5 d. Mass loss was determined gravimetrically before the films were dissolved in 1 mL chloroform, vortexed until dissolved, and syringed through 0.2 µm filters into immediately capped HPLC vials. Weight average molecular mass (M_w) of polymers were determined by size exclusion chromatography (SEC) using a Shimadzu LC-20AD HPLC equipped with RI detector and column heater set at 35°C. Low polydispersity polystyrene standards (Fluka) from 580-400,000 kDa were used for calibration of three linear PLgel (5µm) mixed C columns (Varian, Singapore). HPLC grade chloroform was used as mobile phase at flow rate of 1.0 mL/min. A differential scanning calorimeter (Q500 DSC, TA Instruments) was used to determine the thermal transitions of the films as a function of degradation time. The samples were heated from -30 °C to 80 °C and cooled to -30 °C at a rate 20 °C/min for two consecutive cycles, under pure dry nitrogen at a flow rate of 50 mL/min. The glass transition temperature (T_g) was determined by the signal minima/maxima from the second DSC thermogram obtained, analyzed with TA Universal Analysis software.

2.9 Film Surface and Film Cross-Section Topography

Surface and cross-sectioned PEG incorporated PCTX-PLGA films were coated with platinum for 50 s under a chamber pressure of less than 5 Pa at 20 mA using JEOL JFC-1600 Auto Fine Coater, Japan. Secondary electron images were acquired at 5.0 kV, 12 µA, at a working distance of 8 mm under the Field Emission Scanning Electron Microscopy (FESEM) (JEOL JSM-6340F, Japan). Film cross-sections were prepared by flash freezing the films in Tissue-Tek O.C.T. Compound at -80°C. Embedded film blocks were sliced while frozen at 10 µm and subsequently dried under vacuum at RT.

3.0 Results

3.1 Surface Hydrophilicity: Table 1

The surface properties of the PLGA 53/47 films were characterized using contact angle and wetting tension measurements with distilled water. **Table 1** displays PLGA 53/47 (neat), with 10% paclitaxel, and then mixed with 8k and 35k PEG. Knife casted PLGA 53/47 (neat) had similar values to that of spin-coated PLGA 75/25 and solution casted PLGA 70/30 of 73 ± 2 , 76.1 ± 0.3 , and 78 degrees, respectively [35, 36]. Addition of 10% lipophilic paclitaxel raises the contact angle by 16 degrees and decreases the wetting tension, indicating an increase in surface hydrophobicity. Addition of both PEGs at 15% w/w concentration decreased the contact angle from 89 ± 3 to ~ 50 degrees and improved the wetting tension by an order-of-magnitude. Increasing the PEG concentration to 25% sees no further drop in contact angle, whereas at 50% of PEG, the contact angle decreased by another 10%. For the PEG incorporated films, it was noted that the contact angle continued to decrease over time (on the order of minutes). For reproducible evaluation, all image photos were captured immediately after the water droplet was static, and ripple perturbations had subsided (15-20 s). For comparison, similar contact angles were seen with PLGA surface treatments of chitosan/gelatin coating and oxygen plasma treatment [35, 36].

The PEG % also had an effect on the adhesion of the PLGA films to the substrate used for film casting. When the films were cast on borosilicate glass plates or on polyethylene terephthalate sheets, the films could be peeled off with a metal spatula for the 15% and 25% PEG formulations. PLGA (neat) or with 10% paclitaxel could not be removed from these substrates—Teflon™ plates had to be utilized. 50% PEG films were brittle and could be flaked off the surface, but not peeled.

3.2 Raman Spectra: Figure 1

Pre-processed Raman mapping data from 10% paclitaxel/PLGA, 15% 8k PEG/PLGA, and 15% 35k PEG/PLGA were subjected to BTEM analysis in order to reconstruct the underlying pure component spectra and their associated spatial distributions, which are displayed in Figure 1. The reconstructed pure component spectral estimates via BTEM were then compared to known spectral libraries. It was found that the spectral estimates corresponded to the PLGA 53/47, paclitaxel, amorphous, and crystalline PEG. The BTEM estimate of PLGA 53/47 shows strong

and prominent Raman peaks at 846, 873, 890 cm^{-1} and some additional peaks at 1046, 1095, 1130, 1425, 1454, and 1768 cm^{-1} . The BTEM estimate of paclitaxel shows strong and prominent Raman peaks at 1002 cm^{-1} and some additional bands at 618, 1028, and 1602 cm^{-1} . The BTEM estimates of amorphous and crystalline PEG show prominent Raman peaks at 582, 859, 1139, 1231, 1395, 1469, 1479, 1486 cm^{-1} and 363, 534, 844, 860, 1063, 1124, 1140, 1280 cm^{-1} respectively. The prominent Raman peaks of crystalline PEG at 844 and 860 cm^{-1} has been previously used to differentiate the PEG crystalline phase from its amorphous phase [37]. In the present study, the crystalline phase of PEG was detected in both 15% 8k and 35k PEG films. This indicates that the recrystallization of amorphous PEG has occurred for both systems. However, the ratio of recrystallization of amorphous PEG was somewhat different between these two systems. As shown by the intensities of their score images, the recrystallization of amorphous PEG was more advanced for 15% 35k PEG system compared to 15% 8k PEG.

The Raman mapping and subsequent BTEM analysis also provide the spatial distributions of each constituent used in these systems. As can be seen in Figure 1A, PCTX was distributed homogeneously within PLGA. In Figure 1B (15% 8k PEG), again it can be observed that PCTX is also distributed homogeneously. A closer look also reveals that uniform and homogeneous distribution was observed for PLGA 53/47, PCTX, and amorphous PEG, but not for crystalline PEG, as the recrystallization of amorphous PEG may not occur in a spatially homogenous way but more discretely. Figure 1C (15% 35k PEG), on the other hand, shows that the distribution of all constituents was not uniform. Non-uniform distributions in PEG and paclitaxel became visually apparent at 15% 35k PEG and at the 25% 8k and 35k PEG films (data not shown). Crystallization of amorphous PEG was more pronounced, and paclitaxel was found to preferentially co-localize in the crystalline PEG regions. In the 50% PEG formulations, the brittle films were composed of mostly crystallized PEG.

3.3 Mechanical Properties: Figure 2 and Figure 3A, 3B

To determine the PLGA blended films potential in expanding medical devices, the mechanical properties of elongation and tensile strength at break were determined using 40-50 μm x 1 cm x 8 cm strips, with 5 cm of film between the tensile grips at 0% strain. Figure 2 displays the stress vs. strain curves for four formulations: PLGA (neat), w/10% paclitaxel, w/15% PEG 8k and w/25% PEG 8k (both PEG formulations include 10% w/w paclitaxel). As previously mentioned,

50% PEG formulations were brittle and not sufficiently ductile for tensile analysis. Addition of 10% paclitaxel to PLGA (neat) caused a 2.5 fold reduction in tensile strength at break, probably due to the fact that paclitaxel is crystalline at RT. Addition of PEG reduced the PLGA (neat) break by 35-40% and elongation was even further reduced to 75% for 8k PEG and 95% for 35k PEG. Upon addition of PEG, PLGA w/paclitaxel retained more break strength and elongation than PLGA (neat). Overall, the 35k PEG increased the break when added to the PLGA w/paclitaxel, but elongation was hindered (see Figure 3A and 3B). Elongation was greater for 8k PEG compared to that of the 35k, but still was 2-5 fold less than the PLGA with or without paclitaxel.

3.4 Paclitaxel release from PLGA 53/47 thin films: Figure 4A, 4B and Figure 5A, 5B

Table 2 lists the matrix properties of the films measured for paclitaxel release. The target thickness was between 15-20 μm , which maintains flexibility and was estimated to allow enough drug loading for 30+ days of release. Viscosity differences between mixtures of dissolved PLGA and PEG accounted for the differences in thickness and paclitaxel surface concentration. The 10% paclitaxel was calculated based on the weight of combined PEG and PLGA. As PLGA 53/47 was replaced with increasing amounts of PEG, the solutions became less viscous and dried to thinner films, affecting the paclitaxel surface concentrations. Thus, the paclitaxel surface concentrations were quantified and listed in Table 2, with the results exhibiting higher loading for the 35k PEG formulations. The differences in the paclitaxel surface concentration between the formulations can give different trends when looking at the data in % of release versus $\mu\text{g/d}\cdot\text{cm}^2$ or % of release versus % of release, as seen when Figure 4A was compared to Figure 4B and Figure 5A, respectively.

As a control, 10% paclitaxel with no PEG was prepared to compare the effects of increasing % PEG in PLGA. The hydrophobic paclitaxel was slowly released at an average of $2.2 \mu\text{g/d}\cdot\text{cm}^2$. This accounted for 17 % of the total after 33 days (see Figure 4A, 4B). Addition of 15% 8k PEG increased this to a meager $3.5 \mu\text{g/d}\cdot\text{cm}^2$ for a total of 26% in the same time period. Neither of these formulations displayed any burst release. With 25% 8k PEG, a burst of $56 \pm 12 \mu\text{g/cm}^2$ ($17 \pm 4\%$) was released in 1 hr and $96 \pm 20 \mu\text{g/cm}^2$ ($29 \pm 6\%$) after 1 day. This formulation was observed to have the best overall profile for paclitaxel release of any of the films analyzed. After day 1, $\sim 12 \mu\text{g/d}\cdot\text{cm}^2$ of drug was released on average for the next 12 days before the release

decreased, amounting to $76 \pm 11\%$ of the total drug content after 33 days. The 50% 8k exhibited little controlled release with an 80% burst of drug after the first day.

An increase in the MW PEG had a result contradictory to that of the smaller 8k PEG. At the lower concentration of 15% 35k PEG, 7% burst was quantified after the first day, and then a long sustained release was demonstrated for the next 30 days at an average of $3.8 \mu\text{g/d}\cdot\text{cm}^2$, as seen in Figure 5A and 5B. This was the longest sustained release of any of the formulations. An increase in % PEG merely increased the burst over 2 days, and then exhibited nearly the same flat release profile. From day 2 to 33, the amount of diffusion based release was inverse to the amount of 35k PEG with 104, 91, and $80 \mu\text{g/cm}^2$ paclitaxel for 15, 25, and 50% 35k PEG films. The integrity of the films was noticeably worse with the 25% and 50% PEG, as considerable release standard deviation was noticed from the 6 mm punch-outs. Subsequent analyses from the same film stock did not improve on the release precision.

3.5 Release of PEG from PLGA 53/47 and Mass Loss Composition: Figure 6A and 6B and Table 3

The % mass remaining of the PLGA/PEG films was followed at 4, 10, 15, and 21 days in 37°C PBS with 2% Tween 80 release buffer. After the specified time, films were dried and gravimetric weight measurements were recorded to determine the mass of film remaining. On a separate set of samples, the release of PEG and PLGA 53/47 was followed by NMR quantitation (See Materials and Methods). This data was merged with the HPLC paclitaxel quantitation to determine amount of PEG dissolution and soluble/degraded PLGA 53/47. Figure 6A and Figure 6B plot % cumulative PEG release vs. time for 8k and 35k PEG, and Table 3 gives the % composition of PEG, paclitaxel, and PLGA 53/47 at 4 and 10 days. Day 15 and 21 % mass remaining data displayed only trace increases from day 10 (data not shown).

The largest decreases in residual mass were seen with the highest amounts of % PEG, as expected. An analysis of the mass loss composition and PEG release reveals the large effect that both amphiphilic PEGs had on the release of paclitaxel in the first four days. For example, addition of 25% 8k and 15% 35k increased the paclitaxel release an order of magnitude ($\sim 100 \mu\text{g/cm}^2$) after four days, versus that of the no-PEG control.

Burst and sustained release of paclitaxel formulations correlated to the corresponding PEG release as well. For example, the 35k PEG formulations showed no sustained release of PEG after two days—only a burst was displayed (see Figure 6B). Within the burst time period of 2 days, the majority of the paclitaxel was released concurrently (in the 30 day time frame). After the PEG burst, the paclitaxel release rate was similar to the non-PEG modified film of 10% paclitaxel/PLGA. The 8k PEG formulations were observed to have two small sustained PEG release profiles at the 15% and 25% PEG films, in which paclitaxel release was 2x and 9.5x (at Day 10) that of non-PEG modified film of 10% paclitaxel/PLGA, respectively. The 15% 8k PEG, with the lower PEG crystallinity, had virtually no burst release, while the 25% 8k did, with a more dramatic release of paclitaxel. This suggests that the proportion of PEG crystallinity can influence paclitaxel release.

3.6 PEG Effects on PLGA degradation: Figure 7A, 7B

The MW of the PLGA polymers was expected to decrease faster for the higher % PEG/PLGA, due to the higher wetting tension and osmotic gradient (from the internal PEG bound within the PLGA matrix). When including the typical error of GPC analysis to be around 10-20% for MW, the 8k PEG does not display any accelerating or retarding of the polymer degradation—the degradation trend in the 10% paclitaxel/PLGA formulation was consistent for all 8k PEG formulations in Figure 7A. For the 35k PEG, a slight retardation of the degradation was noticed overall, as seen in Figure 7B. After day 10, all three films continued to have higher M_w averages for the next 4 time points, or 20 days overall, but this could be due to the higher MW fractions inherent in PEG 35k, that overlap the lower MW fractions in PLGA 53/47 (intrinsic viscosity of 1.03, ~150k/142k M_w/M_n). But no trend was noticed with the three 35k PEG containing films.

3.7 Surface and Cross-section topology and the effects of PEG leaching: Figure 8

The cross-section and surface topology of PLGA 53/47 neat and with 15% 8k PEG was visualized at 1000x magnification with the aid of a field emission scanning electron microscope. Formulations without PEG appear smooth with occasional serrations caused by artifacts in the knife caster. Cross-sectioning the PLGA 53/47 neat film at -80°C made the films brittle, as seen in Figure 8A. These films do not form pores before or after 10 days of PBS submersion as seen in Figure 8A and 8B. Figure 8C gives a typical PLGA film incorporating PEG (15% 8k) before

393 PBS buffer immersion and 10 days after (Figure 8D). Even before immersion and polymer
394 degradation, the surface was 'rough' with nano-to-micro size pores, that are likely to be caused
395 by phase separation of the PEG, even though the films appears homogenous with the Raman
396 spectroscopy (see Figure 1). As degradation proceeds within the aqueous buffer and both
397 amorphous and crystalline PEG dissolve, the pores grow larger and are likely to be the first
398 points of PLGA degradation, as seen in the larger pores sizes and channels present in Figure 8D.

4.0 Discussion

4.1 PEG MW and *in vitro* paclitaxel release

By incorporating low and high MW PEG into 10% paclitaxel PLGA films, release of paclitaxel could be correlated with wettability, crystalline PEG, mechanical properties, and MW loss. Earlier work with low-MW PEG showed some limitations. For example, Jackson et al. used 10% 350 MW methoxy-poly(ethylene glycol) in the 100 μ m PLGA films containing from 5-30% w/w paclitaxel. The 15% paclitaxel loaded film in Jackson et al. study was found to have a release of ≤ 3 μ g paclitaxel/day (or about 0.4%/day). The 350 MW methoxy-polyethylene glycol itself was leached out much faster as 75% (or 375 μ g from a 5 mg film) of it was depleted within 72 h [38], similar to our results for the 50% 8k and 35k PEG, although no paclitaxel release was associated with this burst of low MW methoxy-polyethylene glycol. Compared to the 350 MW PEG above, the higher MW 8k and 35k PEG in this study had a more impressive effect on the paclitaxel release, which allowed faster rates of paclitaxel delivery with less paclitaxel loading overall. Higher PEG MW may have a larger paclitaxel loading ratio, increasing the overall solubility in aqueous solution. Paclitaxel partitioning molecules such as PEG and cyclodextrin have been observed to increase the solubility of paclitaxel [39], most likely by both non-specific and specific binding, respectively.

The *in vitro* release conditions were modulated to that of physiological conditions using 2% Tween 80 in PBS buffer at 37°C. The addition of 2% Tween 80 allowed paclitaxel concentrations of ~ 20 μ g/mL, 50 times that of PBS alone (data not shown). Tween 80 has been commonly used in release buffers for paclitaxel for this reason [23, 40]. At the 2% concentration, the paclitaxel solubility allows physiological concentrations of paclitaxel found in serum [41].

The release *in vivo* will depend on the implanted tissue and contact to physiological fluids. Implanted into dense tissues, such as muscle, PLGA films have been found to be retained longer than *in vitro* conditions [42]. If placed in such a dense tissue environment, the PLGA/PEG films would likely have smaller burst release (decreased PEG diffusion and dissolution). An increased rate of paclitaxel from faster degrading PLGA could conceivably occur, as the autocatalytic PLGA oligomers would not be washed away as quickly, as seen in thicker PLGA films [43].

430 ***4.2 Co-localization of crystalline PEG and paclitaxel***

431 The addition of PEG to both biodegradable polymers and non-degradable polymers has
432 confirmed its usefulness in forming pores and increasing the overall porosity, but this can be
433 polymer dependent. For example, when poly-caprolactone films are mixed with PEG, 2-5 μm
434 droplets are formed throughout the film, where the size of the droplets was inversely correlated
435 with the MW of the blended PEG—larger the MW, the smaller the pores [44]. The PLGA/PEG
436 blends used here exhibited a different profile. Spatial distributions of paclitaxel and 15% 8k
437 MW PEG was uniform, but spectral peaks of 800-860 cm^{-1} indicated crystalline PEG among the
438 more common amorphous state as seen from Figure 1B, but also become apparent at the sub-
439 micron level in Figure 8A. Phase separations in PEG with co-localized paclitaxel became
440 apparent at micro-scale at 15% 35k PEG (Figure 1C). The PEG release in Figure 6A and Figure
441 6B, and subsequent composition analysis presented that the films were not leaching PEG in one
442 large bolus for both molecular weights. The 15% 8k exhibited a more gradual PEG release,
443 whereas the other formulations exhibited some level of burst release. The gradual release of
444 PEG in the 15% 8k formulation with a small burst of PEG release did not correlate with a
445 considerable raise in paclitaxel release kinetics (2x vs. 10% paclitaxel/PLGA)

446 The paclitaxel in the PLGA films with no PEG added appears to be homogenously distributed,
447 which accounts for the slow, diffusion based release. When the PEG was added in, it was
448 initially homogenously distributed at the 15% PEG concentration; physically present as
449 amorphous PEG in the Raman mapping of Figure 1, with traces of crystalline PEG observed.
450 Paclitaxel was still homogenously distributed here as well, meaning that there is no partitioning
451 into the PEG (there is probably no phase separation at this loading of PEG). For the 15% 8k
452 PEG, a burst of PEG release (~25% of total) was seen, but this did not correlate with any burst of
453 paclitaxel—the rate of release was equivalent to that of the no-PEG control. The PEG burst
454 likely originates from the amorphous PEG at the PLGA surface—and since paclitaxel was still
455 uniform in the PLGA, the amorphous PEG did not enhance its release.

456 ***4.3 Dissolved crystalline PEG associated with increased paclitaxel burst/release***

457 The 15% 35k, 25%, and 50% PEG formulations demonstrated high burst release rates for the
458 PEG and paclitaxel, and this was due to the probable formation of crystalline PEG at this higher

PEG MW and higher PEG concentration. Figure 1C exhibits the phase separations of crystalline PEG, paclitaxel, and PLGA. Paclitaxel was co-localizing and concentrating within these crystalline PEG phases, and was no longer homogenously distributed throughout the PLGA/PEG film, although it was still present in the PLGA/amorphous PEG phase. These crystalline PEG regions likely dissolve within the first few days, as seen in the increased % PEG dissolved in Figure 6. With a portion of the paclitaxel co-localized in these fast dissolving crystalline-PEG regions, it was released at a much faster rate as well. After the crystalline PEG dissolved away, the paclitaxel release rate reverted to the diffusion based rate seen with the homogenously distributed paclitaxel of PLGA/amorphous PEG. Differential scanning calorimetry supports this observation, as no crystalline PEG was present in PEG/PLGA films after 4 days of release buffer immersion (data not shown).

The presence of crystalline PEG controls the rate of paclitaxel release, and should be optimized when using PEG additives. If the added PEG is amorphous, it does not alter the rate of release from the matrix polymer, as seen in (Figure 4). At the other extreme, a substantially high level of crystalline PEG yielded an unsustainable high initial rate (aka burst release) such as seen for both the 50% 8k and 35k formulations. In our study, the crystallinity was controlled by the amount of PEG and its MW. Other studies have revealed that rate of solvent evaporation can control the amount of crystalline PEG, and subsequently the phase separation pore size in the film. Faster evaporating dichloromethane (DCM, used in our films, bp 40°C) was seen to have less crystallinity than the higher boiling acetone (57°C). Lin and Lee used this technique to increase the film pore size as the crystalline PEG was immediately dissolved [45]. While rarely reported for thin films, the casting parameters such as temperature and pressure can have large effects on the film surface roughness, film porosity, and likely PEG crystallinity as well. For example, evaporative cooling from the DCM solvent would condense water vapor on the film surface if the wet polymer matrices were left exposed to ambient air or were poorly covered. As this could affect PEG distribution and crystallinity, these films were excluded from the study. Film porosity was directly affected by the solvent bp and drying pressure. DCM films required 24 h of drying at atmospheric pressure before they could be exposed to a 55°C vacuum oven. If the films were directly subjected to vacuum, unpredictable foam matrices would occur (data not shown). For higher bp solvents, longer incubations at atmospheric pressures would be needed, and therefore avoided in this study. The concentration of residual DCM was minimized by

employing a vacuum oven at moderate temperatures. The optimized drying procedure yielded <500 ppm and <3 μ g residual DCM, for an assumed 5 mg PLGA film implant (Supplementary Figure 1A inset displays the ^1H NMR DCM peaks at 2 drying conditions). This falls under FDA guidelines of <600 ppm and <6 mg/d residual DCM [46].

The MW degradation supports the PEG-crystalline/paclitaxel phase separation modulated release, as the addition of PEG had only modest influence on the PLGA autocatalytic chain scission. It cannot be said that the amorphous PEG remaining hydrated the PLGA matrix faster on a time scale relevant to PLGA polymer cleavage. With the films in the 15-20 μm thickness, hydration of the samples would be quick regardless of the additive. This has also been demonstrated with other hydrophilic additives in PLGA, such as poly(vinyl alcohol) grafts [47].

4.4 Raman microscopy multivariate analysis compared to coherent anti-stokes Raman scattering

Kang et al have also used a Raman microscopy technique, coherent anti-stokes Raman scattering (CARS), to visualize PEG 2k/paclitaxel domains in PLGA films [29]. However, our Raman technique is quite different. CARS uses non-linear optical imaging whereas our present Raman approach is based on linear optical imaging. Although CARS is a much faster technique compared to the conventional Raman microscope, it also has some drawbacks when used to generate the spatial distribution of PEG, PLGA, and paclitaxel. The approach used here was based on a multivariate analysis or full-spectral range analysis from 300 to 1800 cm^{-1} whereas the CARS images of PEG, PLGA, and paclitaxel were generated either from particular peak positions (i.e. 2890 cm^{-1} for PEG and 2940 cm^{-1} for PLGA) or from a much shorter range of certain spectral band (i.e. 3060-3090 cm^{-1} for paclitaxel). Such overlapping of spectra may yield greater uncertainty in the final spatial distributions.

4.5 Effects of PEG/paclitaxel phase separations on film material properties

When comparing the two molecular weight PEGs, we can assume that the 35k PEG had a more crystalline profile in PLGA than the 8k, as it is not likely to diffuse faster than a smaller MW. While this may have increased paclitaxel burst rates, it was detrimental to the material properties, as film elongation was more compromised in 35k PEG. In a medical device usage, these parameters would need to be carefully optimized. Crystalline PEG/paclitaxel phase separations

were also present at the sub-micron level, as visualized by the sub-micron pits and pores present in the Figure 8 SEM results. If the sub-micron phase separations were distributed over the entire film, the film would appear homogenous on Raman mapping, but the material properties would still be affected, as we see for the 15% 8k PEG formulations when compared to the (neat) PLGA films.

When blended separately with PLGA, PEG and paclitaxel had deleterious effects on both the tensile strength and elongation. This contradicts results published elsewhere, that small 350 MW PEG increases PLGA elongation [38]. This likely is MW dependent, as more elongation was seen for 8k than the 35k PEG. The elongation was greater than 20 %, which would make them a potential film formulation for non-compliant drug eluting angioplasty balloons, which stretch from 10-20 % at maximum inflation pressure. When paclitaxel and PEG were combined together, the deleterious effects were additive for tensile strength. The formation of the crystalline PEG pores probably accounts for the reduced structural integrity. However a substantial amount of elongation was recovered when paclitaxel was blended into the PEG/PLGA thin films. It was most dramatic for the 35k PEGfilms, adding an order of magnitude amount of elongation from no paclitaxel, to films containing 10% paclitaxel. The addition of paclitaxel probably reduces the ratio of crystalline to amorphous PEG. Addition of PEG also added a practical usefulness to the films—it changed the surface energy to a more hydrophilic nature, allowing the films to be peeled off and removed intact from the glass plates and polyethylene terephthalate sheets, which could be medically useful considering the majority of transluminal angioplasty balloons are manufactured from polyethylene terephthalate [48].

5.0 Conclusions

The properties of PLGA films blended with a pore-forming PEG polymer have been described towards their use in controlled paclitaxel delivery. The effect of PEG molar mass and concentration of the release of paclitaxel, as well as on the mechanical properties of the PLGA films are rationalized on the basis of the nature of the PEG and its distribution within the PLGA. Using confocal Raman mapping, we were able to confirm the co-localization of the paclitaxel in the crystalline PEG phase of the phase-separated blends. The crystallized PEG is the phase that leaches out first forming the pores for the burst release of associated paclitaxel. Subsequent release of paclitaxel was by diffusion through the dense polymer phase. When the molar mass of PEG was increased, most of the drug was released by burst release, whose extent correlates to the burst release of the crystalline PEG. The phase separation of crystalline PEG in the blend also lowers tensile strength and elongation to break. In general, the lower molar mass PEG allows for greater range of release rate manipulation. Such blended films hold promise for applications requiring enhanced release rates of hydrophobic drugs from hydrophobic matrices.

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Figure Captions

Figure 1. Raman Mapping of A) 10% paclitaxel/PLGA 53/47 B) 15% 8k PEG/PLGA 53/47 w/paclitaxel C) 15% 35k PEG/PLGA 53/47 w/paclitaxel.

Figure 2. Stress vs strain of PLGA 53/47 (neat), PLGA 53/47 w/10% paclitaxel, and 15%, 25% 8k PEG PLGA 53/47 thin films. * PLGA 53/47 contains 10% paclitaxel.

Figure 3. Mechanical properties of PLGA/PEG thin film with respect to A) Tensile Strength at Break B) % Elongation.

Figure 4. A) % Cumulative release of paclitaxel in 8k PEG/PLGA films B) $\mu\text{g}/\text{cm}^2$ cumulative release of paclitaxel in 8k PEG/PLGA films. *PLGA 53/47 contains 10% paclitaxel.

Figure 5. A) % Cumulative release of paclitaxel in 35k PEG/PLGA films B) $\mu\text{g}/\text{cm}^2$ cumulative release of paclitaxel in 35k PEG/PLGA films. *PLGA 53/47 contains 10% paclitaxel.

Figure 6. Percent cumulative release of PEG in A) 8k PEG/PLGA and B) 35k PEG/PLGA.

Figure 7. Molecular weight decay for A) 8k PEG/PLGA and B) 35k PEG/PLGA. M_w , weight average molar mass.

Figure 8. Scanning electron microscopy of PLGA 53/47 with 10% paclitaxel film surface and cross-section (CS) at A) day 0, B) day 10, C) w/15% 8k at day 0, and D) w/15% 8k at day 10.

Supplementary Figure 1. Schematic of PLGA 53/47 thin film casting onto polyester teraphthalate sheets (PET). The film applicator allows the wet thickness of the homogenous polymer blends to be controlled, which was 300 μm for the PLGA blends. After drying at 55°C under vacuum for 48 h, the dry thickness was 10-20x thinner then the wet thickness, depending on solution composition and polymer concentration.

Supplementary Figure 2. A) ^1H NMR of 15% 8k PEG in PLGA/PEG/Paclitaxel dried film. PLGA protons (α , β , and γ) are labeled under the corresponding ^1H NMR peaks. Inset A) PLGA/PEG/Paclitaxel polymer blends were dried at 55°C to decrease the amount of residual dichloromethane (DCM) located at 5.32 PPM. Drying at 25°C leaves residual amounts of DCM at 0.01% w/w or 1600 ppm. B) ^1H NMR of lyophilized PBS buffer after 4 days of submerged 15% 8k PEG films. PEG 8k integrals were quantified at 3.6 PPM. All samples were dissolved in 1050 mg of CDCl_3 for quantitative analysis, with the 0.03% tetramethylsilane (TMS) peak at 0.0 PPM used as internal standard. See section 2.6 PEG Quantification by ^1H NMR for complete details.

A **B**

A **B**

A **B**

A **B**

Surface

CS

Surface

CS

A

C

B

D

SEI 5.0kV $\times 1,000$ 10 μ m WD 6mmSEI 5.0kV $\times 1,000$ 10 μ m WD 7mmSEI 5.0kV $\times 1,000$ 10 μ m WD 6mmSEI 5.0kV $\times 1,000$ 10 μ m WD 8mm

Homogenous PLGA polymer blends

→
Micrometer dial set
to wet thickness
→

Film Applicator

Macro-view of films after drying in vacuum oven for 48 h @ 55°C.

PCTX-PEG8K-PLGA 15%

PCTX-PEG8K-PLGA 25%

PCTX-PEG8K-PLGA 50%

A **B**

Tables

	dH ₂ O Contact Angle (deg)	Wetting Tension (dyn/cm)
PLGA 53/47	73 ± 2	5 ± 2
w/ 10% paclitaxel	89 ± 3	2 ± 2
w/ 15% PEG 8k	49 ± 2	52 ± 2
w/ 25% PEG 8k	49 ± 1	47 ± 2
w/ 50% PEG 8k	37 ± 1	59 ± 1
w/ 15% PEG 35k	51 ± 2	46 ± 3
w/ 25% PEG 35k	49 ± 2	48 ± 2
w/ 50% PEG 35k	38 ± 2	58 ± 2
PLGA 75/25	76.1 ± 0.3 [35]	NR
w/ surface modified chitosan and gelatin	51.5 ± 0.7 [35]	NR
PLGA 70/30	78 [36]	NR
w/O ₂ plasma treatment	45 [36]	NR

Table 1. Water-in-air dH₂O Contact Angles and Wetting Tension for treated PLGA films. NR = Not Reported.

Film composition	Thickness (μm)	Paclitaxel Surface Concentration (μg/mm ²)	% PEG (by ¹ H NMR)
10% Paclitaxel PLGA 53/47	16 ± 2	4.2 ± 0.2	0
w/15% 8k PEG	16 ± 3	4.3 ± 0.8	16 ± 1
w/25% 8k PEG	16 ± 3	3.3 ± 0.5	25 ± 1
w/50% 8k PEG	13 ± 5	1.9 ± 0.2	50 ± 3
w/15% 35k PEG	21 ± 6	6 ± 2	16 ± 1
w/25% 35k PEG	19 ± 5	5 ± 2	26 ± 1
w/50% 35k PEG	18 ± 3	7 ± 2	51 ± 3

Table 2. Matrix properties of PLGA 53/47 thin films in Figures 4. and 5.

Mass Loss Composition	Day 4				Day 10			
	Total Loss (μg/cm ²)	Ratio of Released PEG / PCTX / PLGA % (μg/cm ²)			Total Loss (μg/cm ²)	Ratio of Released PEG / PCTX / PLGA % (μg/cm ²)		
10% Paclitaxel/PLGA	60	0(0)	15(10)	85(50)	70	0(0)	28(20)	72(50)
w/15% 8k PEG	250	74(186)	4(10)	20(50)	340	75(255)	11(39)	15(50)
w/25% 8k PEG	630	71(448)	21(135)	8(50)	730	68(496)	26(189)	7(50)
w/50% 8k PEG	1210	83(1007)	12(151)	4(50)	1270	83(1054)	13(163)	4(50)
w/15% 35k PEG	330	53(176)	33(108)	15(50)	360	53(176)	33(108)	15(50)
w/25% 35k PEG	580	60(346)	31(180)	9(50)	600	58(346)	34(202)	8(50)
w/50% 35k PEG	1310	78(1020)	18(240)	4(50)	1320	77(1020)	19(247)	4(50)

Table 3. Composition of mass loss at 4 and 10 days in PBS/2% Tween-80 release buffer. PCTX = paclitaxel. Total Loss/cm² (ΔM) = M₀/cm² – M_t/cm² where M₀ was initial mass and M_t was the dried M₀ mass after t days in 37°C PBS/2% Tween 80 release buffer. Standard deviations for all values are ≤ 10%. Ratio of released of PEG, paclitaxel, and PLGA was calculated from the soluble fractions by NMR (PEG and PLGA) and HPLC (paclitaxel).

Homogenous PLGA polymer blends

→
Micrometer dial set
to wet thickness →

Film Applicator

Macro-view of films after drying in vacuum oven for 48 h @ 55°C.

PCTX-PEG8K-PLGA 15%

PCTX-PEG8K-PLGA 25%

PCTX-PEG8K-PLGA 50%

A **B**