Direct UV-triggered thiol-ene cross-linking of electrospun polyester fibers from unsaturated poly(macrolactone)s and their drug loading by solvent swelling

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Abstract. Electrospinning is considered a relative simple and versatile technique to form high porosity porous scaffolds with micron to nanoscale fibers for biomedical applications. Here, electrospinning of unsaturated aliphatic polyglobalide (PGI) into well-defined fibers with an average diameter of 9 μm is demonstrated. Addition of a dithiol crosslinker and a photoinitiator to the polymer solution enabled the UV-triggered intra-crosslinking of the fibers during the spinning process. The in-situ crosslinking of the fibers resulted in amorphous material able to swell up to 14% in tetrahydrofuran (THF) without losing the fiber morphology. Seeding Mesenchymal Stem Cells (MSCs) onto both crosslinked and non-crosslinked PGI fibers proved their compatibility with MSCs and suitability as scaffolds for cell growth and proliferation of MSCs. Moreover, the ability to directly load crosslinked PGI with hydrophobic molecules by soaking the fiber mesh in solution is shown with Rhodamine B and Indomethacin, a hydrophobic anti-inflammatory drug. This marks an advantage over conventional aliphatic polyesters and opens opportunities for the design of drug loaded polyester scaffolds for biomedical applications or tissue engineering.

Introduction

Aliphatic polyesters are widely used in several medical applications, such as sutures,¹ bone screws,^{2,3} tissue engineering (TE) scaffolds,²⁻⁶ and drug delivery systems.⁷ Arguably, poly(ϵ -caprolactone) (PCL) and poly(lactide/glycolide) (PLGA), obtained by ring-opening polymerization, are among the most versatile biomedical polyesters, due to their synthetic accessibility, biocompatibility, and mechanical strength.⁸⁻¹¹ Moreover, these materials are highly processible into applicable medical formats and devices. One of the most promising techniques for processing bioresorbable polyesters is electrospinning.¹²⁻¹⁶ Electrospinning is considered a

relative simple and versatile technique and has received a great interest in the area of tissue engineering due to the ability to form high porosity porous scaffolds with micron to nanoscale fibers similar to the natural extracellular matrix (ECM).^{15,17} These fibrous degradable scaffolds provide mechanical support to host cells and create conditions for their growth and proliferation, degrading as cells produce their own ECM.^{13,17} Numerous natural and synthetic homo- and co-polymers have been electrospun, including PCL and PLGA,^{9,18} to engineer scaffolds for several different tissues, such as vasculature,¹⁹ neural,¹⁰ tendon/ligament,²⁰ meniscus,²¹ and bone.²²

Currently there is a demand for next generation biopolymers for electrospinning that offer additional functionality for immobilizing bioactive cues or cross-linking to enable post-spinning manipulation such as drug loading. While fiber functionalization via click-chemistry has been explored and reviewed,^{23,24} only a few examples of *in-situ* crosslinked electrospun fibers have been disclosed to date. In one example, Ellison reported a process in which a mixture of thiol- and ene-functional monomers was spun and simultaneously UV-crosslinked.²⁵ Hoogenboom and Sanyal produced crosslinked oxazoline fibres by a UV-triggered thiol-ene reaction,²⁶ while Theato used the same technique to crosslink polybutadiene fibres.²⁷ To the best of our knowledge there is no example of cross-linked electrospun polyester fibers matching the versatility of PCL or PLGA. One barrier, is the quite challenging chemistry to introduce functional groups along the polyester chain via ring opening polymerization (ROP),^{28,29} which typically requires rigorous multi-step procedures to derivatize lactide or lactone monomers.²⁸⁻³¹ Unsaturated natural macrolactones such as globalide could overcome these restrictions. Typically they are polymerized via enzymatic ROP³²⁻³⁵ or recently by (organo)catalytic strategies,³⁶⁻⁴⁰ producing polymers with main-chain double bonds as anchor points for post-polymerization functionalization and crosslinking via thiol-ene chemistry.^{28,32,41,42}

Here we report, for the first time, the electrospinning of poly(globalide) to produce bioresorbable microfibers (PGL-F). We further take advantage of the main chain unsaturation by electrospinning PGL with *in-situ* UV-initiated thiol-ene crosslinking to produce fiber meshes capable of swelling in organic solvents without losing their fibrillar structure. We demonstrate the potential of these materials as bioresorbable scaffolds as well as the ability to load them with hydrophobic compounds by simple solvent soaking.

Experimental Section

Materials. Globalide was purchased from Symrise. Novozyme 435 (Candida Antarctica Lipase B immobilized on cross-linked polyacrylate beads) was purchased from Novozymes A/S. Ethylene glycol bis(3-mercapto propionate) was purchased from Wako. Pentanedithiol, dipheyl (2,4,6-trimethylbenzoyl) phenone oxide, 2-hydroxy-2-methylpropiophenone, 2,2 dimethoxy – 2 – phenyl acetophenone, dichloromethane (DCM), dry toluene and PCL (M_w 70 – 90 kDa) were purchased from Sigma–Aldrich. Dulbecco’s modified eagle medium containing high glucose (DMEM+ GlutaMAX), Fetal bovine serum (FBS), penicillin/streptomycin and insulin, transferrin, selenium premix (ITS) were purchased from Biosciences. Amphotericin B, sodium pyruvate, bovine serum albumin (BSA), dexamethasone, L-ascorbic acid 2-phosphate, linoleic acid, L-proline, and phosphate-buffered saline (PBS) were purchased from Sigma-Aldrich. Transforming growth factor β 3 (TGF β 3) was purchased from Prospecc Biosciences.

General Methods

Gel Permeation Chromatography (GPC) measurements were performed using an Agilent 1200 series instrument equipped with GPC control software. All measurements were carried out using a Polymer Laboratories Gel 5 μm Mixed-C 300 x 7.5 mm column, at 40 °C with DAD and RID detection. Tetrahydrofuran (THF) was used as the eluent at a flow rate of 1 mL/min. The molecular weights of all polymers were calculated based on polystyrene standards. For Scanning Electronic Microscopy (SEM), samples were sputter-coated with gold. The images were obtained using a Hitachi variable pressure SEM, model S-3400N, ranging acceleration voltage between 10-20 kV. The diameters of the fibers were measured by choosing randomly 20 different fibers in each image (at 500x magnification), using the software of the microscope. The FT-Raman measurements were performed on PerkinElmer – Raman station 400F, with a laser source of 785 nm. 16 scans were recorded for each spectrum. To characterize the influence of crosslinking within the scaffolds on their mechanical properties, tensile strength and Young's Modulus of the samples were measured. The analysis was performed, in triplicate, using a Zwick/Roell model Z2 with loading of a cell of 50N at 5 mm/min, using samples cut in dog-bone shape. X-Ray Diffraction (XRD) was carried out on a Bruker AXS D8 Advance with a 3kW tube with a copper anode using the K-alpha line. The sample was mounted on a six-axis goniometer and a locked coupled scan was used. The samples were then scanned through an angle theta and the detector was maintained at twice that angle theta. The melting point (T_m) and the melting enthalpy (ΔH_m) of the samples were measured on TA instruments Q200 DSC at heating rate of 5 °C per minute under a nitrogen atmosphere from -60 °C to 120 °C using aluminum pans.

Synthesis of polyglobalide (PGI) via enzymatic ring opening polymerization

A typical procedure for the polymerization of PGI: Globalide (2.16 g; 9.5 mmol), Novozym 435 (80 mg) and toluene (1.8 g) were placed in a Schlenk flask, which was purged with nitrogen. Then the mixture was stirred and heated at 60 °C for 4 hours in an oil bath. To separate and inhibit the enzymes, DCM was added and Novozym 435 was then removed by filtration. Cold methanol was used to precipitate the polymer. This was then vacuum-dried at room temperature for 24 hours. Typical yields were around 60% and M_n 25,000 g/mol (SEC trace and $^1\text{H-NMR}$ see Figure S1 and S2, ESI).

Electrospinning of PGI and PGI-X

The electrospinning of PGI and PGI-X was performed using a Spraybase electrospinning machine with a stationary collector. PGI was dissolved in DCM or THF in concentrations ranging from 10 – 65% wt., a voltage range of 9-16 kV, flow rate 50-200 $\mu\text{L}/\text{min}$, and distance of the tip 10-20 cm.

The optimized electrospinning parameters for PGI were used as a reference to electrospun PGI-X. For this step PGI was dissolved in DCM at concentrations ranging from 50 – 60%wt., a voltage range 8-15 kV, flow rate 50-300 $\mu\text{l}/\text{min}$, and distance of the tip 15-20 cm were used. Two different reagents were applied as cross-linkers, ethylene glycol bis(mercapto propionate) (X-1) and 1,5- pentanedithiol (Xl-2), both were added to the polymer solutions at the proportion of 20% wt./wt. As photo-initiators two different solutions were tested, the 2,2 dimethoxy-2-phenyl acetophenone (P1) and (2,4,6 – trimethylbenzoyl) phenone oxide with 2-hydroxy-2-methylpropiophenone (P2) in a 1:1 wt/wt. The PGI samples were collected on aluminum foil and

PGI-X samples were collected in methanol. All the samples were then vacuum-dried for 48 hours and stored in a desiccator until further use.

X-Ray Diffraction (XRD)

XRD was carried out on a Bruker AXS D8 Advance with a 3kW tube with a copper anode using the K-alpha line. The sample was mounted on a six-axis goniometer and a locked coupled scan was used. The samples were then scanned through an angle theta and the detector was maintained at twice that angle theta.

Degree of Swelling

The dried samples of PGI-X were cut with dimension of 1x1 cm and weighted and immersed in 20 mL of DCM. The wet samples were blot dried with a filter paper and weighed again. The degree of swelling of the samples was defined using the following equation

$$SD = \left(\frac{M2 - M1}{M1} \right) \times 100$$

where M2 is the mass of swelled sample and M1 is the mass of dried samples.

Degradation of Electrospun PGI and PGI-X

To evaluate the degradation of the fibers, samples with dimensions of 1x1 cm were weighed and sterilized using UV-light for two minutes on each side. The samples were incubated in a PBS solution (0.01M, pH 7.4) for 90 days at 37 °C; three samples were used for each experimental point. After the hydrolytic degradation, the samples were rinsed with 50 mL of distilled water and dried under vacuum over 48 h. Degradation level was measured by the mass difference of the samples and converted to percentage of the initial weight.

Dye encapsulation

A sample of PGI-X with dimensions of 1x1 cm was immersed in a THF Rhodamine-B 0.1 wt.% solution for 10 min and dried under vacuum overnight. The dried sample was washed five times with 10 mL of water:ethanol 1:1 solution until an optically clear washing solution was obtained.

Indomethacin encapsulation

Drug loading capacity of the PGI-X was studied using Indomethacin (IND) as a hydrophobic model drug. The samples were prepared by cutting discs (8 mm diameter) from an electrospun mesh. In one method, 10% wt. of IND was incorporated in the polymer spinning solution and the IND loaded PGI-X collected as described. In the second method, 8 mm in diameter discs of PGI-X (n=3) were immersed for 6 h in solutions of IND in DCM at concentrations of 10 and 20%wt., respectively. To verify the drug loading capacity by swelling, aliquots of the drug solution before and after the swelling process were collected and analysed by high-performance liquid

chromatography (HPLC, Agilent 1120 Compact LC with a Phenomenex Gemini 5u C18 110 , LC 250x4.6mm Column). Ten microliters of the samples were injected and a solution of acetonitrile/PBS buffer pH 8 (50:50, v/v) was used as a mobile phase, the detector wavelength used was 254 nm, flow rate of 1 mL/min and run time of 10 min. The calibration curve was prepared using solutions of IND in the release medium ranging from 0.098 to 196 mg/mL.

Drug Release studies

The drug release performance of PGI-XI and PGI samples were analysed by HPLC as described earlier. The samples (n=5) were placed in Eppendorf tubes containing 2 mL of medium release solution (PBS and methanol 80:20, v/v) and then incubated at 37°C in a water bath shaker. The release medium was completely removed at predetermined time point and replaced with fresh solution.

Cell viability assays

Bone marrow derived porcine stem cells (MSCs) were isolated as previously described² and expanded in growth media consisting of high-glucose Dulbecco's modified Eagle's medium (DMEM) GlutaMAX supplemented with 10 % fetal bovine serum (FBS), penicillin (100 U/mL)-streptomycin (100 µg/mL) and 0.25 µg/mL amphotericin B (all Gibco, Biosciences) at 20% pO₂. Electrospun scaffolds (Ø 8 mm) were punched out from the electrospun mesh and sterilized by ethylene oxide (EtO). To prepare for cell seeding, scaffolds were hydrated by progressive washes in ethanol and several washes with ultra-pure water. Scaffolds were incubated in expansion media overnight before cell seeding. At the end of passage 2, cells were trypsinized and seeded

onto one side of the electrospun scaffolds at a density of 100,000 cells per scaffold. Cell-seeded scaffolds were maintained expansion medium at 20 % pO₂ for 7 days, with medium changed twice. MSC-seeded scaffolds were cultured on custom-made wells suspended above the underlying tissue culture surface.

Cell Viability and Metabolic Activity Assays

Live/dead imaging was performed to qualitatively evaluate cell viability. Cell-seeded scaffolds were stained with 4 mM calcein-AM (green: 'live') and 2 mM ethidium homodimer-1 (red: 'dead') in phenol-free DMEM for 1 h at 37 °C protected from light (Live/dead viability/cytotoxicity assay, Invitrogen). Prior staining and before imaging samples were washed with PBS. Samples were examined under a scanning confocal microscope (Olympus FV1000) at an excitation and emission wavelengths of 515 and 615 nm. Cell metabolic activity was quantified via reduction of Alamar Blue following manufacturer's instructions. The reduction of alamar blue was measured after 1 day in culture. Cells plated on tissue culture plates (TCP) were used as control (n = 5). The absorbance was read at 570 nm using 600 nm as a reference wavelength.

To assess cell proliferation, cell-seeded scaffolds were collected at day 1, 3, 7 and 14, blot dried and stored at -80°C. Scaffolds were digested overnight at 60°C using 3.88 U/ml papain (n = 4). DNA content was quantified via Picogreen assay (Quant-iT PicoGreen dsDNA Assay Kit, Invitrogen) according to manufacturer's protocol. The fluorescence emission intensity was measured at 528 nm on a plate reader (BioTek).

Statistics

Statistical difference was established at $p < 0.05$ by ANOVA followed by Tukey's multiple comparisons test (GraphPad Prism 5, GraphPad Software). All data are reported as means $\pm$ standard deviation.

Results and Discussion

Electrospinning operating parameters such as flow rate, intensity of the electric potential and distance between the electrode and the collector can influence the obtained results. Moreover, solution properties such as viscosity, conductivity, dielectric constant, solvent boiling point and surface tension, are important parameters that affect the experimental outcome.^{43,44} These parameters must be optimized for any new polymeric material. In this work, the enzymatic polymerization of globalide (GI) was applied resulting in an M_n of 25,000 g/mol ($\bar{D} = 2.1$). It should be noted that the preferred molecular weight of, for example, PCl in electrospinning is much higher ($\geq 80,000$ g/mol) to meet the required solution viscosity.^{45,46} The intrinsically higher solution viscosity of PGI compared to PCl for a comparable M_n allowed us to formulate solutions with a sufficient viscosity at a concentration of > 30 wt%. Below this concentration, the electrospinning process was unsuccessful irrespective of the other processing conditions.

Figure 1. Schematic illustration of the fabrication of crosslinked polyglobalide microfibers using in-situ UV thiol-ene crosslinking during electrospinning of polyglobalide.

As a first set-up, to optimize the parameters to electrospin PGI, the polymer was dissolved in dichloromethane (DCM) and extruded at various flow rates. It was observed that the polymer concentration influenced directly the fiber morphology and diameter (Figure S3, ESI). Comparing the images, at 40 wt% PGI concentrations (Figure 3A, B and C) beads occurred and the heterogeneity in fiber diameters tended to increase. This could be correlated to the viscoelastic and electrostatic forces to the surface tension, which results in the minimization of the surface area that tends to convert the liquid jet into spherical drops.⁴³ More homogeneous fibers were produced at 50 wt% polymer concentrations. A slower polymer feed rate allowed an increased time for solvent evaporation producing more well-defined microfibers (Figure 3E and F). Changing the solvent to THF also resulted in inhomogeneous fibers with uneven surfaces and

regions of fused fibers, due to the ineffective solvent evaporation during the fiber forming process. Optimal fiber characteristics were obtained using DCM, a voltage of 8 kV, working distance of 15 cm, flow rate of 50 $\mu\text{l}/\text{min}$, and polymer concentration of 50%wt., producing fibers of an average diameter of $7.89 \pm 0.97 \mu\text{m}$ (n=20).

Figure 3. SEM images of PGI-F obtained under different electrospinning conditions. The voltage (8 kV) and distance from the tip to the collector (15 cm) was kept constant while varying the concentration of the polymer solution and the flow rate. 40 wt.% in DCM with flow rate of 100 $\mu\text{l}/\text{min}$ (A), 50 $\mu\text{l}/\text{min}$ (B) and 25 $\mu\text{l}/\text{min}$ (C) and 50 wt% in DCM with flow rate of 100 $\mu\text{l}/\text{min}$ (D), 50 $\mu\text{l}/\text{min}$ (E) and 25 $\mu\text{l}/\text{min}$ (F).

We next investigated the possibility of *in-situ* cross-linking of the fibers. Following previous work within the group on the functionalization of PGI with multi-functional thiols,⁴² we decided to test the incorporation of ethylene glycol bis(mercaptopropionate) (X1-1) with a commonly

used photoinitiator, 2,2 dimethoxy-2-phenyl acetophenone (PI-1). These were added to the polymer solution prior to electrospinning otherwise employing the previously determined optimized conditions. The solution jet and the collector was irradiated by UV light (365 nm) and the fibers collected on aluminium foil. It was observed that the fibers were inhomogeneous and were fused together (Figure 4A). In addition, the polymer solution was not stable and started to crosslink inside the syringe prior to electrospinning. Replacing PI-1 by a 1:1 wt/wt mixture of photoinitiators diphenyl(2,4,6-trimethylbenzoyl)phosphine oxide and 2-hydroxy-2-methylpropiophenone (PI-2) resulted in a more stable polymer solution, however fused fibers were still observed on the collector plate (Figure 3B). Moreover, polymer fibers were still soluble in THF suggesting incompletely crosslinking. Therefore, XI-1 was replaced by 1,5-pentanedithiol (XI-2). The samples produced with the combination of XI-2 and PI-2 presented a better fiber morphology with less conglutinated networks (Figure 4C), however, again issues were observed with fused fibers. To overcome this, methanol, a non-solvent for PGI was used to collect the fibers directly after they passed through the UV light. This technique was first described by Theato to prevent fibers fusing on the collector for crosslinked electrospun polybutadiene fibres.²⁷ Under these conditions a homogeneous fiber (diameters of $9.2 \pm 1.6 \mu\text{m}$, $n=30$) morphology was obtained similar to the non-cross-linked fibers (Figure 4D).

Figure 4. SEM images of PGI-X fibers: (A) collected on aluminum foil using XI-1 and P-1, (B) collected on aluminum foil using XI-1 and P-2, (C) collected on aluminum foil using XI-2 and P-2 and (D) collected in methanol using XI-2 and P-2.

First evidence of a successful cross-linking reaction was obtained from the fact that the fibers were not soluble in THF anymore. Spectroscopically, cross-linking was confirmed from the characteristic Raman double bond bands (Figure 5) around 1660 cm^{-1} , which were significantly reduced for the cross-linked fibers (denoted as PGI-X) compared to the non-crosslinked fibers (denoted as PGI-F). Moreover, PGI-X samples display a signal at 1000 cm^{-1} characteristic of C-S bonds, which is not present in the spectrum of the PGI-F (Figure 5).⁴²

Figure 5. FT-Raman spectra of PGI-F (black) and PGI-X (red).

As a consequence of the crosslinking the degree of crystallinity of electrospun fibers was significantly reduced as evident from differential scanning calorimetry (DSC) and Wide Angle X-Ray Scattering (WAXS). While DSC melting peaks in the bulk PGI as well as in the PGI-F are located around 40°C, only a small residual melting transition is present in PGI-X (Figure S4, ESI). Similarly, WAXS spectra of bulk PGI and PGI-F display reflection peaks at 2θ values at 21 and 23, in agreement with the semicrystalline polymer structure, while no such peaks can be identified in PGI-X (Figure S5, ESI). In agreement with previous studies on cross-linked PGI bars, cross-linking significantly reduces the crystallinity by disturbance of the crystalline domains, so that nearly fully amorphous fibers were obtained.³² On the other hand, preliminary measurements confirm that the crosslinking resulted in an increase in the mechanical properties. The tensile strength and Young's modulus increased 194% and 40% respectively, however, more detailed studies are required to fully monitor mechanical properties of the materials (Figures S6-S8, ESI).

One important criterion to determine the feasibility of a tissue engineering material is the hydrolytic degradation rate. Original work showed that bars of poly(macrolactone)s were neither enzymatically or hydrolytically degradable due to their hydrophobic character as well as high crystallinity. However, when electrospun we observed hydrolytic degradability of both PGI-F and PGI-X microfibers, which were attributed to the higher surface area to volume ratio of the microfibers over previously tested materials. For PGI-F a small mass loss of 9.6% ($\pm 1.6\%$) of a 1 x 1 cm mesh sample in PBS buffer solution was found within the first 30 days. Over the next 30 days a similar mass loss rate was found after which the degradation slowed to a mass loss of 27.6% ($\pm 4.6\%$) after 90 days (Figure S9, ESI). PGI-X degraded slightly faster than PGI-F probably due to the amorphous character of the sample reducing 14.7% ($\pm 3.8\%$) of its initial mass over 30

days. The following mass loss was constant at 10% every 30 days after this, to a total mass loss of 34.3% ($\pm 1.5\%$) after 90 days.

Ultimately, the feasibility of using PGI for biomedical applications lies in its biocompatibility and nontoxicity. Adult mesenchymal stem cells (MSCs) have been used in preclinical models for tissue engineering of bone, cartilage, muscle, and other mesenchymal tissues with significant promise.⁴⁷ MSCs were seeded on PGI-F and PGI-X fiber meshes to assess their viability, metabolic activity and proliferation, to validate their use for tissue engineering applications. MSCs were seeded at a density of 1×10^5 cells/scaffold onto one side of the PGI-F and PGI-X fiber meshes. Live/dead staining and confocal imaging was performed to qualitatively evaluate cell viability (Figure 6A and B). MSCs remained viable on PGI-F and PGI-X electrospun fiber scaffolds after 24 h in culture. We observed minimal cell death in both substrates. Cell metabolic activity was measured using alamarBlue reduction assay. No significant difference between PGI-F and PGI-X in terms of cell viability according to their cell metabolic activity was detected (Figure 6C). We included a positive (cells on tissue culture plastic (TCP)) and a negative control (wells with no cells, labelled 'blank'). Long term viability and proliferation was quantified by measuring the DNA content on both PGI-F and PGI-X, using pico green assay for DNA quantification. In both scaffolds, DNA content was higher after 14 days of culture compared to day 1 (Figure 6D). All together these results confirm that PGI-F and PGI-X support cell viability and proliferation.

Figure 6. Viability and proliferation of MSCs on PGI and PGI-X. Representative live/dead images of cells at day 1 in (A) PGI and (B) PGI-X (live: green; dead: red, indicated with arrows). Alamar blue reduction readings in the scaffolds after 1 day in culture are shown in (C), blank and cells in tissue culture plates (TCP) are included as controls. DNA amount at day 1 and day 14 are plotted in (D). Data is reported as mean $\pm$ standard deviation; * $P < 0.05$ ($n = 5$).

These first results highlight that PGI eletrospun fibers are a potential alternative scaffold material to PCL or PGLA in tissue engineering applications. Moreover, the possibility of crosslinking PGI opens opportunities not achievable with conventional polyesters. SEM micrographs in Figure 6 demonstrate that, while some deformation of the fibers is apparent, the fibrillar structure

and the fiber dimensions remain unchanged even after evaporation of the THF. While PGI-F and PCL fibers dissolve in THF (Figure S10, ESI), PGI-X swell up to 14% and retains its structural integrity. This can potentially be exploited to load the hydrophobic fibers with hydrophobic active ingredients such as drugs to facilitate or support tissue regeneration. We demonstrated the feasibility of this process by the loading of PGI-X with Rhodamine B as a model molecule. In this process, a PGI-X fiber mesh was placed in a THF solution of Rhodamine B. After removal from the solution the mesh shows the typical red color of the dye (Figure 7C). The fiber mesh was then extensively washed with water:ethanol 1:1 to remove all surface adsorbed Rhodamine B until the washing solution was optically free of color (Figure 7G). The fact that the extensively washed fiber mesh still contains Rhodamine B suggests the incorporation of the dye into the fiber bulk by the soaking process.

Figure 7. SEM images of PGI-X before (A) and after swelling with THF (B), as well as PGI-X encapsulated Rhodamine-B before (E) and after (F) washing with DI water/ethanol 1:1 solution.

To further demonstrate the drug loading capacity and efficiency of PGI-X fibers, loading of the hydrophobic anti-inflammatory drug IND was studied. In one approach, loading was achieved in a conventional process by adding IND to the spinning solution. A drawback of this approach is that the maximum loading capacity of the drug in the fibers is limited by its solubility in the spinning solution, in this case it was 10% (wt/wt) of IND relative to the total mass of PGI used. Increasing the loading capacity is not easily possible as increasing the amount of solvent would compromise the spinning outcome. In the second approach, PGI-X fibres were soaked in a dichloromethane (DCM) solution of IND at concentrations of 10 (PGI-X-10) and 20% wt/v (PGI-X-20). The actual loading was determined by monitoring the IND concentration in the solvent before and after loading by HPLC. The results shows that 21 ± 2 mg IND per 100 mg PGI could be loaded in the PGI-X-10 sample, while 35 ± 4 mg per 100 mg PGI was loaded in the PGI-X-20, which is significantly higher than by the direct spinning approach (10 mg IND/100 mg PGI). To quantify how much IND was indeed located inside the fibres, the samples were placed in a buffer/methanol solution and the IND release recorded by HPLC (Figure 9). An initial burst release regime comprising release of physisorbed drug and diffusion of drug near the surface is seen for the first 120-180 min. for all samples.⁴⁸ The burst release from PGI-X-10 and PGI-X-20 was around 65 $\mu\text{g}/\text{mg}$ PGI and 140 $\mu\text{g}/\text{mg}$ PGI, respectively, which were higher than for the PGI-F sample (10 $\mu\text{g}/\text{mg}$ PGI) due to greater amount of physisorbed IND on the fibre surfaces. By subtracting the burst release from the total amount of IND loaded into the samples, the total amounts of IND incorporated in the bulk of the fibers can be calculated which will only be released upon fibre erosion (not further studied here),^{49,50} *i.e.* 90 $\mu\text{g}/\text{mg}$ PGI for PGI-F, 145 $\mu\text{g}/\text{mg}$ PGI for PGI-X-10, and 205 $\mu\text{g}/\text{mg}$ PGI for PGI-X-20. These experiments highlight not only that

a higher drug loading is achievable by the swelling approach using crosslinked fibres but also that the total amount of drug can be controlled by the drug concentration in the swelling solution.

Figure 9. Release profiles of indomethacin loaded fibers in PBS and methanol 80:20, v/v encapsulate during electrospinning (PGI-F-Ind.) and by swelling in DCM at 10 (PGI-X-10) and 20% wt/v (PGI-X-20).

Conclusions

We have demonstrated that it is possible to electrospin unsaturated PGI and by *in-situ* UV-triggered thiol-ene reaction produce crosslinked fibers. Initial biocompatibility and degradation tests confirm the viability of these materials for biomedical applications such as tissue engineering and drug delivery systems as an alternative to commercial aliphatic polyesters. The benefit of crosslinking was highlighted by the possibility of solvent swelling under retention of

the fiber morphology. This opens possibilities to load fibers with active ingredients post spinning thereby omitting the need to optimize spinning protocols for each drug/polymer formulation. Moreover, higher loading capacity as well as the opportunity to vary loading amounts by their concentration in the swelling medium mark an advantage over conventional polyester fibers.

ASSOCIATED CONTENT

Supporting Information. The following files are available free of charge.

Additional thermo and mechanical analysis, fiber diameters and swelling images (PDF)

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ACKNOWLEDGMENT

F.C.S.O. acknowledges funding from the Brazilian Government-CAPES Grand Number N⁰ 13695/2013-04. S.D.K. acknowledges support from European Union's Horizon 2020 Framework Program for Research, Technological Development and Demonstration (Marie Curie Intra-European Fellowship for Career Development, 656259). The authors acknowledge support from Spraybase and the Science Foundation Ireland Research Centre CURAM (Grant Number 13/RC/2073). This work was supported by the Irish Research Council (IRC) [IRCSET P/G S/Ship.13]. C.J.K. acknowledges funding from European Union for a Marie Curie European Reintegration Grant under H2020 (Project Reference 659715), RCSI's Office of Research and Innovation Seed Fund Award (Grant Number GR 14-0963) and Science Foundation Ireland (AMBER, SFI/12/RC/2278). G.P.D and T.dS acknowledge funding from Science Foundation

Ireland (AMBER, SFI/12/RC/2278). The authors would like to thank Dr. Brendan Twamley from Trinity College Dublin for the XRD analysis and Dr. Larisa Florea from Dublin City University for the Raman spectroscopy analysis.

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