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European Journal of Pharmaceutics and Biopharmaceutics 68 (2008) 82–89
European Journal of Pharmaceutics and Biopharmaceutics
www.elsevier.com/locate/ejpb

Research paper

# Controlling protein release from scaffolds using polymer blends and composites

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Received 23 November 2006; accepted in revised form 24 May 2007
Available online 18 July 2007

**Abstract**

We report the development of three protein loaded polymer blend and composite materials that modify the release kinetics of the protein from poly(DL-lactic acid) ($P_{DL}LA$) scaffolds. $P_{DL}LA$ has been combined with either poly(ethylene glycol) (PEG), poly(caprolactone) (PCL) microparticles or calcium alginate fibres using supercritical $CO_2$ ($scCO_2$) processing to form single and dual protein release scaffolds. $P_{DL}LA$ was blended with the hydrophilic polymer PEG using $scCO_2$ to increase the water uptake of the resultant scaffold and modify the release kinetics of an encapsulated protein. This was demonstrated by the more rapid release of the protein when compared to the release rate from $P_{DL}LA$ only scaffolds. For the $P_{DL}LA$/alginate scaffolds, the protein loaded alginate fibres were processed into porous protein loaded $P_{DL}LA$ scaffolds using $scCO_2$ to produce dual release kinetics from the scaffolds. Protein release from the hydrophilic alginate fibres was more rapid in the initial stages, complementing the slower release from the slower degrading $P_{DL}LA$ scaffolds. In contrast, when protein loaded PCL particles were loaded into $P_{DL}LA$ scaffolds, the rate of protein release was retarded from the slow degrading PCL phase.


*Keywords:* Tissue engineering; Scaffolds; Protein delivery; Growth factors; Supercritical fluid

## 1. Introduction

Many tissue engineering strategies involve the use of a biodegradable, porous scaffold fabricated from a synthetic polymer to act as a support for the growth and proliferation of cells [1]. However, in addition to their traditional role as cell supports, many emerging strategies utilise these synthetic scaffolds as carriers for the controlled delivery of protein growth factors that can induce tissue regeneration by instigating and controlling cellular processes [2–4]. There are a number of challenges that must be overcome in order to achieve controlled release from these highly porous structures. For example, it is desirable for any carrier device to be able to mimic the protein release profile and kinetics found *in vivo* [5]. To complicate matters, the desired protein release kinetics may differ dependent upon the growth factor(s) used and ultimately the application or target tissue [6]. As a result, some degree of flexibility in the release kinetics of growth factors is highly desirable in any polymeric device.

One of the main challenges in controlling release kinetics lies in how best to manipulate the mechanisms of protein release from the polymer matrix. Synthetic polymer scaffolds normally control the release of proteins by two different methods. Initially, the protein diffuses through the porous matrix as the scaffolds take on water from its environment causing the most exposed surface protein to be washed out (known as the burst phase). This burst phase is characteristically followed by a lag phase before the polymer starts to degrade, causing the release of the

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doi:10.1016/j.ejpb.2007.05.023

remaining protein at a rate dependent upon the speed of polymer degradation. However, synthetic polymers such as poly(DLlactic acid) ($P_{DL}LA$) are hydrophobic, slowing the rate of water ingress and taking up to 6 months to degrade slowing the release of any entrapped proteins [7]. Therefore, the addition of hydrophilic polymers has been suggested as a method of increasing both the water uptake and degradation rate of polymers, thus enhancing the dissolution of drugs [8]. Several methods have been used to achieve this by incorporating a hydrophilic component into the PLA material. These include: the high temperature plasticisation of PLA with poly(ethylene glycol) (PEG) [9], co-polymerisation of poly(L-lactide) ($P_LLA$) and PEG [10] and chemical modification through photo-grafting of the hydrophilic component to the PLA material [11]. However, the use of high temperature, solvent and chemical modification techniques to combine polymers is not compatible with labile proteins, thus preventing the incorporation of protein growth factors into either the polymer blending or scaffold fabrication step. In addition, the use of solvents and toxic chemicals in scaffold fabrication should be minimised due to potentially high residues being left in the final scaffold [12].

One method of plasticising and blending two polymers without the use of high temperatures or toxic solvents is by using supercritical $CO_2$ ($scCO_2$) processing. When raised above its critical parameters (73.8 bar, 31.1 °C) $scCO_2$ can dissolve into the amorphous regions of polymers such as $P_{DL}LA$ plasticising them at low temperatures in a solvent-free environment. Upon decompression, the $CO_2$ bubbles nucleate to form pores that become permanent as the polymer solidifies. This has permitted the incorporation of not only proteins [13,14,2,3] but also mammalian cells [15] into the scaffold fabrication step. Because at low molecular weights PEG either exists as a liquid or has a low melting point, it can be blended into the $P_{DL}LA$ material along with the protein in a single $scCO_2$ processing step without the use of high temperatures or solvents. Therefore, we propose the use of a novel polymer blending method to incorporate PEG into $P_{DL}LA$ scaffolds in order to increase the hydrophilicity of the scaffolds and modify protein release kinetics.

Whilst providing a potential solution to the modification of release kinetics for a single protein, the $P_{DL}LA$/PEG blend would only exist as a single phase system and therefore is not capable of controlling the release of two separate proteins. It may be that a number of growth factors are needed at different stages throughout the regeneration process, requiring the independent control of two or more growth factor types. For example, for the regeneration of skin, fibroblastic growth factor (FGF) and keratinocyte growth factor (KGF) are required for cell proliferation, whilst VEGF is required to instigate angiogenesis [16]. It is also considered that the dual release of both transforming growth factor-β (TGF-β) and insulin-like growth factor-1 (IGF-1) could induce the proliferation of chondrocytes and the synthesis of type II collagen, respectively, in the repair of articular cartilage [17]. Combined delivery of bone morphogenetic protein 2 (BMP-2) and IGF-1 from gelatine coatings showed that varying temporal delivery of multiple growth factors significantly affected cell response [18,19]. In the first *in vivo* study of its kind, the dual release of two separate growth factors from the same scaffold, each with its own distinct release kinetics, was able to both induce and sustain angiogenesis leading to the formation of mature blood vessels [20]. In this example a PLGA scaffold containing vascular endothelial growth factor (VEGF) was combined with PLGA microparticles containing platelet-derived growth factor (PDGF) to provide dual growth factor release from the same structure. Whilst the initial release of VEGF instigated the angiogenic process, the additional release of PDGF leads to the maturation of the blood vessels. This study highlights the importance of dual growth factor release although it was limited by the use of only a single polymer, poly(lactic-co-glycolic acid) (PLGA).

We hypothesised that instead of using a single polymer in two different forms to entrap the proteins, two separate polymers with totally different properties could be combined to form a dual release scaffold with distinct protein release kinetics. We have tested this hypothesis by combining $scCO_2$-fabricated protein loaded $P_{DL}LA$ scaffolds with either; cross-linked protein-entrapped alginate fibres to speed up protein release or, slow degrading poly(caprolactone) (PCL) protein loaded microparticles to retard protein release. A calcium alginate/$P_{DL}LA$ composite material fabricated in $scCO_2$ has already been demonstrated to modify the release of alginate-entrapped bovine serum albumin (BSA) [21]. This work utilised a novel ion-exchange surface entrapment method to incorporate the protein into the alginate fibres before mixing them with $P_{DL}LA$ powder and subsequent composite scaffold fabrication in $scCO_2$ [22]. PCL is a semi-crystalline polymer that has been used in numerous biomedical applications due to its low toxicity, biodegradability and mechanical properties [23]. It is suitable for this study as in contrast to alginate, it degrades slowly due to the tight packing of its crystalline structure, slowing water ingress and any subsequent protein release [24]. Furthermore, the PCL used in this study is not plasticised under the same $scCO_2$ conditions used to plasticise the $P_{DL}LA$ material, and thus the solvent-entrapped protein is maintained within the microparticles during the processing step.

## 2. Methods

### 2.1. Preparation of $P_{DL}LA$/PEG blends and protein loaded scaffolds using supercritical $CO_2$

$P_{DL}LA$ with a weight average molecular weight (MW) of 52,000 Da (10 g, Purac, The Netherlands) was placed inside a stainless steel 60 ml pressure vessel along with either PEG 400, PEG 750 or PEG 2000 (Sigma, UK) in sufficient quantities to make a final ratio of 1% w/w and 5% w/w PEG. BSA powder (1 g; Sigma, UK) was added to each mixture

inside the vessel and pressurised with $CO_2$ (200 bar, 35 °C) (food grade; Cryoservice, UK) to plasticise the polymers. The contents of the vessel were then mixed with a paddle stirrer for 3 h at this pressure before decompression of the vessel and removal of the blended material. To confirm that blending of the two polymers had taken place, the glass transition temperatures of both pure $P_{DL}LA$ and the PEG/$P_{DL}LA$ blended samples (without protein) were measured using a Perkin-Elmer DSC7 calorimeter. The DSC scans were carried out at a heating rate of 6 °C a minute under a nitrogen gas flow on samples weighing approximately 5 g. The $P_{DL}LA$/PEG/protein blends were then re-processed inside custom-made PTFE moulds for 1 h in $scCO_2$ (200 bar, 35 °C). After a 1-h decompression cycle, the porous, protein loaded scaffolds were removed and the non-porous skin cut away before incubation in a phosphate-buffered saline solution (pH 7.13) at 37 °C (5% $CO_2$).

### 2.2. Bradford assay for BSA release from $P_{DL}LA$/PEG scaffolds

To measure protein release from the $P_{DL}LA$/PEG composite scaffolds the Bradford protein concentration was used [25]. The BSA containing scaffolds were suspended in 2 ml Tris buffer (25 mM containing 25 mM NaCl and 25 mM HCl) and incubated at 37 °C. Scaffolds were removed from the incubator at time intervals and a small sample taken (10 μl in triplicate) and added to a 96-well plate. The Bradford reagent (200 μl) (Bio-Rad, UK) was then added to each well before incubating the plate in the dark at room temperature for 15 min. Absorbance was measured at 595 nm after shaking for 30 s and the data compared to known protein standards between 0.01 and 0.5 mg/ml to calculate the concentration of protein in the sample.

### 2.3. Water uptake measurement in $P_{DL}LA$/PEG blended scaffolds

Water uptake was assessed by submerging the $P_{DL}LA$/PEG (1% w/w and 5% w/w PEG) blended scaffolds in water for 1 h at room temperature (24 °C). The scaffolds were first weighed and then submerged in 10 ml of distilled water for between 0 and 150 h before blotting on filter paper and re-weighing. The net change in weight was taken as the level of water uptake within the scaffolds.

### 2.4. Fabrication of $P_{DL}LA$/alginate composite dual release scaffolds

$P_{DL}LA$/alginate composite scaffolds were loaded with a solution of ribonuclease (Sigma, UK) and horseradish peroxidase (HRP) (Sigma, UK) by combining both a $scCO_2$ mixing technique and an alginate entrapment technique as previously described [13,22]. Briefly, a ribonuclease-rich solution was added to $P_{DL}LA$ powder to give 13 mg of protein per 130 mg of polymer and the mixture freeze-dried overnight to remove the water. Alginate fibres (Advanced Medical Solutions, UK) of approx. 1 mm in thickness were pre-swollen in a sodium-rich NaCl/$CaCl_2$ solution (NaCl/$CaCl_2$ ratio 20:1, NaCl concentration 10% w/v) for 5 min before exposure to a 5-mg/ml solution of HRP for 30 min. The fibres were then immersed in a barium chloride solution (5% w/v) for 15 min, followed by washing with distilled water and drying in air at room temperature. The HRP loaded fibres were then cut into fibrils of 1–2 mm in length, mixed with the $P_{DL}LA$/ribonuclease powder (13 mg alginate per 130 mg $P_{DL}LA$) and processed using $scCO_2$ (200 bar/35 °C) for 1 h in a clamp-sealed 60 ml pressure vessel. After a 1-h decompression cycle the scaffolds were taken out of the pressure vessel and the non-porous skin removed before assay.

### 2.5. Fabrication of $P_{DL}LA$/PCL composite dual release scaffolds

Protein loaded (HRP) PCL microparticles were fabricated by using a single emulsion solvent entrapment technique. In order to provide sufficient particles for 10 scaffolds, PCL (130 mg; MW 60,000 Da; Sigma, UK) was dissolved in dichloromethane (DCM) (2 ml) before the addition of 500 μl of a DCM solution, containing PEG 400 (30% w/v) and vortex mixing for 10 s. HRP powder (6.5 mg; Sigma, UK) was dissolved in 500 rmul of the DCM/PEG400 solution (containing 20 μl of FITC; Molecular Probes, UK) and added to the dissolved polymer before vortex mixing for a further 10 s. A solution of poly(vinyl alcohol) (PVA) (0.3% w/v) was then added to the protein/polymer solution before vortex mixing for 20 s. An additional 100 ml of the 0.3% PVA solution was then added before a 4-h evaporation process to remove the DCM. The particles were filtered and washed three times to remove any excess PVA before freeze drying overnight to remove any moisture. As previously described, a ribonuclease-rich solution was also freeze-dried overnight with $P_{DL}LA$ powder to give 13 mg of protein per 130 mg of $P_{DL}LA$. The HRP loaded microparticles were then mixed with the $P_{DL}LA$-ribonuclease powder (13 mg particles per 130 mg $P_{DL}LA$) and processed using $scCO_2$ (200 bar/35 °C) in a clamp-sealed 60 ml pressure vessel for 1 h. After a 1-h decompression cycle the scaffolds were taken out of the pressure vessel and the non-porous skin removed before assay.

### 2.6. Assay for horseradish peroxidase (HRP) release from composite scaffolds

To measure protein activity and thus release from the $P_{DLLA}$/alginate and $P_{DL}LA$/PCL composite scaffolds, a simple colorimetric activity assay for HRP was used. The HRP containing scaffolds were suspended in 2 ml Tris buffer (25 mM containing 25 mM NaCl and 25 mM HCl) and incubated at 37 °C. Aliquots (10 μl) of buffer solution were taken from each vial at set time intervals and were added to a 96-well plate in triplicate. To induce the colour change

3,3′,5,5′-tetramethylbenzidine supersensitive ELISA (100 μl; Sigma, UK) substrate was added to each well before 2 M NaOH (100 μl) was added to stop the reaction. Absorbance was measured immediately at $\lambda$ 450 nm on a Lucy 1.0 plate reader (Anthos). The amount of HRP within the buffer was determined using a standard curve of known concentrations of HRP (0–100 μg/ml) versus average absorbance (nm).

### 2.7. Assay for ribonuclease release from composite scaffolds

Ribonuclease activity was measured by a UV spectrophotometric method previously described [14]. In brief, 10-μl samples of Tris buffer (25 mM containing 25 mM NaCl and 25 mM HCl) were removed from the scaffolds at set time points and added to 2.5 ml of the cytidine-2′,2′-monophosphate substrate (0.1 mg/ml in distilled $H_2O$) (Sigma, UK). The rate of substrate conversion to cytidine-3′-phosphate was measured by the change in absorbance at 284 nm. The values for ribonuclease activity were compared to the activity of known concentrations (0.01–1 mg/ml) in order to calculate the % loading of the protein released.

### 2.8. Scanning electron microscopy (SEM)

To prepare the samples for SEM, the composite scaffolds were gold coated using a Balzers Union SCD030 sputter coater, before being scanned at 20 kV with a Philips 505 scanning electron microscope to view the pore structure of the $P_{DL}LA$-based scaffolds and confirm the presence of the alginate fibres in the $P_{DL}LA$/alginate composite scaffolds. The size of the PCL particles was determined using SEM by measuring the diameter (at the widest point) of 100 particles taken at random and calculating a mean value.

## 3. Results and discussion

### 3.1. $P_{DL}LA$/PEG blended scaffolds

In order to achieve an efficient mixture of $P_{DL}LA$ and PEG, the two polymers were blended under $scCO_2$ conditions. To confirm that blending had been achieved the glass transition temperature ($T_g$) of the foamed composite materials was measured using differential scanning calorimetry (DSC). The downward shift in $T_g$ seen in the $P_{DL}LA$/PEG composite material when compared to the $P_{DL}LA$ material (Fig. 1a) indicates that the PEG 400 has blended with $P_{DL}LA$. In agreement with the previous literature, this plasticising effect was also shown to be controllable by altering either the PEG content or molecular weight (Fig. 1b) [9]. By decreasing the molecular weight or increasing the content of PEG in the blend, the $T_g$ is shown to fall further as the plasticising effect of the PEG is increased. The pore structures of the $P_{DL}LA$/PEG blends (1% and 5% PEG 2000) were then observed and compared by

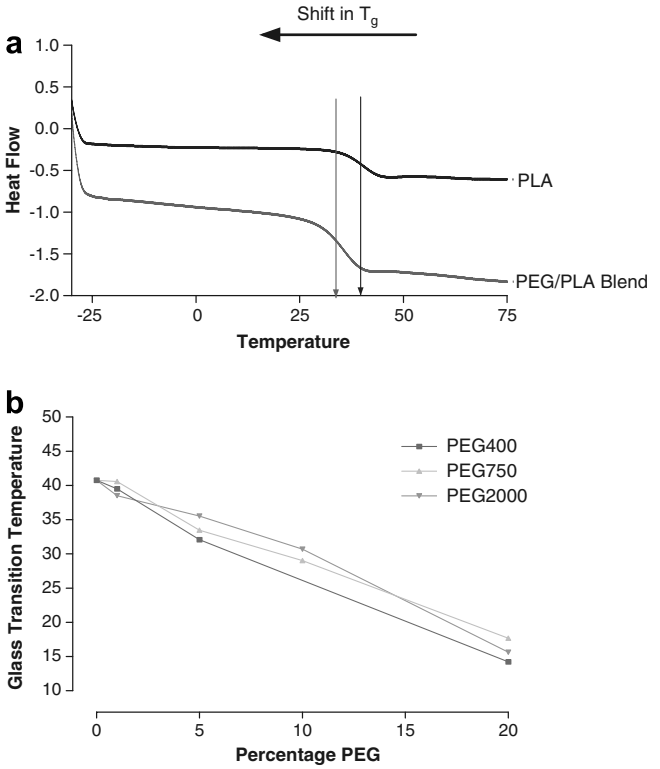


Fig. 1. Confirmation of $P_{DL}LA$/PEG blending, with (a) a DSC thermogram showing the shift in the glass transition temperature ($T_g$) of $P_{DL}LA$ after the incorporation of 5% w/w PEG 400 and (b) a decrease in the $T_g$ seen with increasing PEG content in the PEG/$P_{DL}LA$ blends, due to the PEG acting as a plasticiser of the $P_{DL}LA$.

SEM (Fig. 2). The comparative SEM images show that $P_{DL}LA$ scaffolds containing 1% w/w PEG 2000 (Fig. 2a) are visually indistinguishable from those containing 5% w/w PEG 2000 (Fig. 2b). This suggests that in these quantities and even at a molecular weight of 2000 Da, the PEG has not interfered with the pore formation process in the $P_{DL}LA$ scaffolds.

The hydrophilic characteristics of PEG were used in an attempt to combat the highly hydrophobic nature of the $P_{DL}LA$ material and increase water uptake, thus modifying the release characteristics of the entrapped BSA. Water uptake experiments were carried out on the scaffolds made from different $P_{DL}LA$/PEG blends by measuring the weight change of the scaffolds after being immersed in distilled water (Fig. 3). These data indicate that most water uptake occurs during the first day of exposure to the water. The scaffolds fabricated with PEG 400 show the highest affinity for water uptake at 1%; scaffolds fabricated with PEG 750 and PEG 2000 each have a lower affinity for water uptake at 1% PEG addition. However, there is no obvious molecular weight effect with 5% PEG content and water uptake is not increased by this further addition of PEG.

The effect of this water uptake in $P_{DL}LA$ scaffolds blended with 1% w/w PEG on protein release kinetics was then measured by a controlled release assay for entrapped BSA (Fig. 4). These data correlates with the

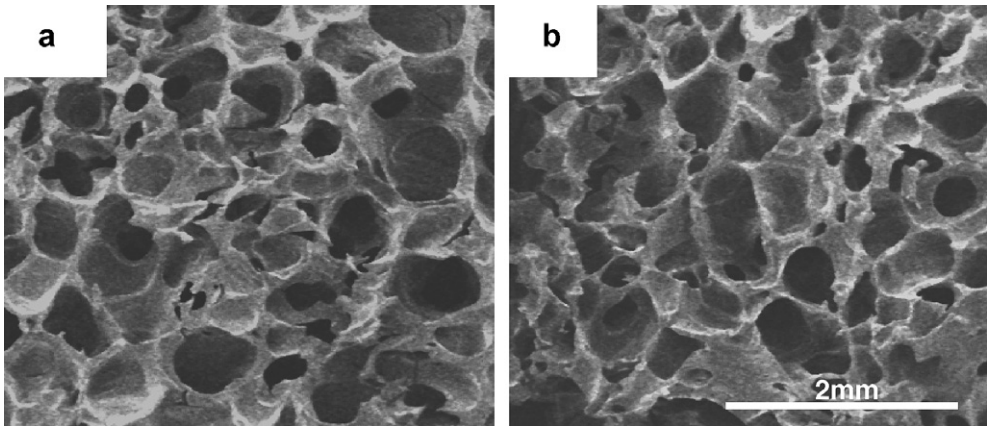


Fig. 2. Scanning electron micrographs of $P_{DL}LA$ scaffolds showing the porous structures created by blending or mixing two polymers into composite protein release scaffolds. (a) Contains 1% w/w PEG (MW = 2000) and (b) contains 5% w/w PEG (MW = 2000). No significant difference was observed in mean pore size for the $P_{DL}LA$ and $P_{DL}LA$/PEG blends at low molecular weights (data not shown).

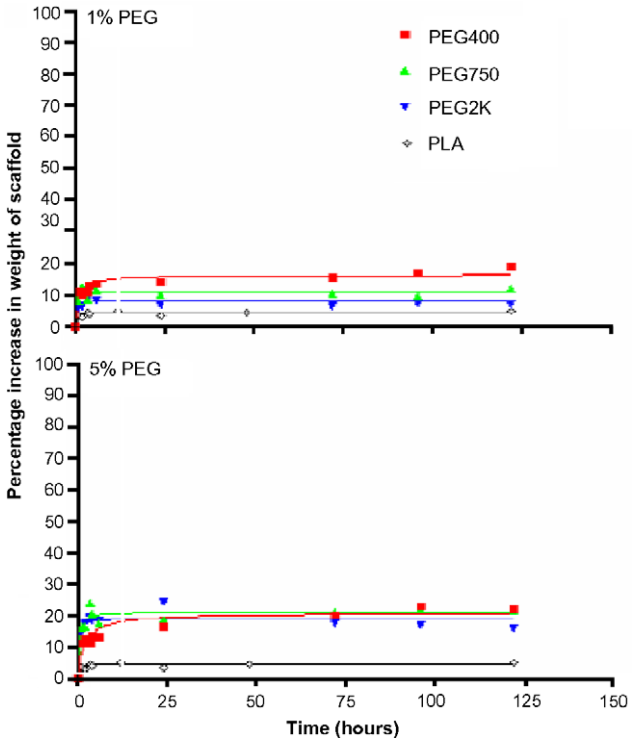


Fig. 3. Water uptake (and thus the bulk hydrophilicity) of the $P_{DL}LA$/PEG blended scaffolds fabricated in supercritical $CO_2$. The greatest affinity for water uptake occurred with the addition of 1% w/w of PEG 400; increased molecular weight appeared to decrease water uptake. Further addition of PEG (5% w/w) did not improve the water uptake of the scaffolds.

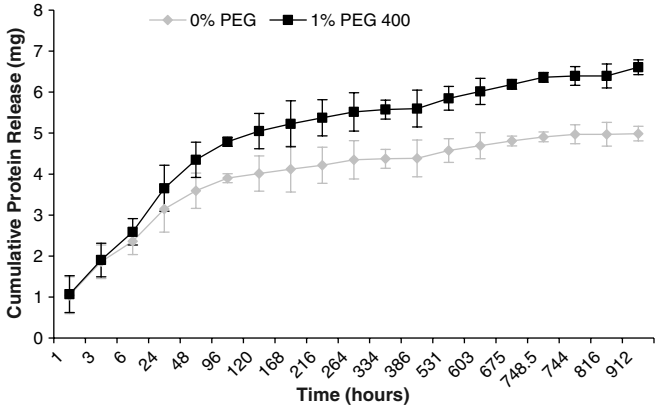


Fig. 4. BSA release profiles over 1000 h released from $P_{DL}LA$ scaffolds containing either 0% w/w PEG or 1% w/w PEG 400 as measured by a Bradford protein concentration assay. This demonstrates that the release kinetics of the protein can be controlled by the inclusion of PEG in the blend.

water uptake data shown in Fig. 4 and proves the direct link between increasing water uptake and more rapid drug release [10]. A relatively low concentration of PEG 400 is sufficient to significantly speed up the release of the protein when compared to a $P_{DL}LA$ scaffold which does not contain PEG. In addition, by altering either the molecular weight or % content of PEG, the release kinetics can be modified to suit the application. In addition to the modification of the release kinetics, this $scCO_2$ mixing technique permits the formation of polymer blends without the use of solvents or high temperatures, facilitating the incorporation of sensitive proteins and providing a complete composite material for subsequent scaffold formation. It is therefore feasible that polymer blending, protein entrapment and scaffold fabrication could all potentially be achieved using a single $scCO_2$ processing step in the future.

### 3.2. $P_{DL}LA$/alginate composite dual release scaffolds

Next, we adapted the supercritical fluid mixing technique to create scaffolds in which two entrapped proteins can be released with independent release kinetics. In the future such systems could be used in tissue regeneration applications in which two growth factors need to be released at different times and concentrations from the same scaffold. Our first approach was to form a composite of $P_{DL}LA$ with preformed alginate fibres. The composite

scaffold system comprised horseradish peroxidase (HRP) loaded alginate fibres incorporated into a $P_{DL}LA$ foam containing ribonuclease. The alginate was mixed with the $P_{DL}LA$ and it was observed that fibres were present in both the centre of the scaffold for sustained release of the HRP (Fig. 5a) and also protruding from the surface to instigate a larger burst release of the protein (Fig. 5b).

The effect of using alginate fibres is to increase the release of HRP during the initial burst phase. This first dual release study demonstrates the applicability of alginate fibres as a protein release mechanism, with approximately 15% more HRP released than ribonuclease from the $P_{DL}LA$ matrix during the initial burst phase (Fig. 5c). This is likely to be a result of the significant proportion of alginate fibres protruding from the surface of the scaffold. Immediate exposure of the hydrophilic alginate fibres to the aqueous environment appears to have facilitated a more rapid release of the HRP when compared to the ribonuclease diffusing through the hydrophobic $P_{DL}LA$ matrix. This concurs with previous data where the alginate fibres have released the protein more quickly when not within the confines of a $P_{DL}LA$ matrix and a greater surface area is exposed to the aqueous environment [21]. This trend continues up to 20 days where only 28% of the ribonuclease has been released compared to 43% of the HRP. From 25 to 30 days onwards, the bulk erosion of the $P_{DL}LA$ scaffold has most likely caused the faster dissolution of the remaining entrapped ribonuclease culminating in just short of 90% release for both proteins at 100 days. These release kinetics provide a suitable controlled release system for the delivery of protein growth factors or drugs when it is required that one protein be released in higher concentrations for the first 20 days followed by a more equal release of the two proteins from then on in. One potential application for such a system could be in the regeneration of bone tissue where a concentrated release of VEGF is required initially to induce angiogenesis, but a constant supply of BMP-2 is desirable to trigger the formation of new bone [26]. Alternatively, these release kinetics could be further modified by changing variables such as the ionic concentration of the pre-swelling solutions and the protein concentration as previously demonstrated [21,22] or by manipulation of the loading/distribution of the alginate fibres within the $P_{DL}LA$ matrix. Furthermore, this method of releasing two proteins from a single structure has the additional advantage that no solvents are required during the scaffold fabrication process, which is in direct contrast with other dual release strategies [20,27].

### 3.3. $P_{DL}LA$/PCL composite dual release scaffolds

Our second approach to composite formation used two polymers that are both plasticised by $scCO_2$. However, one of the polymers, PCL, requires higher temperatures to melt in the supercritical fluid than the second polymer, $P_{DL}LA$. Hence, we were able to incorporate a protein (HRP) into the PCL material and form particles using a single emulsion entrapment method before the $scCO_2$ processing

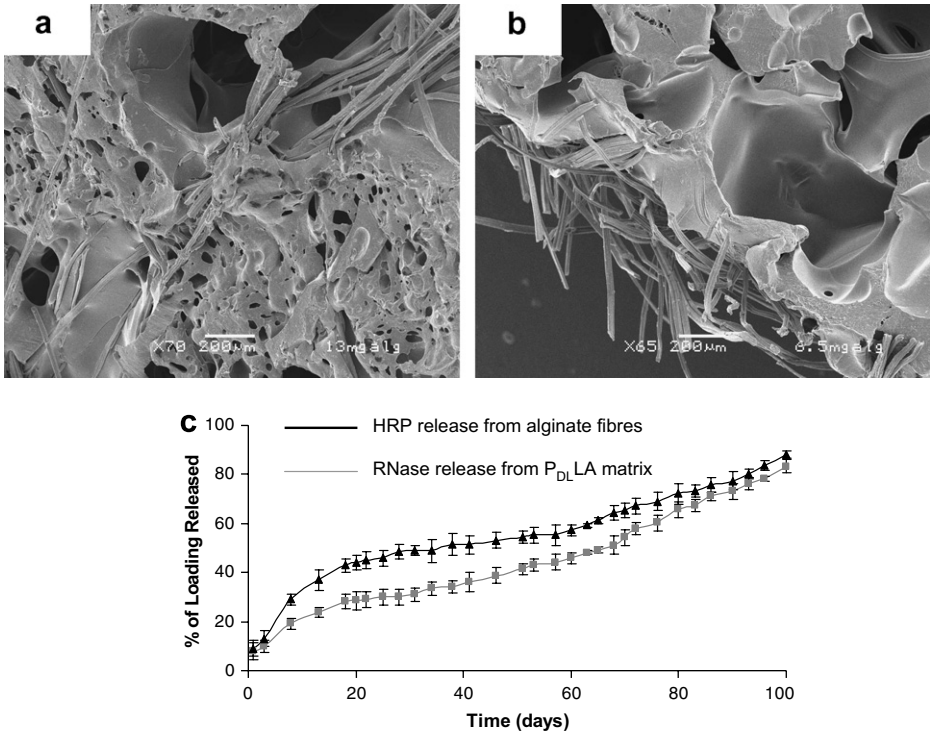


Fig. 5. Images and dual release profiles for composite $P_{DL}LA$/alginate scaffolds. SEM images indicate the presence of alginate fibres in the centre of the scaffolds (a) and also protruding from the scaffold surface (b). The release of both ribonuclease from the porous $P_{DL}LA$ material and HRP from the alginate fibres was measured by protein activity assays at specific time intervals to create a release profile for each protein (c).

conditions were then moderated such that the PCL particles were unaffected but the $P_{DL}LA$ phase could be formed into a scaffold containing a second protein (ribonuclease). Hence a composite was formed with very slow release PCL particles embedded within the $P_{DL}LA$ scaffold (Fig. 6a). PCL particles containing HRP (mean diameter, $350 \pm 150$ μm) were tagged with FITC to confirm the distribution of the particles post-$scCO_2$ fabrication (Fig. 6b). These images show that the particles have remained separate from the $P_{DL}LA$ structure and are well distributed throughout the scaffold. This provides evidence that the $scCO_2$ processing step has not caused the particles to plasticise and become incorporated into the $P_{DL}LA$ phase, thus preventing the formation of a single phase protein release system.

A controlled release study showed that the PCL particles released only 32% of the total protein (HRP) inside 80 days compared to 98% of the ribonuclease (Fig. 6c). Indeed, more than half of the total HRP released from the PCL particles was after 80 days indicating that the PCL was degrading very slowly and retarding the release of the protein. This is in direct contrast to the ribonuclease loaded $P_{DL}LA$ scaffolds where, after the initial burst release, there was a steady release of protein into the buffer, thus indicating that the $P_{DL}LA$ was degrading more quickly than the PCL, as expected. Therefore this system provides a method of releasing two separate proteins with completely different release kinetics from a single structure. The release of HRP from the $P_{DL}LA$ embedded PCL particles is in direct contrast to both protein release from the alginate fibres and previously developed composite systems whereby the proteins display a more sustained release profile throughout [17,27]. Thus, the $P_{DL}LA$/PCL composite scaffolds provide an alternative dual release system for when an application requires the release of a single protein followed by the latent release of a second protein.

## 4. Conclusions

In summary, we have demonstrated that the release kinetics of either a single protein or two separate proteins can be controlled by using simple methods of combining different polymers into composite release systems. Small changes to the ratio of a $P_{DL}LA$/PEG blend or a change in the molecular weight of the PEG can modify the protein release kinetics considerably, making this a highly tuneable system. What is more, by combining $P_{DL}LA$ with another synthetic or natural polymer, a two phase composite material can be formed that can control the release of two proteins each with a distinct release profile. This results in the ability to modify the single or dual release of growth factors to suit the application.

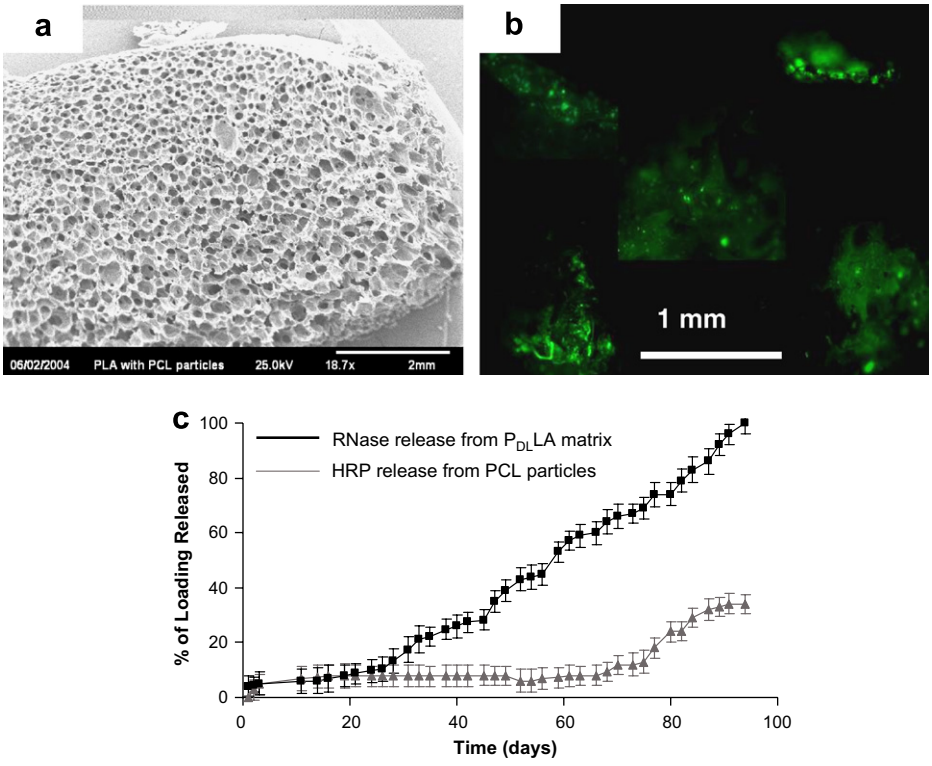


Fig. 6. Images and dual release profiles for composite $P_{DL}LA$/PCL scaffolds. The SEM of the $P_{DL}LA$ scaffold loaded with PCL microparticles (a) shows the porous structure of the composite device and (b) the PCL microparticles stained with the fluorescent FITC dye (indicated by the small bright green dots) are shown to be distributed throughout the scaffold. The release of both ribonuclease from the porous $P_{DL}LA$ material and HRP from the PCL particles was measured by protein activity assays at specific time intervals to create a release profile for each protein (c).

## Acknowledgements

We thank Martin Dellar, Richard Wilson and Peter Fields for manufacture of the $scCO_2$ equipment. Additional thanks also go to the BBSRC for funding. Steve Howdle is a 'Royal Society Wolfson Research Merit Award' holder.

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