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Novel gradient casting method provides high-throughput assessment of blended polyester poly(lactic-*co*-glycolic acid) thin films for parameter optimization

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ABSTRACT

Pure polymer films cannot meet the diverse range of controlled release and material properties demanded for the fabrication of medical implants or other devices. Additives are added to modulate and optimize thin films for the desired qualities. To characterize the property trends that depend on additive concentration, an assay was designed which involved casting a single polyester poly(lactic-*co*-glycolic acid) (PLGA) film that blends a linear gradient of any PLGA-soluble additive desired. Four gradient PLGA films were produced by blending polyethylene glycol or the more hydrophobic polypropylene glycol. The films were made using a custom glass gradient maker in conjunction with a 180 cm film applicator. These films were characterized in terms of thickness, percent additive, total polymer (PLGA + additive), and controlled drug release using drug-like fluorescent molecules such as coumarin 6 (COU) or fluorescein diacetate (FDAc). Material properties of elongation and modulus were also accessed. Linear gradients of additives were readily generated, with phase separation being the limiting factor. Additive concentration had a Pearson's correlation factor (R) of >0.93 with respect to the per cent total release after 30 days for all gradients characterized. Release of COU had a near zero-order release over the same time period, suggesting that coumarin analogs may be suitable for use in PLGA/polyethylene glycol or PLGA/polypropylene glycol matrices, with each having unique material properties while allowing tuneable drug release. The gradient casting method described has considerable potential in offering higher throughput for optimizing film or coating material properties for medical implants or other devices.

1. Introduction

When engineering polymeric thin films for medical devices, the research and design (R&D) team must optimize numerous parameters. It is rare for a pure polymer to meet all the considerations required for the function of a device.

To develop the thin films for a specific application, the neat polymer must have various additives incorporated into polymer solutions or melts to meet the final design specifications [1]. These additives are used to modify properties such as controlled drug release [2], surface tension [3], mechanical properties [4,5], adhesion [6], etc. These modifications need to be assessed empirically, hence significant resources in labour and materials are often needed. Complicating the assessment is the influence of the parameters on one another. One additive included to improve a specific property may be deleterious to other functions. For example, adding porogens (pore-forming additives) in thin films increases surface area and diffusional drug release as desired. However, the inclusion may drastically change the mechanical properties to an extent where the thin film is no longer suitable for the intended purpose.

At present, optimizing a film with permutations of several additives is a considerable undertaking which may hinder progress in the R&D process. To address this problem, we have successfully developed a novel procedure of thin film casting that produces additive gradients from 0% to 50%. As displayed in Fig. 1, the design of the custom gradient caster allows up to 100 ml of mixed solution to be cast in one session. The casting allows an ascending polymer ratio vs. length, with maximum lengths of 180 cm possible. The method was designed to provide enough material for analysis using the multiple procedures required for characterization, including mechanical testing, controlled drug release, proton nuclear magnetic resonance (^{1}H NMR) analysis, thickness measurements, etc. The advantages of this technique include lower material investment, labour efficiency, and a higher rate of throughput. Moreover, trends dependent on additive concentration in material and film properties are quickly identified.

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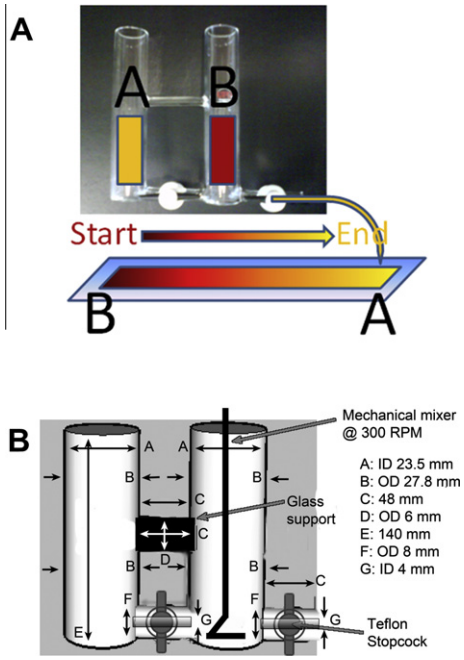

Fig. 1. (A) Diagram of gradient casting. As the two stopcocks are opened in the gradient caster, solution B immediately flows out and solution A is mixed in increasing concentrations in chamber B. (B) Dimensions of gradient caster. ID: inner diameter. OD: outer diameter. All glassware was constructed from laboratory-grade borosilicate glass.

When thin films of fixed additive amounts are synthesized, it is unlikely that the initial preparation will match the sought material parameters. This will necessitate another round of film casting, synthesis, or both. In the system described here, additive concentration will be dependent on the length for gradient cast films; one simply has to return to the original film and sample from a different section along the gradient.

Our process is a straightforward approach that provides greater film area than other recently published gradient methods based on controlled evaporative spin coating or surface-plasma-generated methods [7,8]. It has similarities in design and features to the methodology utilized in casting gradient polyacrylamide gels for protein characterization [9]. In the present study it was employed to cast thin films of polyester poly(lactic-*co*-glycolic acid) (PLGA).

Polyesters are a well-known biodegradable polymers employed in thin film drug delivery systems [10]. Commercially available polyesters consist of polycaprolactone (PCL), polylactic acid (PLLA) and the more common PLGA. They have been used in various applications such as the controlled delivery of drugs [11], antibiotics [12], and vaccines [13], and also tissue engineering [14] and bone defect healing [15]. PLGA has found favour due to its biodegradability, biocompatibility and approval for parenteral use by regulatory authorities around the world. Numerous active pharmaceutical drugs such as growth factors, antibiotics and anti-cancer drugs have been incorporated into PLGA-based platforms with considerable therapeutic effect [16,17].

Polyester/hydrophobic drug formulations utilize a number of methods and additives to modulate their release, including particulate leaching [18,19], matrix foaming [20], and additive incorporation such as polyethylene glycol (PEG) and polypropylene glycol (PPG). Etanidazole pressed discs-PEG [21], stent coatings [22], and spray-dried films [23] have all utilized low-molecular-weight PEG (2–4 kDa) to modify drug release or acted as a versatile plasticizer for PLGA [22,24]. Incorporation of PEG or PPG into similar block copolymer polyesters can also affect mechanical or release properties, respectively [25,26].

The effect on mechanical properties by the incorporation of PEG into PLGA thin films has been seen to be detrimental when mixed in high concentrations, limiting its use as a drug release modulator [2].

As a first application of our gradient knife-casting method, we hypothesized that the more hydrophobic cousin of PEG, PPG, would retain such material properties as high modulus, elongation, and low amounts of phase separation when incorporated into PLGA, yet allow an increase in overall drug release due to its water miscibility. Herein we incorporated 4000 Da PEG (PEG 4 K), 4000 Da PPG (PPG 4 K) into thin films of PLGA 53/47 with an intrinsic viscosity of 1.03 dl g^{-1}, with a molecular weight of ~100 kDa (PLGA 100 K), through gradient films. Fluorescein diacetate (FDAc) and coumarin-6 (COU) were used as model drugs for controlled release. We have previously published how FDAc can be used as a high-throughput screen for paclitaxel release in PLGA thin films, and is therefore a good rationale for choice in this study [27].

2. Materials and methods

2.1. Materials

PLGA 53/47 (with intrinsic viscosity of 1.03 dl g^{-1} was purchased from Purac, the Netherlands. 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 and polypropylene glycol of molecular weight of 4000 g mol^{-1}, and polysorbate 80 (Tween 80) were purchased from Sigma–Aldrich, Singapore. Rhodamine 6 g, COU and FDAc were purchased from TCI Japan, 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. Gradient casting thin films

Gradient films were produced using the gradient caster in Fig. 1. Initially 20 ml of the more viscous solution (15% PLGA (w/v DCM)) was poured into Chamber B, and the additive in Chamber A, i.e. 20 ml 15% PEG 4000 (w/v DCM). Each well contained 65 mg (in 20 ml) of FDAc or COU for later release studies. The gradient maker was tilted at a 10% incline for faster flow rate and fixed to the film applicator with flow rate adjusted by the Teflon stopcock. Gradient mixing was initiated after a few seconds (~5–10 cm) of knife casting pure PLGA/drug film – in this amount of time, the viscous solution was allowed to fill the entire 8 cm width of the knife caster. The chamber A valve was then opened to begin mixing with chamber B. Chamber B mixing was performed using a battery-operated "milk frother" (Ikea, Singapore) modified for overhead mixing and taped into place. Gradient solutions were poured (rate of ~20 ml min^{-1}) directly into the film applicator within a fume extractor hood. Film applicator height was set at 500 μm and the flowing viscous gradients were directly cast onto 50 μm polyethylene teraphthalate sheets at 20 mm s^{-1}, room temperature (RT), employing a S125 knife caster, capable of 180 cm length films (MTL Systems Pte Ltd, Singapore). DCM was evaporated at RT for 24 h in a fume hood, followed by vacuum oven (<10 Torr) at 55 °C for 48 h. Punchouts of 6 mm diameter (using a simple paper punch) were taken every 5 or 10 cm for characterization.

2.3. PLGA 100 K, PEG 4 K, and PPG 4 K quantification by ^{1}H NMR

Dried (6 mm diameter × 3 pieces) punch-outs were dissolved in 1050 ± 10 μg (700 μl) of $CDCl_3$, vortexed, and centrifuged at 10,000 rpm for 5 min prior to transferring the supernatant into

NMR tubes. ^{1}H NMR spectra were recorded on a Bruker Advance Spectrometer at 400 MHz using the signal of tetramethysilane (TMS) present in deuterated chloroform at 0.03% as an internal standard. ^{1}H NMR (400 MHz, $CDCl_3$, δ) 1.5–1.7 (bs, PLGA 3H, —C(=O)—CH(*CH*3)—O—C(=O)—CH2—O), 3.45–3.85 (bs, PEG 4H, —O—*CH2*—C*H2*—O—), 3.2–3.8 (bs, PPG 3H, —O— (*CH*1—CH3) —*CH2*—O—), 4.6–5.0 (bs, PLGA 2H, —C(=O)—CH(CH3)—O—C(=O) C*H2*—O—), 5.0–5.3 (bs, PLGA 1H, —C(=O)—C*H*(CH3)—O—C(=O)— CH2—O—). ^{1}H NMR error was calculated by the combined standard deviations (within each gradient composition) of the integrated CHCl3 peaks (weighing error) and the standard deviations of the lactide/glycolide ratios (integration and machine error).

2.4. High-throughput screening of fluorescent dyes

High-throughput FDAc quantification has been previously published [27]. Briefly, FDAc incorporated into the 6 mm diameter PLGA discs were immersed in 200 μl of PBS/2% Tween 80 solution (release buffer), within a 96-well Costar flat black polystyrene flat-bottomed plate. Samples were assayed in pentaplicates and stored in a 37 °C incubator. Aliquots of 20 μl were drawn out of the release plate, placed into a separate black read plate and diluted with 180 μl of 100 mM NaOH, instantly yielding fluorescein (excitation/emission: 490/520). The amount of hydrophobic dye released was quantified using three calibration curves (with separate gain settings) spanning three orders of magnitude: 0.01–10 μg ml^{-1}. The release plate had the remaining solution carefully drawn out, and replaced with another 200 μl of release buffer.

2.5. Film mechanical properties

Polymer solutions of 15% w/v in DCM were prepared with PLGA 53/47 + 0–15% PEG 4 K or PPG 4 K in 3 ml of DCM. For example, a 5% PEG 4 K/PLGA solution was dissolved in 3 ml DCM overnight with 425 mg of PLGA 53/47 and 25 mg of PEG 4 K. Film applicator height was set at 500 μm and the viscous solution was cast onto Teflon-coated glass plates at an applicator speed of 50 mm s^{-1} at RT in a fume hood. DCM was evaporated at RT for 24 h followed by placing in a vacuum oven at 55 °C for 2 days. The dried 40–50 μm thin films were sliced into rectangular strips (8 × 1 cm) according to ASTM D882 [28]. Each rectangular film was fixed to Instron Model 5567 rubber-coated grips with a load cell capacity of 10 N, pulled at rate of 5 mm min^{-1} (10% min^{-1}) and analyzed with Bluehill software version 3.00. The modulus and elongation at break were prepared and characterized perpendicular to the casting direction in pentaplicate. No isotropic effects on the mechanical properties were investigated.

3. Results

3.1. Casting of films

During our initial trials of gradient casting, we observed that the polymer solution viscosity was a particularly important factor in obtaining reproducible gradients. The more viscous solution, 15% w/v PLGA 100 K (complex viscosity, η*, of 8.6 ± Pa s, similar to honey) had to be placed in Chamber B (see Fig. 1 and Supplementary Video 1) for consistent mixing. Solutions of 15% PEG 4 K or PPG 4 K (Chamber A) were ~10–100 times less viscous. Gradient casters employed in polyacrylamide gel electrophoresis use magnetic stir bars in Chamber B mixing. This was not practical for the present study since it was not convenient to use a magnetic stir plate with the gradient caster mounted to the knife caster. In addition, the mixing of viscous polymer solutions is often problematic with magnetic stir bars. To achieve polymer mixing, a domestic battery operated "milk frother" was adapted as a miniature overhead stirrer (see Supplementary Video 1). It must be noted that the battery-operated "milk frother" is only suitable for nonflammable solvents, such as DCM. As the gradient solution exited the gradient caster, it poured directly into the film applicator, which spread the wet film 80 mm wide, and 0.5 mm thick on polyethylene teraphthalate films.

3.2. Limitations on linear gradients

The viscosity of the additives was the limiting factor on the linear concentration range generated. After 100 cm of gradient casting, the additives PEG 4 K and PPG 4 K concentration was greater than 50%. The thinned gradient solutions produced at these additive concentrations were not containable within the film applicator (see Supplementary Video 1 at 90–100 cm). This affected film thicknesses, as they were no longer uniform, and other characterization results were erratic as well, i.e. ^{1}H NMR, controlled drug release. Generally, films lengths longer than 100 cm (>50% additive) were discarded.

3.3. Polymer composition by quantitative ^{1}H NMR analysis

Polymer constituents within the gradient films were determined by quantitative NMR, as outlined by Rizzo and Pinciroli [29]. With tetramethylsilane as the internal standard, PLGA 100 K, PEG 4 K, PPG 4 K, and FDAc were determined within a 5–8% degree of error (see Section 2 for error calculation). COU displayed ^{1}H NMR peaks with low S/N ratios, and was therefore determined by fluorescence quantitation after total dissolution of the films in acetone. Figs. 2A–5A display the individual compositions of the gradients films with respect to length. Polymer trends were as expected; PLGA 100 K started out at 4000–4500 μg cm^{-2} and decreased with an increase in linear length, whereas additives increased from 0 to 2000 μg cm^{-2} after 90–100 cm. Ratios of the fluorescent drug mimics/total polymer (PLGA 100 K + additive) were kept at ~2.1% for both PLGA 100 K and PEG 4 K (or PPK 4 K) in both gradient casting chambers to keep the drug percentage the same across all gradients. This ranged from 100 to 70 μg cm^{-2} drug amounts from 0 to 100 cm length, respectively.

3.4. Percentage of additive and thickness with respect to gradient length

Figs. 2B–5B display both the percentage of additive and thickness of the films with respect to gradient length. Generally, the percentage of additive was linear ($R^2 \geqslant 0.95$) with respect to gradient length after the chamber A valve was opened and mixing was initiated. Analysis of the slope demonstrates that the percentage increased by 0.6 ± 0.1% cm^{-1} for all gradients made. An exception to this observation was the PLGA 100 K/PPG 4 K/COU gradient (Fig. 4B), where the gradient reaches a plateau after 50 cm of 36% PPG 4 K. This was attributed to gross phase separation of the PPG 4 K film after ~40% w/w mixing. PPG 4 K, which is a liquid at room temperature, decreased after 50 cm as the phase separated liquid likely had some evaporation in the vacuum oven. This was apparent in the films as well, as they took on a heterogenous appearance. Thickness measurements were estimated by the ^{1}H NMR composition data combined with the known densities of PLGA 100 K, PPG 4 K, and PEG 4 K. The volumes of the individual components were calculated from the ^{1}H NMR integrations and their known densities. With the exact surface areas known from the 6 mm punch-outs, the thickness could then be estimated. These values never varied more than ±10% of measurements made with a micrometer screw gauge. While this method ignores partial molar volume effects, the slight decrease in accuracy is justified by the ease in computation, savings in labour, and decreased variability from operator error. Thickness of the films decreased over the gradient length,

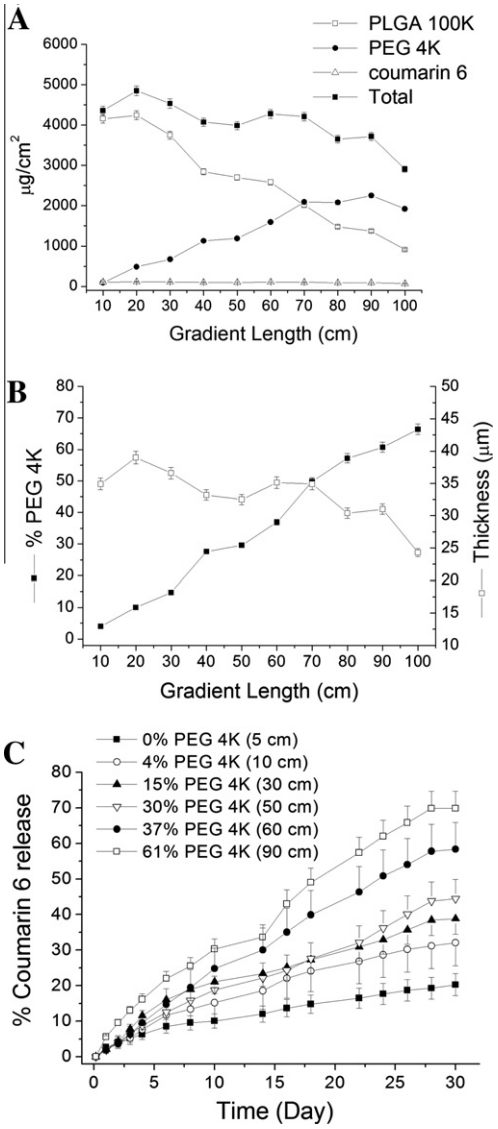

Fig. 2. PEG 4 K and COU. (A) Thin film composition by gradient length. (B) Percentage of PEG 4 K additive and thickness of film. (C) Controlled release of COU from 0 to 61% additive. Some error bars have omitted for clarity.

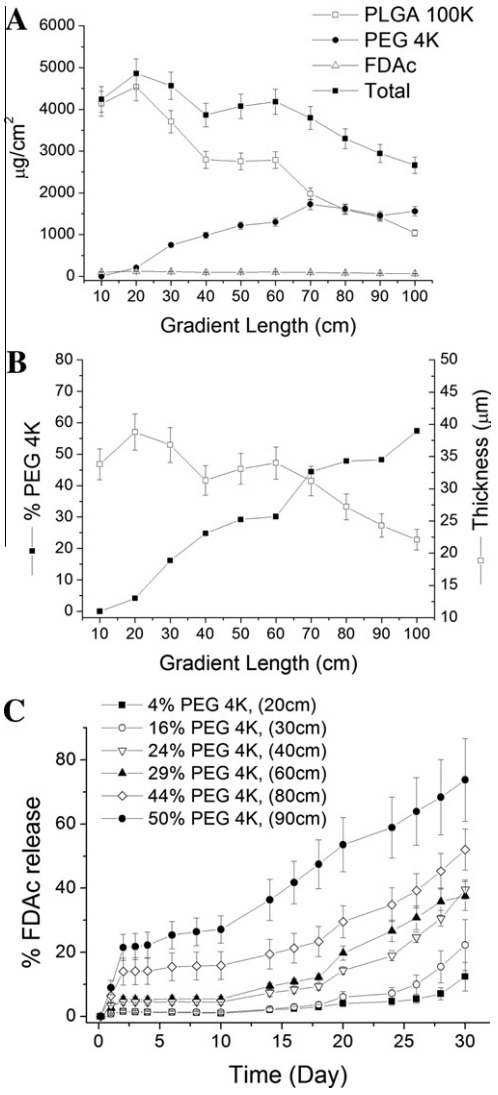

Fig. 3. PEG 4 K and FDAc. (A) Thin film composition by gradient length. (B) Percentage of PEG 4 K additive and thickness of film. (C) Controlled release of FDAc from 0 to 50% additive.

which was associated with the thinner viscosities of the gradient solutions as additive concentration increased and PLGA 100 K decreased.

3.5. Controlled release of PLGA 100 K/PEG 4 K thin films: COU vs, FDAc release

The gradient caster combined with film applicator allowed 0 to 50% PLGA 100 K/additive films to be synthesized in increments of less than 1% additive cm^{-1}. This potentially provides ~50 films of varying PLGA/additive content in a single casting. This provided an extensive range for determining additive effects on controlled drug release. To limit band broadening deviations in additive percent from the applicator (perpendicular to the casting direction) at the film edges, all samples taken from a 4×1 cm (width $\times$ length) box centered at the gradient length being characterized. At every 5–10 cm gradient length, eight 6 mm diameter punchouts were collected. Five of the samples were used for controlled drug delivery in a high-throughput screening assay within 96-well plates [27]. Fig. 2C displays the COU release kinetics at several additive concentrations for the PLGA 100 K/PEG 4 K gradient. With no burst release, this gradient yielded the most linear drug release ($R^2 > 0.95$ for all plotted), ranging from $0.55 \pm 0.02\%$ day^{-1} @ 0% PEG 4 K to $2.21 \pm 0.07\%$ day^{-1} @ 61% PEG 4 K. As an estimate, 1% drug $day^{-1} \approx 1$ µg drug cm^{-2} day^{-1}, FDAc exhibited a dissimilar release profile than COU (see Fig. 3C), which is likely to be due to FDAc's non-polar profile in comparison to COU's polar amine group. Up until 30% PEG 4 K additive, little burst release of FDAc was found, whereas greater than 30% additive, burst release was apparent. This was followed by a lag effect for 10 days, then increasing release as the PLGA 100 K gradually underwent bulk degradation [30–32]. Burst release after 30% additive was likely due to phase separation of the PEG 4 K within the PLGA 100 K matrix, as previously reported [23,25]. Fig. 6A displays the total release of drug after 30 days vs. percentage PEG 4 K additive. This analysis displays how COU was released faster under most circumstances than FDAc. Pearson's correlation coefficient, r, was >0.93 for both the FDAc and COU PLGA 100 K/PEG 4 K gradients, indicating a near-perfect positive correlation between additive percentage and total release over 30 days. The linear fit predicts the total release for both drugs (after 30 days) as a function of additive percentage. Interestingly, the linear fits intersect, predicting that at 37% PEG 4 K, the two drugs

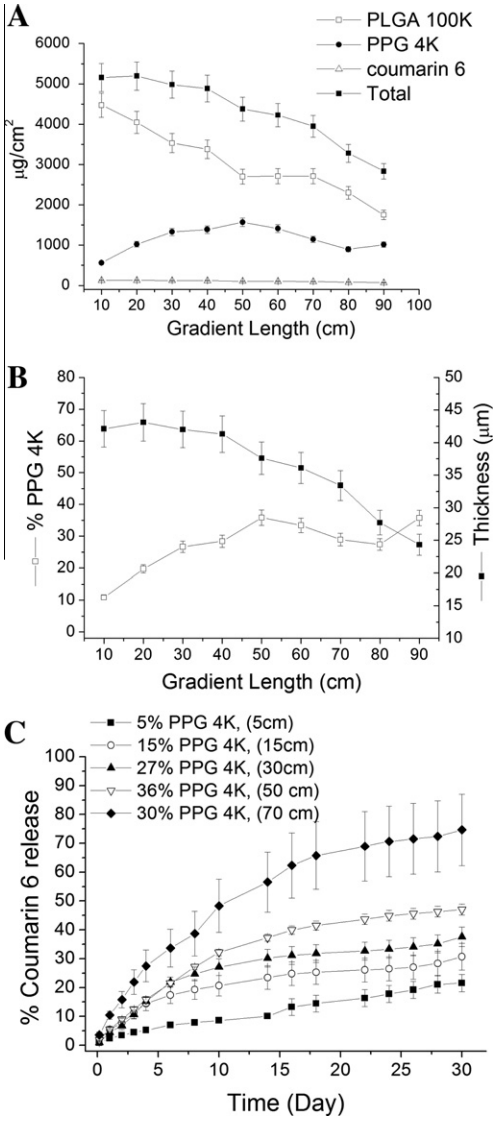

Fig. 4. PPG 4 K and COU. (A) Thin film composition by gradient length. (B) Percentage of PEG 4 K additive and thickness of film. (C) Controlled release of COU from 0 to 50% additive.

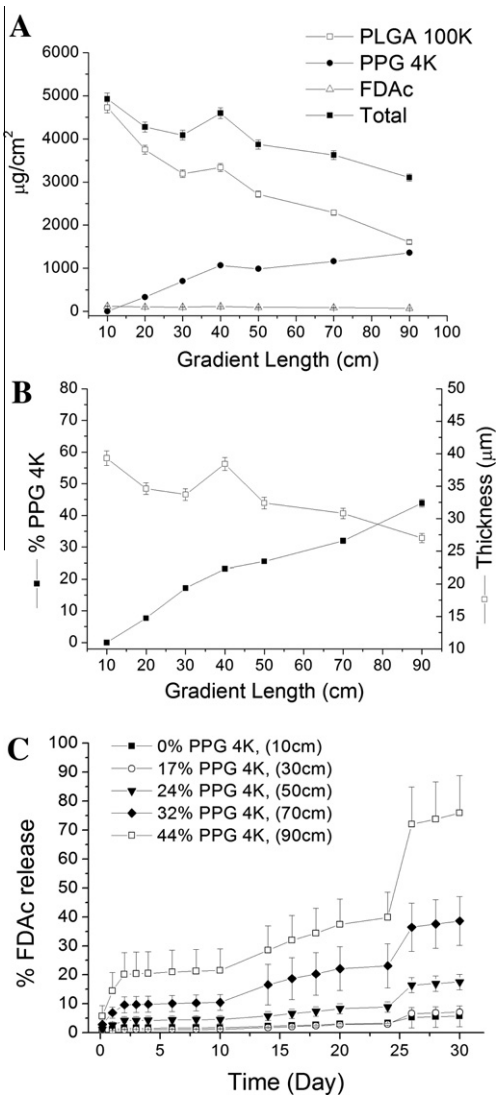

Fig. 5. PPG 4 K and FDAc. (A) Thin film composition by gradient length. (B) Percentage of PEG 4 K additive and thickness of film. (C) Controlled release of coumarin 6 from 0 to 44% additive. Some error bars have been omitted for clarity.

would have 54% release after 30 days, assuming similar release conditions. Similar analyses could be performed at any time point in the release experiment to estimate what additive concentration would be required for a sought-after cumulative release.

3.6. Controlled release of PLGA 100 K/COU thin films: PEG 4 K vs. PPG 4 K

When incorporating additives for tuning the material properties, having a technique to monitor trends is paramount. Ideally, the concentration of additive has a high correlation to the optimized parameters, moreover the less additive needed, the better. Generally, as more is incorporated, the resulting material properties will vary substantially to the expected trend of the addition. The controlled release of COU was compared across the two additives, PEG 4 K and PPG 4 K, as seen in Figs. 2C and 4C. Functionally, less PEG 4 K additive was needed for the same amount of total release after 30 days vs. that of PPG 4 K. For example, to achieve 40% release of COU over 30 days, the linear fits in Fig. 6B predicts a requirement of 16% of PEG 4 K vs. 35% of PPG 4 K. In this analysis, only additive percentages, with no visual phase separation (<40% for the PPG 4 K/COU gradient), are accounted for. Phase separation of the PPG 4 K films had a more profound impact on the PLGA 100 K release properties. In the PLGA 100 K/PPG 4 K gradient (Fig. 4C) a higher release at the 70 cm film was observed despite ^{1}H NMR calculating a lower additive concentration than earlier gradient positions (Fig. 4B). Gradient lengths longer than 60 cm yielded heterogenous films for both PLGA 100 K/PPG 4 K gradients. Since PPG 4 K was a liquid at RT, phase separation has more consequences on the controlled drug release than that of PEG 4 K, which simply forms solid amorphous and crystalline PEG 4 K domains [2]. In addition, the liquid PPG 4 K was more likely to evaporate in the vacuum oven, and thus give large standard deviations in controlled release (Fig. 4C at 70 cm) and display erratic bursts of release (Fig. 5C at 70 and 90 cm). The abrupt jumps in FDAc release at 10–14 days and 25–27 days was likely due to the PPG 4 K and FDAc phase separating together (See Fig. 5C). Such jumps in release were previously for seen high concentrations of PEG and paclitaxel that were phase separated together in PLGA films as well [2].

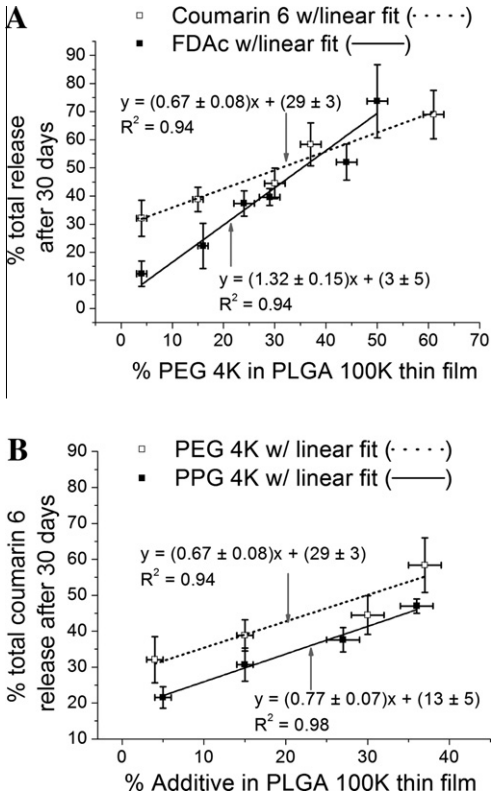

Fig. 6. Statistical comparison between (A) two drug compounds in a similar thin film matrix and (B) COU in PLGA 100 K thin films with two different additives.

3.7. Mechanical properties of PLGA 4 K thin films with PEG 4 K and PPK 4 K additives

PLGA 100 K thin films were incorporated with 5, 10, and 15% PEG 4 K and PPG 4 K. The mechanical properties were assessed according to ASTM standard D882 [28]. Figure 7A displays the typical stress vs. strain curves for PEG 4 K. Addition of low molecular weight PEG additive acted as an effective plasticizer for the PLGA 100 K films – the addition of 5% PEG 4 K decreased the elongation before break and modulus by ~40% as shown in Fig. 7B and C. Our previous results have revealed that the changes in elongation and modulus are dependent on the PEG MW – higher molecular weights decrease the elongation, but raise the modulus [2]. In this manuscript, we chose a lower MW PEG (4 kDa), as no phase separation with the PLGA matrix was noticed upto 1:1 PEG:PLGA ratios. In contrast, 8 and 35 kDa phases separate much lower than 1:1 PEG:PLGA ratios, causing heterogeneous films and errant mechanical properties [2].

As displayed, increasing percentages of PEG 4 K tended to restore the elongation of the neat PLGA, but severely reduced the modulus. The PPG additive displayed a different profile. The addition of 5% PPG produced brittle PLGA 100 K thin films that were difficult to handle, and also significantly increased the modulus over the original neat PLGA film ($P > 0.95$), as seen in Fig. 7C. Increasing the PPG 4 K to 15% w/w restored some of the elongation, but continued to raise the modulus overall.

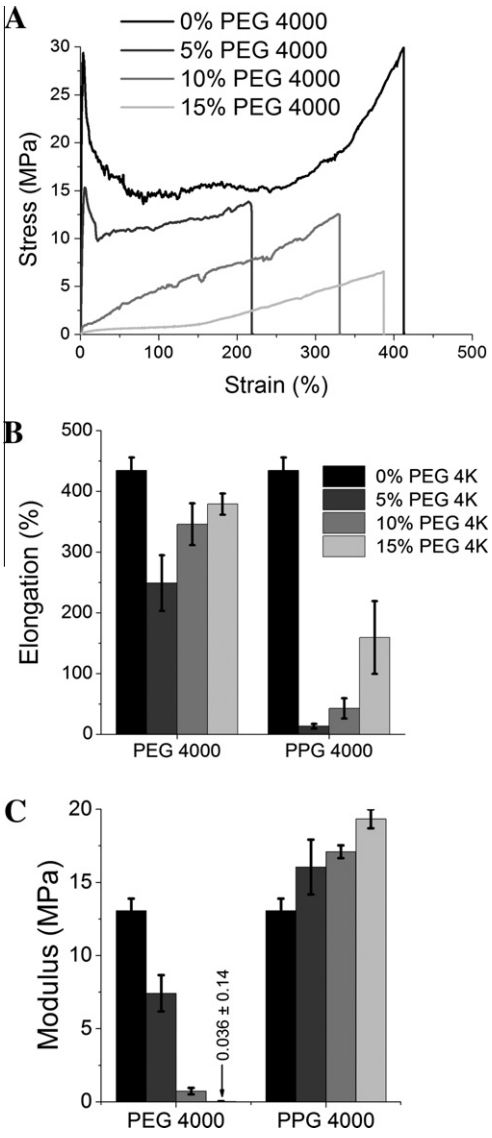

Fig. 7. Mechanical properties of PLGA 100 K/PEG 4 K thin films. (A) Stress vs. strain curves of PLGA 100 K films with 0, 5, 10, and 15% PEG 4 K. Comparison of (B) elongation percentage and (C) modulus of PLGA 100 K films with 0, 5, 10, and 15% PEG 4 K and PPG 4 K.

4. Discussion

4.1. High-throughput rationale

Herein we have described a method to make PLGA thin films that incorporate an increasing linear gradient of any matrix-soluble additive. Such a method offers higher throughput in optimizing thin film properties by quickly identifying trends with the production of a single film. This procedure would benefit applications where blending of PLGA or other suitable thin films are currently under investigation, such as optimizing a cell attachment material [33], miscibility studies [34], incorporation of nanoparticles [35,36] or surface modification [37]. Where controlled drug release is concerned, it was found that our previously developed protocol for high-throughput screening paclitaxel with the mimic FDAc provided a synergistic productivity when combined with the gradient thin films [27]. For example, if one is limited to small amounts of labelled paclitaxel (radio-, fluoro-, etc.) that need to be released at specific rates, initial screening can be combine with the gradient method described here, with the use of FDAc in place of the paclitaxel.

4.2. Benefits towards controlled drug release R&D

Concerning controlled drug release, the gradient method described offers high-throughput results for three specific objectives:

(i) effects of additives, shown by example in this paper, (ii) determining matrix solubility and release kinetics of drugs within predetermined thin film matrices, and (iii) optimizing thin films where formulations needing three or more excipients are needed.

The study of gradient films, for example, where two or more controlled drug releases are needed simultaneously from the same matrix, will be much more convenient when employing this method. Such dual release paradigms are investigated for increased bioavailability [38], synergistic tumour treatments [39], and cardiovascular medical devices [40]. By producing the films in gradients, trends can be rapidly identified and analyzed, such as that shown in Fig. 6A. These trends can then predict when two drugs are likely to have the same cumulative release, hence the worker has control over the release time wanted, the matrix concentration chosen, and the dosage of drug. This assumes, though, that neither drug would affect the others release and controlled release has a known dependence on amount of drug loading. In the case of the latter, several researchers provide empirical evidence to support this under certain conditions [41–43].

4.3. PEG 4 K and PPG 4 K additives in PLGA 100 K

While optimizing the gradient casting procedure, many common additives were assessed. These included polyester wax (better known as carbowax), 25 kDa branched polyethylenimine, glycerol, and triethyl citrate. Ultimately, they failed due to gross phase separation, amine-catalyzed destruction, or no clear benefit to controlled drug release (glycerol and triethyl citrate), respectively (data not shown). Polyethylene glycol and polypropylene glycol both displayed the ability to tune the controlled release of two hydrophobic drugs COU and FDAc.

PEG and propylene glycol are often used as plasticizers, while also providing increasing rates of drug release for drug-dosing patches or thin films [44–46]. Plasticizers are dispersants utilized to increase the fluidity or flexibility of polymers. The PEG 4 K exhibited the typical properties expected; a decrease in modulus (Fig. 7C) with an increase in drug release (Figs. 2C and 3C). PPG 4 K displayed similar release properties, yet had remarkably different mechanical properties. The PPG dispersant acted as a stiffener, making the films difficult to peel from the Teflon coated plates without exhibiting brittleness. With the use of PEG–PPG–PEG block polymers or "poloxamers" (aka Pluronic®), one should be able to tune the mechanical properties of PLGA 100 K between those displayed for PEG 4 K and PPG 4 K.

As mentioned, PEG 4 K and PPG 4 K both modulate drug release in a similar fashion, yet have very different effects on material properties. In our previously published manuscript, we noted that PEG had little to no impact on the PLGA degradation, as measured by MW vs. time [2]. At day 0, the PLGA MW was ~100 kDa before and after casting, which drops to ~40 kDa after day 10, 20 kDa by day 20, and under 10 kDa after day 30.

Alternatively to poloxamers, the PEG and PPG additives could be used simultaneously to control drug release, along with the extra ability to modulate the mechanical properties. This should allow medical devices to be constructed to a specific release rate with defined plasticity/stiffness. The high-throughput casting method described would indeed aid the optimization of such a combination of additives.

4.4. Coumarin derivatives in PLGA 100 K/PEG 4 K or PPG 4 K matrices

Based on the near zero-order release results found with the COU fluorescent dye, one can speculate that the many coumarin derivatives may be suitable for encapsulation into PLGA 100 K/PEG 4 K or PLGA 100 K/PPG 4 K matrices; as Fig. 6B suggests it can be "tuned" for specific release kinetics. Many drugs are derivatives of the coumarin pharmacocore, sharing similar chemical properties. Coumarin derivatives are a popular drug scaffold and are continually being developed into more potent drugs; indeed, they are already well known in their role as vitamin K antagonists, such as the anti-coagulants Warfarin and Tioclomarol, but also exist as antibiotics (Novobiocin, amino-coumarin), and anti-aggressive drugs (Batoprazine) [47–49].

5. Conclusions

A novel method of gradient casting PLGA thin films has been presented that allows trends in controlled drug release and other material properties to be identified in a high-throughput manner. The effectiveness of the approach was demonstrated with one well known biodegradable polymer (PLGA), two common additives (PEG and PPG 4 K), and two fluorescent molecules that mimic the properties of some hydrophobic drugs. Numerous additives for PLGA 53/47 (intrinsic viscosity of 1.03 dl g^{-1}) matrices were attempted. Of these PEG 4000 and PPG 4000 were able to have a positive correlation coefficient when comparing concentration and release of drug-like fluorescent molecules of FDAc and COU. Plots of additive concentration vs. percentage total release for any given time period allow one to make predictions of matrices with two eluting fluorescent drugs or compare additives against one another to optimize material properties. Despite increasing rates of drug delivery, both additives had unique material properties when incorporated into PLGA thin films – PEG 4 K was found to be a typical plasticizer and PPG 4 K tended to stiffen the films.

Appendix A. Figures with essential colour discrimination

Certain figures in this article, particularly Fig. 1, is difficult to interpret in black and white. The full colour image can be found in the on-line version, at doi:10.1016/j.actbio.2012.01.014.

Appendix B. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.actbio.2012.01.014.

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