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Encapsulation of T4 *bacteriophage* in electrospun poly(ethylene oxide)/cellulose diacetate fibers

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ABSTRACT

Phage therapy is a potentially beneficial approach to food preservation and storage. Sustained delivery of bacteriophage can prevent bacterial growth on contaminated food surfaces. Using coaxial electrospinning bacteriophage can be encapsulated in electrospun fibers with high viability. The resulting bio-based electrospun fibers may have potential as a food packaging material. In the present work, T4 bacteriophage (T4 phage) was incorporated into core/shell electrospun fibers made from poly(ethylene oxide) (PEO), cellulose diacetate (CDA), and their blends. Fibers prepared using PEO as the shell polymer showed an immediate burst release of T4 phage upon submersion in buffer. The blending of CDA with PEO significantly decreased the rate of phage release, with no released T4 phage being detected from the solely CDA fibers. Increasing the PEO molecular weight increased the electrospun fiber diameter and viscosity of the releasing medium, which resulted in a relatively slower T4 phage release profile. SEM analyses of the electrospun fiber morphologies were in good agreement with the T4 phage release profiles. Depending on the PEO/CDA ratio, the post-release electrospun fiber morphologies varied from discontinuous fibers to minimally swollen fibers. From these results it is suggested that the T4 phage release mechanism is through solvent activation/polymer dissolution in the case of the PEO fibers and/or by diffusion control from the PEO/CDA blend fibers.

1. Introduction

The clinical benefits of bacteriophage or phage therapy have begun to attract considerable attention, and bacteriophage therapy has recently been suggested as a possible alternative to chemical antibiotics (Ma et al., 2008; Puapermpoonsiri, Spencer, & van der Walle, 2009). Bacteriophage have been used to treat *P. aeruginosa* ear infections in domestic dogs (Soothill, Hawkins, Anggard, & Harper, 2004), *Escherichia coli* infections in calves and lambs (Smith & Huggins, 1983), *Salmonella* colonization on broiler chickens (Atterbury et al., 2007) and *Campylobacter* on the surfaces of meat (Goode, Allen, & Barrow, 2003). Typically, bacteriophages are either administered by oral gavages in the case of infected animals or by simple spraying techniques in the treatment of surface infection.

In the surface treatment of bacterial infected meat, the timing of phage dosing is critical. For example, too early an inoculation can result in a dramatic reduction in bacteriophage efficiency and activity or even in complete treatment failure (Payne & Jansen, 2003). As compared to conventional drugs bacteriophage operate under different pharmacokinetic principles; bacteriophage are living organisms that interact, replicate, and evolve (Greer, 2005; Payne & Jansen, 2003). In the treatment of infection with bacteriophage there is an increase in phage number via rapid viral self-replication. Therefore, in principle only a small initial inocula would only be needed to set out an active phage therapy (Ackermann & DuBow, 1987; Greer, 2005). Thus, there is a strong desire to develop a polymeric matrix delivery device that can encapsulate bacteriophage in a viable mode and liberate them in a slow controlled fashion.

Electrospun biopolymer fibers are considered a reliable carrier/delivery device for many bioactive agents (Andrady, 2008; Seeram, Fujihara, Teo, Lim, & Ma, 2005). The advantage of electrospinning to encapsulate drugs and other biomolecules is that electrospun nanofibrous mats possess large surface areas, with good dimensional stability and are relatively easy to produce (Sorrentino, Gorrasi, & Vittoria, 2007). Bacteriophage has been incorporated in electrospun fibers using aqueous suspensions of M13 phage in polyvinyl pyrrolidone (Lee & Belcher, 2004), as well as T4, T7, and lambda phage in polyvinyl alcohol (Salalha, Kuhn, Dror, & Zussman, 2006). These phage encapsulated water-soluble fibers exhibited an instant release mechanism; however the liberated phage demonstrated low bacterial host infectability (Salalha et al., 2006). The loss in phage activity was attributed to the simple suspension electrospinning process. The rapid evaporation of water during the single nozzle electrospinning process leads to a drastic change in the osmotic environment of the electrospinning

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polymer solution/forming fibers, resulting in phage dehydration and deactivation (Salalha et al., 2006; Zhang et al., 2006).

Recently coaxial electrospinning has been shown as a viable method to encapsulate sensitive biomolecules in electrospun nanofibers; bioactive agents such as enzymes and bacteria have been successfully incorporated into coaxial electrospun fibers (Dror, Kuhn, Avrahami, & Zussman, 2008; Zhang et al., 2006). Using this technique, the easily denatured biological agents are allocated to the core of the bicomponent fibers and thereby protected from rapid dehydration by the outer-layer polymer shell (Klein et al., 2009). Moreover, bioagent release can be controlled by changing the properties of the polymer shell (Srikar, Yarin, Egaridis, Bazilevsky, & Kelley, 2008; Xu et al., 2005). Hydrophilic polymer shells readily dissolve in aqueous environments and instantly release core components, whereas hydrophobic polymer shells do not, and can retain encapsulated components for a prolonged period (Srikar et al., 2008). Similarly, increasing the polymer shell thickness decreases the release rate of the encapsulated components (Zhang et al., 2006). The shell thickness can be increased by increasing the polymer concentration, polymer molecular weight, and/or feeding rate of the polymer shell solution during electrospinning (Andrady, 2008; Huanga, Zhangb, Kotakic, & Ramakrishna, 2003; Zhang et al., 2006).

To overcome the sensitivity of T4 phage to electrospinning and increase phage viability the applicability of coaxial electrospinning was investigated. To our knowledge, no reports on encapsulating T4 phage in electrospun fibers using coaxial electrospinning have been reported. Although the use of water-soluble polymer electrospun fibers enables the rapid and immediate leaching of biomolecules such as T4 phage, it limits their practical application in long-term uses such as food-packaging materials. To overcome this, blends of hydrophilic/hydrophobic poly(ethylene oxide) (PEO)/cellulose diacetate (CDA) were used as the shell layer for the coaxial electrospun fibers. The effect of polymer blending on fiber morphology and the rate of T4 phage release was studied.

2. Experimental

2.1. Materials

Poly(ethylene oxide) (PEO) with number average molecular weights (M_n) of 100,000, 300,000, and 600,000, and cellulose diacetate (CDA) with M_n = 30,000 and degree of substitution (DS) of 2.5 were purchased from Sigma–Aldrich and used as received. *Escherichia coli* bacteria (*E. coli*) strains and wild type T4 phage were kindly provided by the laboratory of Prof. Griffiths, University of Guelph, Canada.

Tryptic soy agar (TSA—solid media) and tryptic soy broth (TSB—liquid media) were prepared by dissolving 30 g of each powder in 1 L of distilled water. Tryptic soy broth semisolid (TSB_{SS}) was made by dissolving 30 g of TSB and 4 g of agarose in 1 L of distilled water. Storage media buffer (SM) for storing T4 phage was made by dissolving 5.8 g of NaCl, 2 g of $MgSO_4{\cdot}7H_2O$, 50 mL of 1 M Tris–hydrochloride (pH 7.5) and 1 mL of 10% (w/v) gelatine in 1 L of distilled water. All the above reagents and media were purchased from Sigma–Aldrich and were autoclaved prior to use.

3. Methods

3.1. Phage propagation and plaque assay

The bacteria host for phage T4 propagation was grown by dispersing 100 μL of *E. coli* in 5 mL of TSB media with shaking at 120 rpm/min at 37 °C overnight. 100 μL of this culture was then mixed with 100 μL of T4 phage stock solution. This mixture was incubated at 37 °C for 20 min and was mixed with 4 mL of partially cooled TSB_{SS}. This was then poured onto a cooled TSA plate and incubated at 37 °C overnight. The resultant T4 phage lawn was identified as a clear transparent plate in comparison to the control cloudy plate (100 μL of neat *E. coli*). Subsequently, 5 mL of SM buffer was used to flood the plates and then chilled at 4 °C for 1 h. The storage medium containing phage and TSB_{SS} was then centrifuged for 20 min at 6800 rpm and 4 °C. The supernatant was then passed through a 0.45 μm sterile filter and the lytic activity of the phage was determined by plaque assay test.

In the plaque assay test, 10 fold serial dilutions (1:9 dilution) of the propagated T4 phage were prepared. Then, 100 μL aliquots of each dilution were added to an equal volume of *E. coli* bacterial culture. Each mixture was then added to 4 mL of partially cooled TSB_{SS}, poured onto a TSA plate, and stored at 37 °C overnight. A negative control, bacterial culture without phage was prepared in the same manner. Following overnight incubation, the number of plaques was counted for each dilution, and used to calculate the number of plaque forming units (PFU). Phage activity was determined to be 10^8 PFU/mL.

3.2. Phage fluorescence labeling

A 10 mg excess of fluorescein isothiocyanate (FITC) was added to 10 mL of freshly propagated filtered bacteriophage T4 equilibrated in storage media buffer at pH = 7.5, and agitated continuously for 2 h at room temperature. Following agitation, the resulting suspension of bacteriophage was dialyzed (500 Da cut-off; Spectra/Por FloatA-Lyzer, G2) in SM buffer, pH 7.5. The dialysis buffer was exchanged every 6 h over a 24 h period to remove any free FITC.

3.3. Coaxial electrospinning

Coaxial electrospinning was performed using PEO and PEO/CDA blend solutions (chloroform:methanol; 98:2 %wt) as the shell and a T4 phage/buffer suspension as the core. The flow rates of both the core and shell solutions were controlled by two syringe pumps: 0.01 mL/min for the shell and 0.001 mL/min for the core. The electrostatic field used was 1 kV/cm, and the distance between the spinneret and collector plate was 20 cm. The fibers were collected on a sterilized aluminum plate, which was then freeze dried overnight to avoid fiber dissolution due to presence of buffer in the core and immediately tested for phage activity. This was done for all blends, but was only a concern for the 100% PEO fibers.

3.4. In vitro release

The bacetriophage T4 was released from the electrospun fibers by submerging 1.0 g of the electrospun fiber mat in a test tube containing 10 mL SM buffer (pH = 7.5), which was shaken at 120 rpm and 37 °C. At predetermined time intervals, 50 μL of the release buffer was removed and subjected to plaque assaying using the above mentioned serial dilution test. Every 50 μL aliquot removed was replaced with 50 μL of fresh SM buffer. The calculated T4 phage activity was expressed as PFU/mL. To calculate the activity percentage, the PFU/mL values were converted to log value.

3.5. Thermal analysis

Thermal characteristics of all components were studied using differential scanning calorimetry (DSC) (TA Instruments Q 1000 DSC). In a typical experiment ~5.0 mg samples were accurately weighed, placed in aluminum pans and heated under a nitrogen atmosphere from −90 to 220 °C at 10 °C/min. A heat-cool-heat program cycle was used and the glass-transition temperature (T_g) and

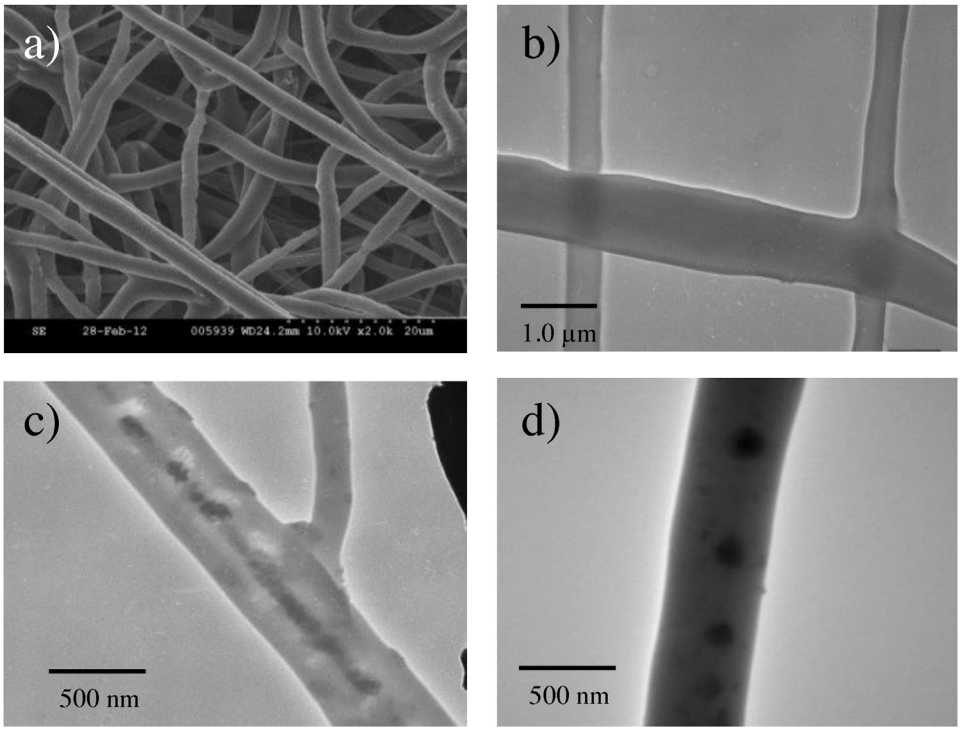

Fig. 1. (a) SEM image of coaxial electrospun PEO fibers, (b) TEM image of solution electrospun PEO fiber, (c) TEM image of coaxial electrospun PEO fiber showing core/shell morphology, and (d) TEM image of coaxial electrospun PEO fibers showing encapsulated T4 phage in the core of the fibers.

melting temperature (T_m) were recorded as the midpoint temperature of the heat capacity transition and the peak temperature, respectively from the second heating run. Samples were run in duplicate and were within experimental error of each other (1.0 °C).

3.6. Microscopic analysis

The morphology of the electrospun fibers was examined by scanning electron microscopy (SEM). Fibers were mounted on an aluminum stub, coated with a 5 nm gold layer, and imaged using a Hitachi S-2600 N SEM operating at 25 kV. For transmission electron microscopy (TEM) analysis the specimens were prepared by direct deposition of the electrospun fiber onto a carbon film coated copper grid. Images were collected using a Hitachi H-800 TEM at 100 kV without any sample staining. The encapsulated fluorescein-labeled bacteriophages were viewed with a Leica SP5X white light laser confocal microscope system. The fluorescein conjugates were excited with an Argon laser set to 488 nm and with an emission bandwidth of 521–616 nm. Leica (SP5X) 20× dry objectives were used and the pinhole was automatically adjusted for optimal performance.

4. Results and discussion

4.1. Encapsulation and release of T4 phage from electrospun PEO fibers

Core/shell electrospun fibers were successfully produced by coaxial electrospinning using a PEO/chloroform solution for the shell and a T4 phage/buffer suspension for the core. To ensure complete encapsulation of the T4 phage within the core of the fiber, the feeding ratio of the core was set to ten times slower than that of the polymer shell solution (Dror et al., 2008; Klein et al., 2009). Fig. 1 clearly shows that uniform beaded-free fibers with core/shell morphology were produced where the T4 phage were incorporated in the core of the fibers. Incorporation of the T4 phage in the core of the fibers should dramatically enhance the viability of the phage after electrospinning. It is anticipated that solvent evaporation from the core is very slow and therefore, the drastic changes in the osmotic environment that occur in solution electrospinning should not be observed. Incorporation of the bacteriophage in the core of the electrospun fibers dramatically enhanced the viability of the bacteriophage during storage. The activity of dried bacteriophage powder decreased rapidly from 10^8 to 10^2 PFU/mL after 1 day of storage, whereas the fiber encapsulated bacteriophage took more than 4 weeks to reach the same decrease in activity (Korehei & Kadla, 2013).

Encapsulated bioactive components in the core of core/shell fibers usually demonstrate a more sustained release profile as compared to those randomly distributed throughout a fiber (Zhang et al., 2006). However, despite the core/shell structure, a rapid release of T4 phage was observed when the PEO/T4 phage fibers were subjected to in vitro release in aqueous buffer medium (pH 7.5). Fig. 2 shows that 100% T4 phage release is obtained within 30 min of immersion of the PEO/T4 electrospun fibers in aqueous buffer. The burst release of T4 phage is attributed to the high hydrophilicity of PEO, which rapidly dissolves in buffer; T4 phage liberation occurs through solvent activation followed by PEO fiber dissolution. The dissolution rate of electrospun fibers is usually influenced by polymer fiber diameter (Zhang et al., 2006). Therefore, variation in PEO fiber diameter could be used to affect the release behavior of T4 phage.

4.2. Effect of PEO fiber diameter and solution viscosity on the release profiles of T4 phage

In coaxial electrospinning, the diameter of the electrospun fiber can be influenced by several factors, including feeding ratio of the core and shell solutions, polymer concentration, and/or polymer molecular weight (Andrady, 2008; Liao, Chew, & Leong, 2006; Seeram et al., 2005). While keeping all other variables constant, an increase in PEO molecular weight (M_n) from 100k to 600k increased

Table 1
Effect of polymer molecular weight and concentration on fiber diameter, solution viscosity and on release of T4 bacetriophage.

PEO (M_n)	Concentration (%wt)	Fiber diameter (μm)	Viscosity (Pa s) at shear rate 10 s^{-1} [a]	Release at 10 min, log of PFU/mL (STD)	Release at 10 min (%)
100k	9.0	1.35 ± 0.03	0.13 ± 0.01	8.56 ± 0.02	92
100k	11	2.39 ± 0.02	0.36 ± 0.03	7.58 ± 0.15	83
300k	2.5	1.80 ± 0.02	0.37 ± 0.01	7.76 ± 0.03	85
600k	1.5	2.48 ± 0.05	0.65 ± 0.06	7.19 ± 0.14	78

[a] Measurements made on electrospun PEO fibers exposed to buffer medium for 10 min at which time a 1 mL aliquot (non-dissolved material remained settled at the bottom of the test tube) was removed and placed between the cone and plate geometries.

the fiber diameter from 1.35 to 2.48 μm (Table 1). This resulted in a relatively slower release rate of T4 phage (inset Fig. 2), corresponding to almost an order of magnitude drop in phage activity at 10 min (Table 1). Here, the hydrophilic PEO carriers pass through a fast process of chain unfolding from the semicrystalline phase to the amorphous one, which leads to complete polymer chain disentanglement (Sperling, 2005). Increasing thickness of polymer shell layer retards the process of polymer chain unfolding and disentanglement and therefore a relatively slower release rate of T4 phage was observed.

In order to better understand the effect of fiber diameter on T4 phage release rate, an attempt was made to increase the 100k PEO fiber diameters by increasing PEO concentration. Increasing the 100k PEO concentration from 9 to 11 wt% increased the fiber diameter from 1.35 to 2.39 μm, similar to that obtained using the 600k PEO (2.48 μm). However the release profile was only slightly slower (these results were confirmed in multiple experiments). Therefore, the release rate of T4 phage from PEO electrospun fibers appears to be not only governed by fiber diameter, but may also be affected by the viscosity of dissolving polymer solution.

As the PEO electrospun fibers dissolve during the in vitro release the viscosity of the releasing buffer medium is enhanced and dependent on PEO molecular weight. Steady state rheology measurements showed an increase in viscosity from 0.13 to 0.65 (Pa s) as PEO molecular weight increased from 100 to 600k, respectively (Table 1). In general, the motility of bacteriophage and their ability to lyse bacteria decrease as solution viscosity increases (Hanlon et al., 2001). Here, the rate of diffusion and thus activity of the T4 phage shifted to a slower release profile as the PEO solution viscosity increased (Fig. 2). Increasing the polymer concentration of the 100k PEO from 9 to 11 wt% increased the viscosity from 0.13 to 0.36 Pa s, similar to that of the 300k PEO system (0.37 Pa s). This is consistent with the similar release profiles observed for the 100k (11 wt%) and 300k (2.5 wt%) PEO fibers (Fig. 2). Therefore, the viscosity of the swollen fibers and releasing environment could have a significant impact on the release profile of T4 phage.

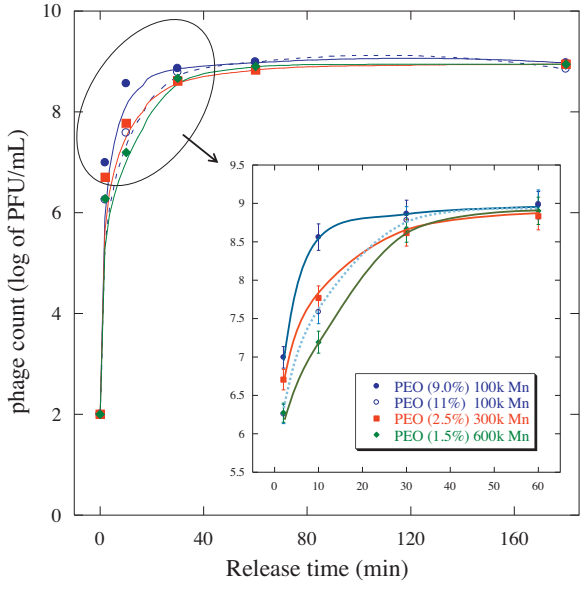

Fig. 2. T4 phage release profiles from PEO coaxial electrospun fibers prepared using different PEO molecular weights and concentrations. For all experiments $n = 3$, and the error bars indicate one standard deviation; Phage counts of zero were recorded at 100 PFU/ml (or 2 log of PFU/mL), which is the limit of the detection in the assay test.

The changes in PEO fiber morphology during the in vitro release experiments and release of T4 phage were also observed to be dependent on polymer molecular weight. Fig. 3 shows SEM images of fibers submerged in buffer medium at various time intervals. In the first 5 min, the PEO electrospun fibers began to swell. Swelling was found to be slower as fiber diameter increased; 100k (9 wt%) PEO fibers appearing more dissolved at 5 min than the corresponding 600k PEO fibers. After 10 min of immersion in the buffer medium, the low molecular weight PEO (100k) electropsun fibers were dissolved and isolated as polymer films after freeze-drying. By contrast, swollen electrospun fibers were still observed for the PEO 300 and 600k fibers as evident by some traces of a fibrous structure. After 30 min of immersion all of the electrospun fibers morphologies were isolated as polymer films, consistent with the complete release of T4 phage. These observed morphological changes in the PEO fibers are in good agreement with the measured T4 phage release profiles.

4.3. *Effect of blending hydrophilic/hydrophobic polymer on release rate of T4 phage*

The high hydrophilicity of PEO resulted in rapid fiber dissolution and immediate leaching of T4 phage into the surrounding buffer medium; a burst release was exhibited over a period of 30 min that resulted in almost 100% release of the T4 phage. To avoid the rapid release of T4 phage, the hydrophilicity of the shell polymer layer was decreased through the blending with hydrophobic cellulose diacetate (CDA). When CDA fibers were coaxially electrospun with a core containing T4 phage, the resulting fiber did not exhibit any measurable phage activity or release even after two months of submersion in buffer medium (Supplemental data). SEM images of the CDA fibers before and after immersion in buffer medium did not reveal any fiber expansion or degradation. This is in agreement with the known properties of CDA, which can maintain its molecular integrity for a very long period (>2 years) before being hydrolytically degraded (Heinze & Liebert, 2004). Unlike the release of small molecules, a controlled release of large biological compounds such as T4 phage requires relatively large fiber expansion and/or the creation of large macro-pores in the fiber. Therefore, by manipulating the ratio of hydrophilicity/hydrophobicity polymers in the shell layer a controlled diffusional release as a result of fiber swelling may be obtained. Thus, a series of hydrophilic/hydrophobic polymer (PEO/CDA) blends were prepared in an attempt to alter fiber morphology and provide better control over the release of T4 phage.

The blending of CDA with PEO led to a reduction in the release rate of T4 phage in all formulations as compared to the pure PEO

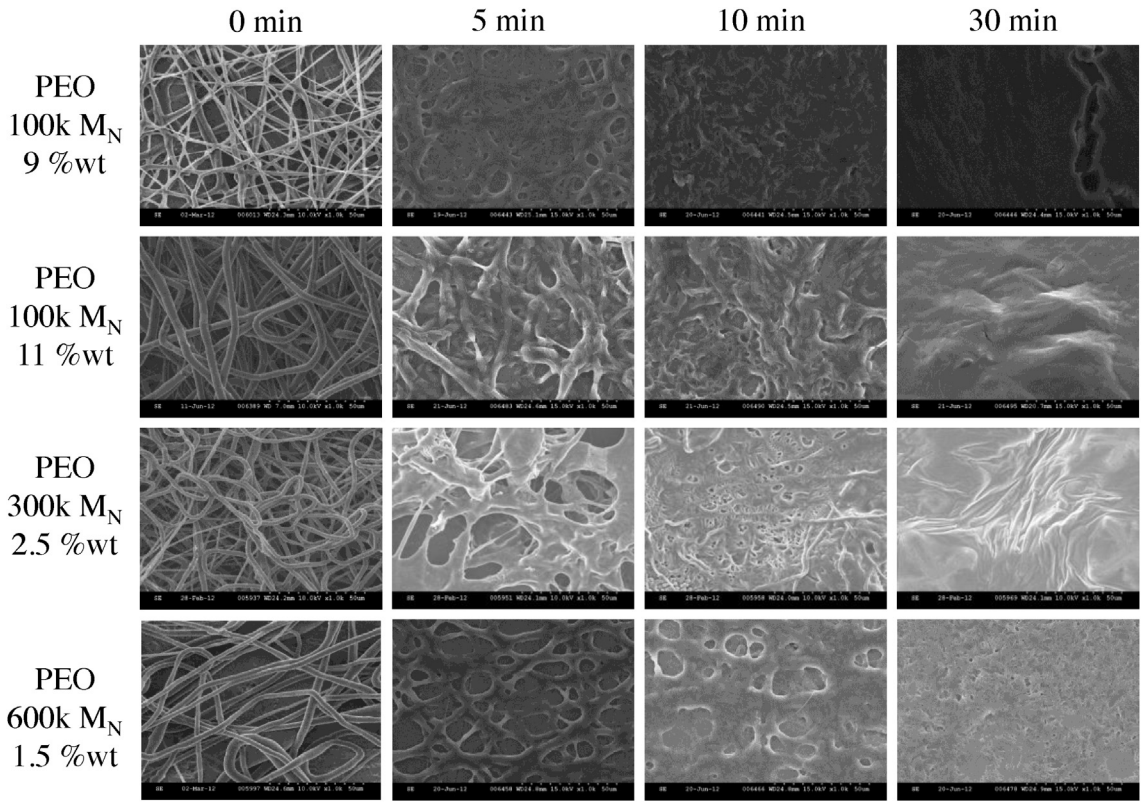

Fig. 3. SEM images of T4 phage loaded coaxial electrospun PEO fibers before and during the in vitro release experiments. Samples were freeze-dried prior to SEM analysis. Images were obtained at a magnification of 1.0k.

fiber. As found for the pure PEO fibers the fastest T4 phage release was observed in the CDA/PEO blend fibers prepared using the 100k PEO. Although PEO molecular weight had an effect on the release of T4 phage, the most significant effect was related to the CDA/PEO ratio (Fig. 4).

The observed slower release with the addition of CDA is due to the increased hydrophobicity of the electrospun fibers, which decreases the fiber solubility/swelling in the aqueous media. Here, the addition of CDA improved the structural integrity of the fiber and restricted the electrospun fiber expansion during the post-treatment. Therefore, an improved control of the release of T4 phage was observed in a manner that is independent of loading concentration and core diameter.

By increasing the PEO molecular weight in the blend composition, further increased control over the release profile was possible. The increase in the PEO molecular weight likely leads to greater polymer entanglement (intermolecular interaction) between PEO–PEO and PEO–CDA polymer chains, which results in slower fiber swelling/dissolution and a slower release of T4 phage.

The morphology of all of the polymer blend fibers before and after T4 phage release by submersion in buffer medium was investigated with scanning electron microscopy. The SEM images show uniform bead-free, as-spun fibers for all of the PEO/CDA blends. The morphological structure of the post-release was then monitored for 6 h of immersion in an aqueous buffer (Fig. 5). The SEM images show that the changes in fiber morphology were dependent

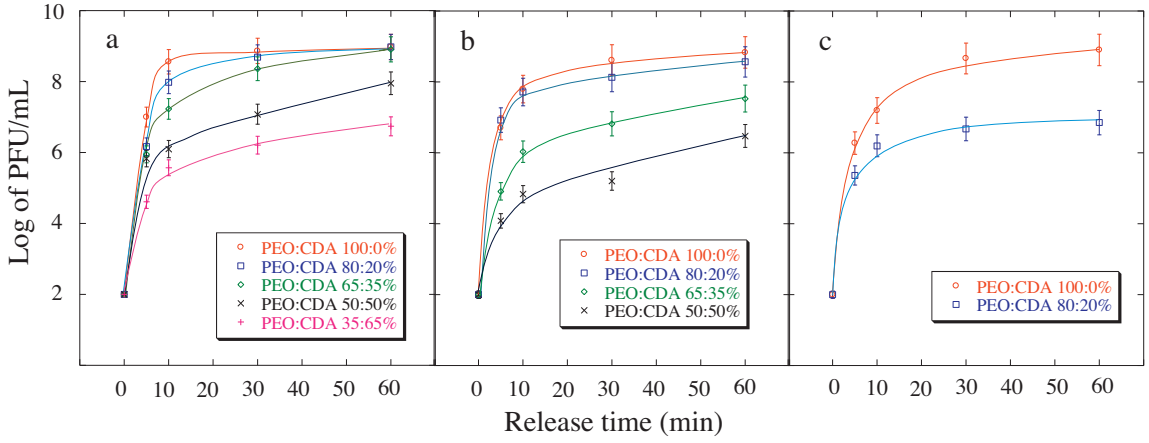

Fig. 4. Release profiles of T4 phage from PEO/CDA blend coaxial electrospun fibers with different PEO molecular weights: (a) 100k, (b) 300k, and (c) 600k. For all experiments $n = 2$, and the error bars represent the average absolute deviation from the mean of each data point. Phage counts of zero were recorded at 2 log of PFU/mL (or 100 PFU/mL) which is the limit of phage detection in assay testing.

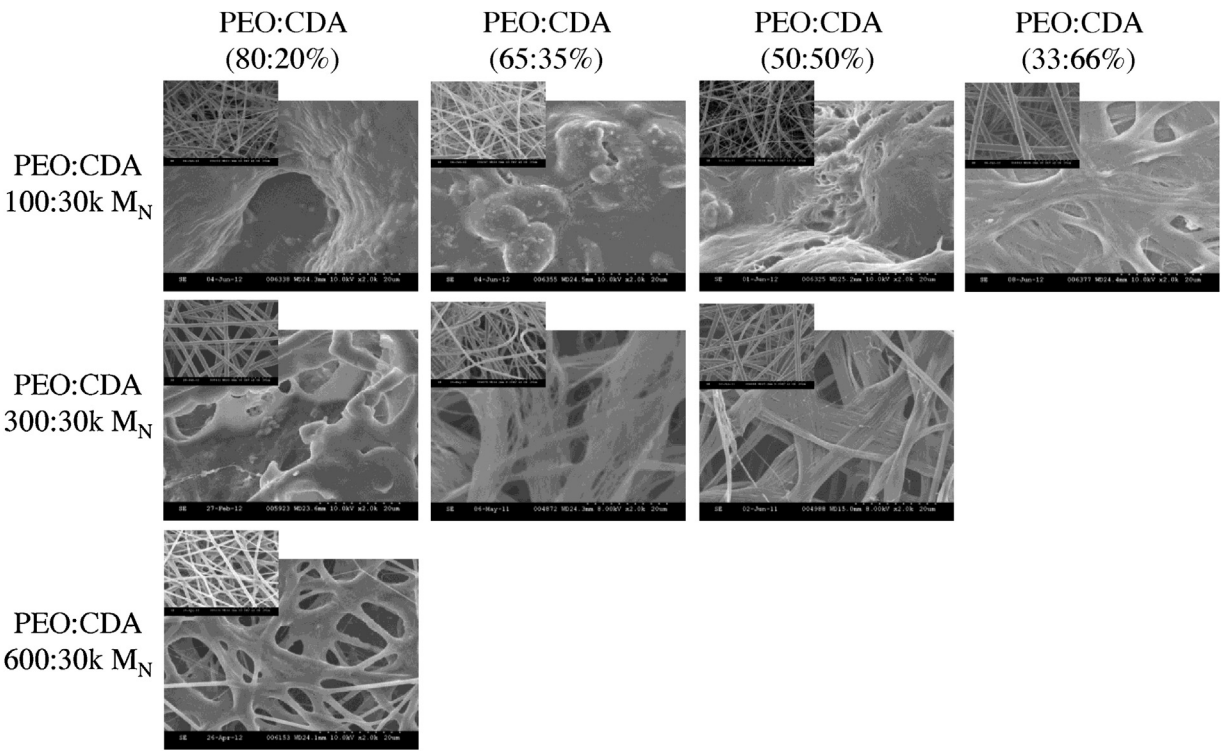

Fig. 5. SEM images of the various PEO:CDA blend coaxial electrospun fiber structures after 6 h immersion in buffer. The small inset images are of the electrospun fiber morphologies before immersion in buffer. All SEM images were obtained at magnification of 2.0k.

on the PEO content in the blend fibers; the higher the PEO content the more significant the change in fiber morphology. In the higher PEO contain blend fibers the morphological structure was again altered to a polymer film as the PEO polymer was dissolved away from the fiber. However, as the CDA content increased, swollen fiber morphology was observed, which was very much dependent on the molecular weight of the PEO used. In the case of the 100k PEO, PEO:CDA ratios of up to 50:50 (wt%) were fully dissolved in the buffer medium, while the 35:65 (PEO:CDA) blends showed evidence of only being swollen in the buffer medium. The behavior of the 300k and 600k PEO/CDA blend fibers was quite different; only the 300k 80:20 (wt%) PEO/CDA blend fibers dissolved, whereas the 65:35 and 50:50 300k PEO/CDA and 80:20 600k PEO/CDA blends retained a highly fibrous morphology. Again, the slow and limited change in fiber morphology for the higher molecular weight PEO blends is attributed to the greater polymer entanglements associated with the increase in PEO molecular weight.

DSC analyses of various PEO/CDA polymer blend electrospun fibers are shown in Fig. 6. The DSC scans show a depression in the heat of fusion and a decrease in the melting temperature of PEO with increasing addition of CDA in the electrospun blend fibers. The decrease in the heat of fusion and melting temperature of the PEO phase implies miscibility and physical interaction (intermolecular forces and/or hydrogen bonding) between the two polymers (Ding, Jiang, & Li, 2001; Jiang, Ding, & Li, 2002; Pielichowska & Pielichowski, 2011).

During the electrospinning process the solvent is rapidly removed and the polymer, PEO undergoes a certain degree of crystallization, which is dependent on the processing environment (Zhao, Wu, Wang, & Huang, 2004). In the presence of CDA, specific intermolecular interactions form between PEO and CDA, which upon solvent removal, i.e. during electrospinning lead to a decrease in the crystallization of the PEO. In the case of miscible polymer blends comprising a crystalline polymer changes in the kinetics of crystallization and degree of crystallinity can occur (Rim & Runt, 1984). The decrease in the equilibrium melting point is largely enthalpic in nature wherein the greater the polymer/polymer interactions, the greater the melting point depression. Although our experiments were not conducted under equilibrium conditions, our findings are in agreement with previous publications on PEO/CDA blends (Ding et al., 2001; Pielichowska & Pielichowski, 2011) and suggest at least partial miscibility between PEO and CDA.

According to Fig. 4 increasing the CDA content in the PEO/CDA electrospun fibers resulted in a decrease in the amount of T4 phage released from the fibers upon immersion in buffer. Moreover, the amount of T4 phage released from the PEO/CDA blend fibers appeared to plateau well below that expected based on

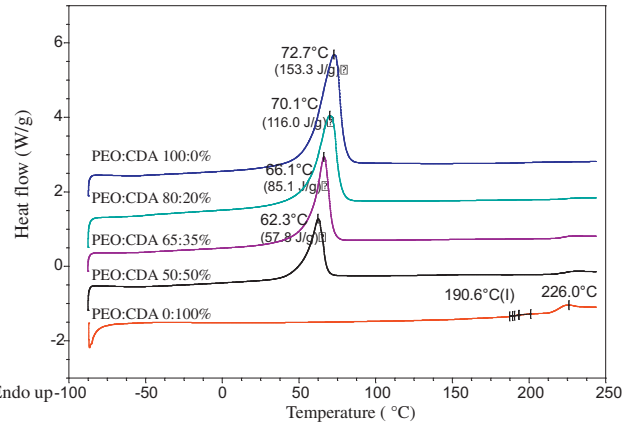

Fig. 6. DSC analysis of 300k PEO:CDA electrospun fibers at different blend ratios. T_g and T_m were recorded as midpoint temperatures of the heat capacity transition and melting peak maximum, respectively, from the second heating runs. Samples were run in duplicate and were within experimental error of each other (1.0 °C). Included on the traces are the PEO melting temperatures and heats of fusion.

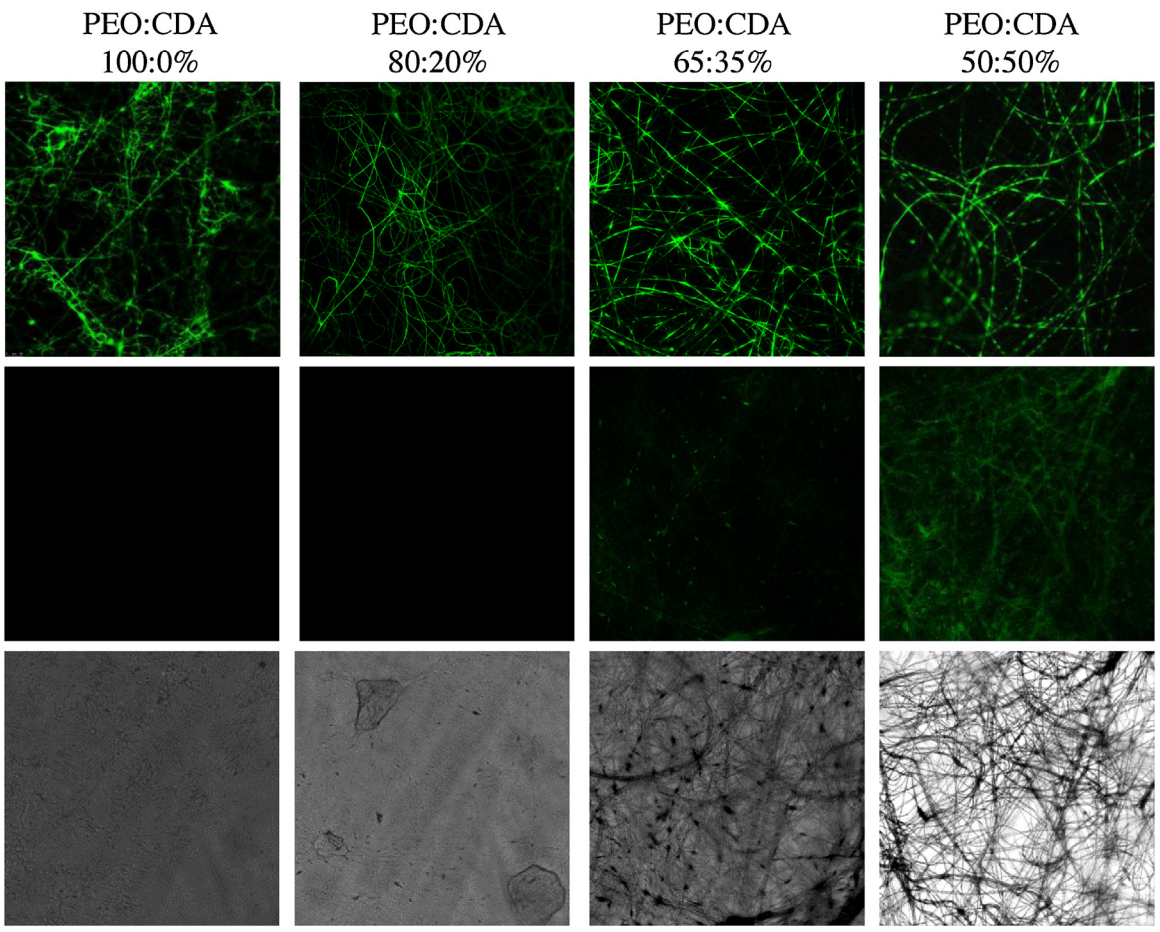

Fig. 7. LSCM images of T4 phage loaded coaxial electrospun 300k PEO:CDA fibers before immersion in buffer (top), after 6 h immersion in buffer (middle), and the corresponding bright field images of the electrospun fibers after immersion (bottom).

the phage loading. Although the SEM images in Fig. 5 show the effect of CDA blending on fiber dissolution/swelling and are somewhat consistent with the decreased rate of phage release, they cannot provide detailed information on the release and release mechanism of the T4 phage. Therefore, laser confocal scanning microscopy (LCSM) was employed to further support the release of the T4 phage from the 300k PEO/CDA electrospun fibers and possibly explain the release mechanism. The 300k PEO blend system was used in this study as it showed the most variation in fiber morphology after immersion in buffer (Fig. 5). In these experiments the T4 phage was labeled with a fluorescent compound, FITC, which fluoresces green when observed using LCSM (Fig. 7). Such fluorescently labeled compounds have been previously used to successfully identify the core/shell structures of relatively large diameter (few hundred micrometers) fibers (Rahman & Mathiowitz, 2004). Our PEO/CDA-phage-FITC electrospun fibers are relatively small (~2 μm in diameter), and as a result details into the core-shell structure could not be clearly identified by our LCSM. Under the confocal microscope all of the fibers exhibited the presence of the FITC-labeled T4 phage throughout the fiber (Fig. 7, top images). However, after immersion in buffer, the electrospun fibers with high PEO content (100 and 80% PEO) revealed no fluorescent signal under LCSM imaging (Fig. 7, middle images). This is consistent with the dissolved SEM images (Fig. 5) and the ~100% release of T4 phage from the electrospun fibers (Fig. 4). As expected the electrospun fibers with higher CDA content, exhibited some fluorescence under LCSM imaging, indicating T4 phage trapped/remaining in the fiber. The fluorescent signal was greater in the PEO/CDA 50:50% blend fibers as compared to the 65:35% blend fibers, consistent with the SEM images in Fig. 5, and release profiles in Fig. 4. This confirms that the release of T4 phage is highly dependent on the degree of swelling of the fiber, which, in turn, depends on the blend ratio of hydrophilic and hydrophobic polymers in the shell layer.

The core/shell structure of the PEO/CDA/T4 phage electrospun fiber is best described as a reservoir–type release design. This design has the advantage of providing a more controlled release that depends on shell thickness, shell permeability, size and amount of loaded bioactive agent, as well as defects in the shell layer such as thin spots and pinholes (Rahman & Mathiowitz, 2004; Zhang et al., 2006). In the polymer blend composition, the observed burst release of the T4 phage from low molecular weight and high PEO content fibers is clearly associated with solvent activation and rapid PEO dissolution. As the CDA content is increased, the fiber dissolution is eliminated, and the fiber swelling behavior plays a primary role in the release of T4 phage. In this case, the underlying release mechanism is likely governed by diffusional mechanisms. The initial instant release could be attributed to imperfections in the core/sheath structure (e.g., spots in the thin shell layer or shell failure), and the release from deeper regions of the electrospun polymer matrix is through diffusion and interconnected macropore network structures. The T4 phage is a fairly large bioactive agent (200 × 400 nm) so its diffusion would be limited in the narrow pores. Therefore, the degree of fiber swelling and the size of the interconnected pores likely play a major role in the release profile of this large biological component.

5. Conclusion

Wild type and fluorescent-labeled T4 bacteriophage were successfully encapsulated in PEO and PEO/CDA blend electrospun core/shell fibers. In vitro release studies revealed the water-soluble PEO fibers exhibited a burst release over a period of 30 min that resulted in almost 100% release of the encapsulated T4 phage. Increasing PEO molecular weight increased the fiber diameter and the viscosity of the releasing media, which resulted in relatively slower release rate of T4 phage. Blending CDA with PEO suppressed the burst release. The release rate was dependent on the CDA:PEO ratio and PEO molecular weight, where increasing both parameters resulted in slower T4 phage release rates. Increasing the CDA content enhanced the hydrophobicity of the electrospun fibers, which resulted in a low degree of fiber swelling of post-release electrospun fibers. SEM and LSCM examination of the post-release fiber morphology indicated that in blends with high PEO content the release mechanism was solvent activation/fiber dissolution, while those with high CDA contents was more likely through a fiber swelling/diffusion mechanism.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.carbpol.2013.03.079.

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