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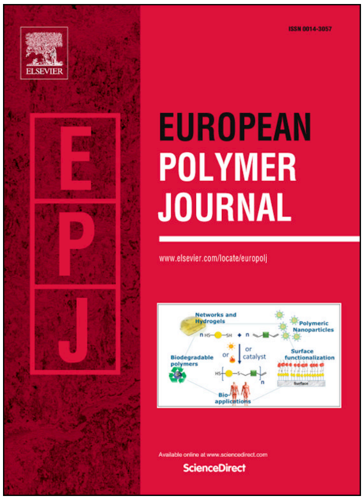

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PII: S0014-3057(18)32203-1
DOI: https://doi.org/10.1016/j.eurpolymj.2018.12.030
Reference: EPJ 8762

To appear in: *European Polymer Journal*

Received Date: 12 November 2018
Revised Date: 11 December 2018
Accepted Date: 18 December 2018

Please cite this article as: Li, Y., Vergaelen, M., Schoolaert, E., Hoogenboom, R., De Clerck, K., Effect of crosslinking stage on photocrosslinking of benzophenone functionalized poly(2-ethyl-2-oxazoline) nanofibers obtained by aqueous electrospinning, *European Polymer Journal* (2018), doi: https://doi.org/10.1016/j.eurpolymj.2018.12.030

Effect of crosslinking stage on photocrosslinking of benzophenone functionalized poly(2-ethyl-2-oxazoline) nanofibers obtained by aqueous electrospinning

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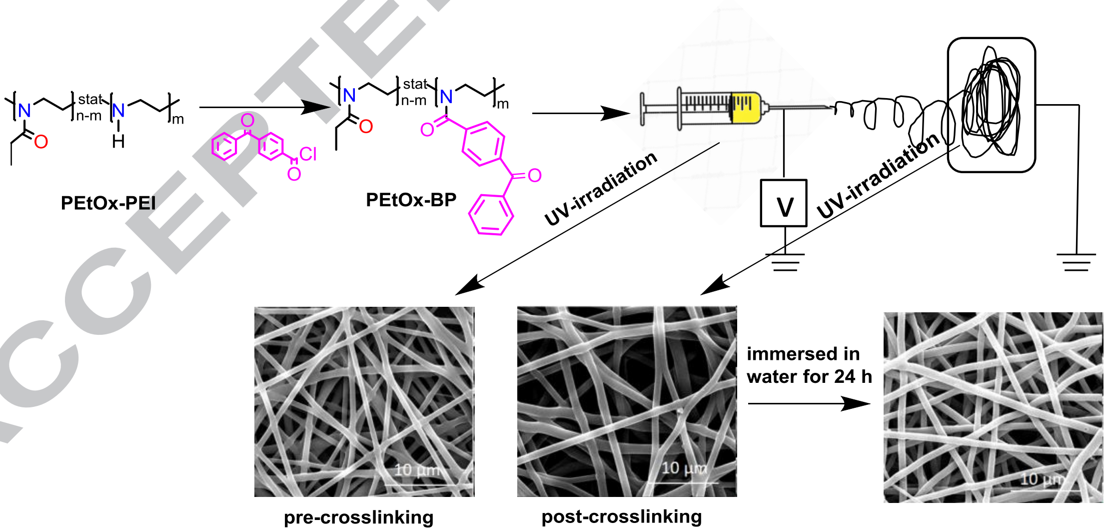
Graphical abstract



Abstract

In this study, benzophenone was introduced onto partially hydrolyzed poly(2-ethyl-2-oxazoline) (PEtOx-PEI) to prepare a poly(2-ethyl-2-oxazoline)-benzophenone (PEtOx-BP) copolymer, which was used to produce water stable nanofibers *via* aqueous electrospinning and photocrosslinking. Three different ultraviolet (UV) irradiation methods, *i.e.* pre-crosslinking before electrospinning, in-situ crosslinking during electrospinning and post-crosslinking after electrospinning, were used to prepare crosslinked nanofibers. The influence of UV-irradiation at these different stages of the nanofiber production process was investigated in terms of alterations in viscosity, nanofiber morphology and water stability of the fibers. It was shown that pre-crosslinking the polymer solutions had a great influence on the solution viscosity which could both positively or negatively alter the stability of the electrospinning process. Whereas the strategy of crosslinking nanofibers during the production process did not lead to uniform nor water-stable nanofibers, the pre-crosslinking and post-crosslinking strategies greatly increased the water stability of the nanofibers. In both techniques the crosslinking density and therefore water solubility can be easily tuned by manipulating the polymer concentration, UV-irradiation time and membrane thickness. Complete insolubility, *i.e.* the formation of crosslinked networks, was achieved by the post-crosslinking strategy. This work provides straightforward methods to increase the water stability of the PEtOx nanofibers, which will definitely be of great value to biomedical applications such as drug delivery and tissue engineering.

Keywords: electrospinning; poly(2-ethyl-2-oxazoline); benzophenone; UV-irradiation; crosslinking; nanofibers

1. Introduction

Electrospun (nano)fibrous membranes are characterized by a small diameter and high porosity resulting in a large specific surface area, which makes them attractive for biomedical applications, including tissue engineering, wound care, and drug delivery[1-8]. Solvent electrospinning is the most commonly applied technique for the production of such nanofibers[9]. For future industrial applications of electrospinning as well as for biomedical applications of the nanofibers, the use of green, non-toxic solvents, such as water, becomes increasingly important[10]. Naturally derived biopolymers, such as collagen, silk fibroin and gelatin, have recently been used to form electrospun nanofibers from aqueous solutions[11-13]. On the other hand, also synthetic, water-soluble polymers, such as poly(2-alkyl/aryl-2-oxazoline)s (PAOx) and poly(N-isopropylacrylamide) (PNIPAM), have been used to prepare nanofibers based on electrospinning from aqueous solvent systems[14, 15]. These synthetic polymer based electrospun materials offer a versatile alternative to naturally derived biopolymers [16-18]. For instance, they have been applied as an artificial scaffold for tissue engineering or as a medium for drug delivery as they are characterized by low cytotoxicity, good cell viability and biocompatibility [19, 20].

The major disadvantage of using water-soluble polymers for electrospinning is, however, the lack of water stability of the produced nanofibers, which impedes their use in humid environments or in direct contact with water. A crosslinking treatment to improve the water stability of the nanofibers is therefore desired. In general, crosslinking of electrospun nanofibers is performed either before or after the production process [21, 22]. Several crosslinking strategies have been reported, including ultraviolet (UV-) irradiation or dehydrothermal treatment (DHT), and using crosslinking agents such as dicumyl peroxide (DCP), glutaraldehyde (GA) and citric acid (CA) [23-26].

This study focuses on poly(2-ethyl-2-oxazoline) (PEtOx), which is a biocompatible, hydrophilic member of the PAOx class and can be synthesized by cationic ring-opening polymerization (CROP) of the corresponding 2-ethyl-2-oxazoline monomer[27]. PEtOx is very hydrophilic, enabling electrospinning from water. Additionally, the polymer is characterized by high thermal and mechanical stability, low toxicity and biocompatibility, making it an interesting material for many applications including biomedicine [28-32]. Aqueous electrospinning of PEtOx was already reported in earlier work [14, 33]. However, the produced PEtOx nanofibers are not stable in moist environments due to their inherent hygroscopic and hydrophilic nature. The nanofibrous membranes should therefore be crosslinked to enable their use in long-term biomedical applications. Hoogenboom et. al. described the successful and straightforward partial hydrolysis of PEtOx to obtain poly(2-ethyl-2-oxazoline)-stat-polyethyleneimine (PEtOx-PEI) copolymers, which contain secondary amine moieties which can be subsequently functionalized[34]. With the help of crosslinking agents, the polymer chains can subsequently be crosslinked *via* these reactive moieties. In previous work, we developed water-stable PEtOx nanofibers *via* in situ photoinitiated radical thiol-ene crosslinking during electrospinning from a DMF/THF solution[16]. Recently, we reported aqueous electrospinning of selenol-functionalized PEtOx which were in situ crosslinked in air during the electrospinning process[35]. However, both approaches have their limitations: the first approach was non-compatible with an aqueous electrospinning process while the PEtOx-PEI-SeH polymer solutions prepared in the second approach were unstable and difficult to be preserved for a long time. Furthermore, the crosslinked nanofibers obtained by these two approaches significantly swelled after being immersed in deionized water. Finally, the reported methods required significant organic synthesis to either prepare a functional 2-oxazoline monomer or a selenolactone reagent. Therefore, in this study, we aimed to develop a simpler, more stable and controllable strategy to enable crosslinking of PEtOx nanofibers electrospun from water.

In this work, the hydrolysis of PEtOx into PEtOx-PEI is followed by covalent attachment of the photoinitiator benzophenone (BP) to the secondary amine groups. Crosslinking is subsequently achieved by UV-irradiation before, during or after the electrospinning process, leading to radical addition of benzophenone to neighboring polymer chains. In general, it is accepted that UV-treatment of benzophenone leads to its radical induced addition to alkyl chains, albeit different mechanisms have been reported [36, 37]. Most reported UV-crosslinking procedures for nanofibers were performed during the electrospinning process [38-40], although there are also a few studies available on UV-crosslinking post nanofiber preparation [26, 41]. The present work studies the three different strategies and, as such, provides insights on the UV-irradiation effects at different stages, *i.e.* before, during and after the nanofiber production process. It is shown that the UV-irradiation time and PEtOx-BP concentration both influence the nanofiber morphology as well as the degree of crosslinking. The present work aims to demonstrate the feasibility of fabricating stable PEtOx nanofibers by a reproducible photo-crosslinking technology.

2. Experimental Section

2.1 Materials

Poly(2-ethyl-2-oxazoline) (PEtOx; tradename Aquazol® 200; M_w 200,000 and Đ 3-4) was supplied by Polymer Chemistry Innovations, Inc. (Tucson, USA). 4-Benzoylbenzoic acid, thionyl chloride (97%), dichloromethane (DCM), hydrochloric acid (HCl, 37%), sodium hydroxide (NaOH), sodium carbonate and toluene were purchased from Sigma Aldrich. All chemical reagents were of analytical grade and used as received.

2.2 Synthesis experimental

Synthesis of poly (2-ethyl-2-oxazoline)-co-polyethyleneimine (PEtOx-PEI)

According to the methods described previously [34], the partial hydrolysis of PEtOx was achieved as follows. First, the freeze dried PEtOx (50 g) polymer was dissolved in deionized water (500 mL) in a three-neck round-bottom flask equipped with a magnetic stirring bar and stirred at room temperature. After dissolution of PEtOx, 50 mL of HCl (37%) was added; the mixed solution was stirred at 75 °C for a reaction time of 2.5 h. Subsequently, the released propionic acid was neutralized (up to pH 9) with 1 mL of a 4 M NaOH solution. The neutralized product was dialyzed for 3 days. Finally, the PEtOx-PEI polymer was freeze dried from water, obtaining a white powder. The degree of hydrolysis in the PEtOx-PEI copolymer was determined by ^{1}H nuclear magnetic resonance (NMR) spectroscopy to be 4.5 mol% (Figure S1), calculated by the integral ratio between the peaks at 2.6−2.8 ppm (PEI backbone) and 3.2−3.5 ppm (PEtOx backbone)[42].

Synthesis of 4-benzoylbenzoyl chloride

In a typical acid chloride synthesis procedure, thionyl chloride (45 mL, 73.40 g, and 0.6169 mol) was added in a single portion to 4-benzoylbenzoic acid (15.01 g, 0.0663 mol) in dry DCM (150 mL), under an argon atmosphere. The reaction mixture was heated to reflux and stirred for 12 h. The resulting clear solution was concentrated by removing the excess thionyl chloride and DCM using a rotary evaporator. Upon removal of the residual amount of thionyl chloride with toluene (100 mL), pure 4-benzoylbenzoyl chloride was obtained as fine white crystals. This product was used as obtained without further purification.

Synthesis of the benzophenone functionalized poly(2-ethyl-2-oxazoline)-stat-(ethylene imine) copolymer (PEtOx-BP)

4-Benzoylbenzoyl chloride (2.49 g, 0.0102 mol) was added to PEtOx-PEI (30 g, 0.0069 mol PEI units) in dry DCM (400 mL), in total 1.43 excess of 4-benzoylbenzoyl chloride was used. Subsequently, 38.66 mL of triethylamine (TEA) was added dropwise. The reaction was stirred at room temperature for 3 days. During the preparation of PEtOx-BP, the flask was covered with aluminum foil to exclude the effects of (UV) light. The DCM was removed by a rotary evaporator, and the polymer was dissolved in deionized water. Then, the polymer was dialyzed and freeze dried. Conversion of PEtOx-PEI with 4-benzoylbenzoyl chloride was determined with ^{1}H NMR analysis to be 99 − 100% by comparing the integrated areas of characteristic signals of 2.6−2.8 ppm (PEI backbone) and 7.4−7.8 ppm (benzene ring) in Figure S1.

Aqueous electrospinning

The PEtOx-BP electrospinning solutions were prepared by dissolving the polymer in deionized water at various concentrations (15−30 wt%). Homogeneous solutions were obtained after stirring overnight at room temperature. During all experimental steps, the PEtOx-BP solutions were covered with aluminum foil. Electrospinning experiments were performed on a mono-nozzle setup with an 18-gauge needle, a tip-to-collector distance of 15 cm and a flow rate of 1 mL/h. The voltage was adapted in the range from 12 kV to 20 kV to enable a stable electrospinning process. To produce larger scale and thicker membranes (A4 size, 5 g/m^2) a rotating drum set-up was used with the same electrospinning conditions. All the electrospinning experiments were conducted at a relative humidity of 40 ± 10% and a temperature of 23 ± 3°C.

UV-irradiation

The applied UV-reactor was constructed from an 11 W UV-lamp. Pre-crosslinking was performed by irradiating the PEtOx-BP solutions prior to electrospinning. A bottle containing 10 mL of polymer solution was put in a 100 mL beaker, and the sides and top of the beaker were covered with aluminum foil. Meanwhile, the beaker was placed on a magnetic stirrer in a climate chamber at 23°C and 40% relative humidity. The UV-lamp was approximately 5 cm from the solution, and UV-treatment was carried out over 3, 6 and 12 hours. Crosslinking during the electrospinning process was carried out by placing two UV-lamps on both sides of the electrospinning jet. The distance between the needle and the UV-lamp was varied from 1cm to 20cm. Post-crosslinking of the produced nanofibers was performed by exposing the membranes to the UV-lamp at a distance of 3cm, under acclimatized conditions with the temperature fixed at 23°C and the relative humidity was varied from 30% to 90% relative humidity. The irradiation times varied from 10 min to 30 min.

2.3 Characterization

Water stability test

All the PEtOx-BP nanofibers (original samples, pre-crosslinked samples, post-crosslinked samples and samples crosslinked during the electrospinning process) were cut into samples with a size of 1 cm x 3 cm and immersed in 3 mL of deionized water at room temperature for prolonged periods of time.

Rheological measurements

The rheological behavior (viscosity) of the PEtOx-BP solutions was determined with an MCR302 rheometer (Anton Paar). The tests were performed with a shear rate ranging from 1 to 10 s^{-1} at a temperature of 25 °C. An upper, rotating measuring plate with a diameter of 25 mm was used together with a fixed parallel plate (diameter of 52 mm).

Scanning electron microscopy (SEM)

The morphology of the electrospun membranes was examined using an FEI Quanta 200F Scanning Electron Microscope (SEM) operating at an accelerating voltage of 20 kV. A gold coating was applied on all the samples using a sputter coater (Balzers Union SKD030) prior to SEM observation. The nanofiber diameters were measured using ImageJ software, and the average fiber diameters and standard deviations were calculated based on the SEM images by performing 50 measurements per sample.

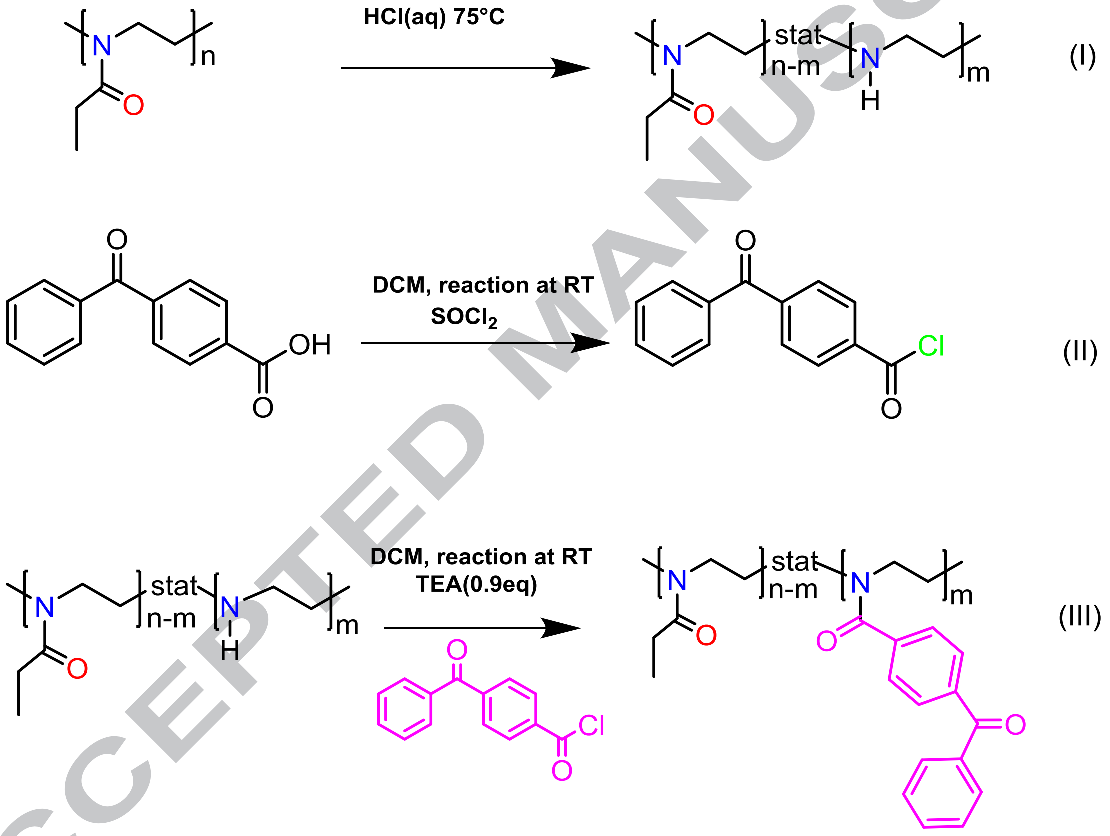
NMR spectroscopy

^1H NMR spectra were obtained on a Bruker Avance 400 MHz spectrometer with $CDCl_3$ as a solvent. Chemical shifts (δ) are expressed in ppm relative to the signal of trimethyl silane (TMS) used as an internal standard.

3. Results and Discussion

3.1 Synthesis of benzophenone functionalized poly(2-ethyl-2-oxazoline) (PEtOx-BP) copolymer

As shown in Scheme 1, the approach for the preparation of PEtOx-BP copolymers consists of a three-step synthetic protocol. In the first step, the partial hydrolysis of a commercial grade PEtOx polymer, *i.e.* Aquazol® 200, was performed under acidic conditions and basic work-up, as described in literature[34] (Step I of Scheme 1). In the second step, 4-benzoylbenzoyl chloride was synthesized to enhance the reactivity of the 4-benzoylbenzoic acid for the coupling onto the synthesized copolymer in step I (Step II of Scheme 1). In the last step, PEtOx-PEI and 4-benzoylbenzoyl chloride were mixed in dry DCM using TEA to deprotonate the secondary amine in the PEtOx-PEI copolymer to yield the PEtOx-BP copolymer (Step III of Scheme 1). The ^{1}H NMR spectrum (Fig. S1) of PEtOx-BP reveals the disappearance of the PEI signals at 2.6−2.8 ppm while new signals appeared at 7.4-7.8 ppm illustrating the successful incorporation of BP. From the ^{1}H NMR spectrum it could be calculated that a PEtOx-BP copolymer was obtained with an approximate content of 4.5 mol% benzophenone.



Scheme 1. (I), Partial hydrolysis of PEtOx, (II) activation of benzophenone acid with thionyl chloride and (III) reaction of PEtOx-PEI with 4-benzoylbenzoyl chloride (II).

3.2 Electrospinning and crosslinking of PEtOx-BP

The synthesized PEtOx-BP was subsequently electrospun from water at different polymer concentrations. As shown in Figure 1, the solution viscosity increased from 38 mPa·s to 3429 mPa·s with increasing PEtOx-BP concentration from 15% to 30% because of the increasing amount of polymer chain entanglements in the solution.

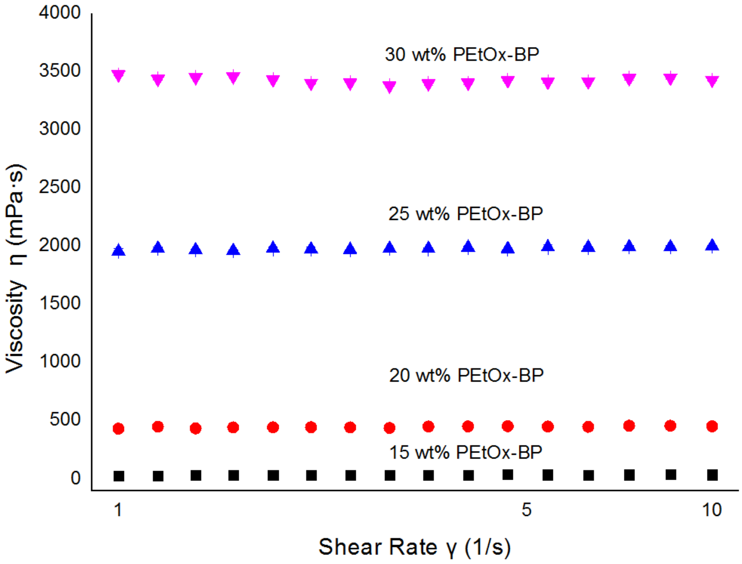


Figure 1. Viscosity versus shear rate plots of 15, 20, 25 and 30 wt% PEtOx-BP.

The respective SEM images (Figure 2) of the PEtOx-BP nanofibers obtained by electrospinning these solutions revealed that not enough chain entanglements were present in the 15 wt% polymer solution, resulting mainly in the formation of beads rather than nanofibers (Figure 2a). When the PEtOx-BP concentration was increased to 20 wt%, the number of beads decreased dramatically, and the formation of elongated beaded and fibrous structures with a diameter of 717 ± 492 nm was observed (Figure 2b). In a typical electrospinning process, the fiber morphology changes from beaded fibers to uniform fibers upon increasing the concentration or viscosity of the polymer solution[43]. Indeed, polymer solutions with 25 wt% and higher resulted in the formation of uniform, bead-free nanofibers (Figure 2c), whereby the nanofiber diameters increased as the concentration increased (from 735 ± 103 nm for 25 wt% to 811 ± 92 nm for 30 wt%). This increase in fiber diameter can be subscribed to the higher solution viscosity at higher polymer concentrations.

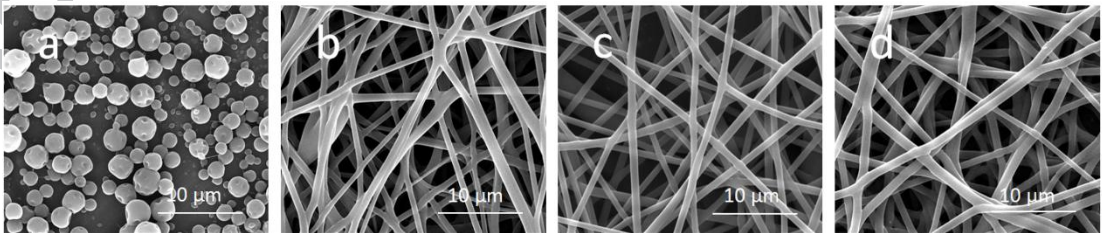


Figure 2. Representative SEM images of PEtOx-BP nanofibers: (a) 15 wt% PEtOx-BP, (b) 20 wt% PEtOx-BP, (c) 25 wt% PEtOx-BP, and (d) 30 wt% PEtOx-BP.

3.2.1 Pre-crosslinking of electrospinning solutions

Pre-crosslinking is realized by UV-irradiation of the PEtOx-BP solutions in a closed cabinet prior to electrospinning. The radical addition of benzophenone to neighboring polymer chains is utilized to covalently crosslink the polymer leading to an increase in molar mass and, as a result, the viscosity of the solutions is increased with longer UV-irradiation times. For example, before UV-irradiation, the viscosity of the 15 wt% PEtOx-BP solution was only 38.3 mPa·s (Figure 3), which inevitably led to the formation of beads during the electrospinning process (Figure 4a). However, upon prolonged UV-irradiation times of 3 hours and 6 hours the viscosity increased to 55.8 and 91 mPa·s respectively, which led to the formation of uniform nanofibers (Figure 4b and c). This viscosity (91 mPa·s) is still much lower than the viscosity needed to obtain uniform nanofibers from the non-crosslinked PEtOx-PEI solutions (1983.7 mPa·s), requiring a polymer concentration of 25 wt%. This difference indicates that the introduction of chemical crosslinks, over physical crosslinks (*i.e.* chain entanglements), enhances the electrospinability of the polymer. Hence, this explains the lower concentration needed for chemically crosslinked BP polymers in comparison to the non-crosslinked PEtOx polymer. Because of the lower viscosity also the nanofiber diameters are smaller (567±65 nm compared to 735 ± 103 nm of nanofibers spun from 25 wt% non-crosslinked PEtOx-PEI solutions). Furthermore, pre-crosslinking leads to the formation of branched polymers with higher molar mass that aids the formation of bead-free fibers[44]. The strategy of pre-crosslinking is thus a good alternative to spin low-viscosity polymer solutions that otherwise need a high polymer concentration for electrospinning. When the UV-irradiation time was further increased to 12 hours, the viscosity of the resulting solution became too high for electrospinning. Although the amount of crosslinking can thus be easily tuned by the UV-irradiation time, the degree of crosslinking is limited to the viscosity window available for stable electrospinning. SEM-images of the 25wt% and 30wt% pre-crosslinked nanofibers can be found in Figure S2.

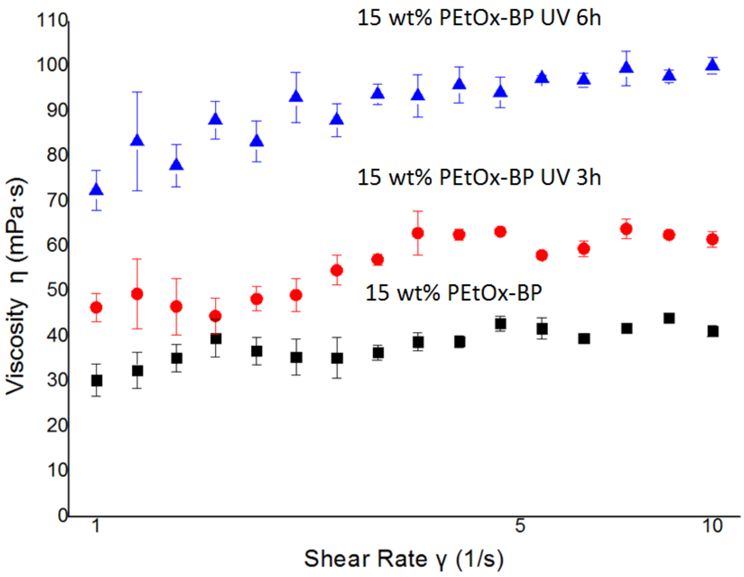


Figure 3. Plots of viscosity versus shear rate for 15 wt% PEtOx-BP solution and pre-crosslinking from 15 wt% PEtOx-BP solution of 3 and 6 hours UV-irradiation.

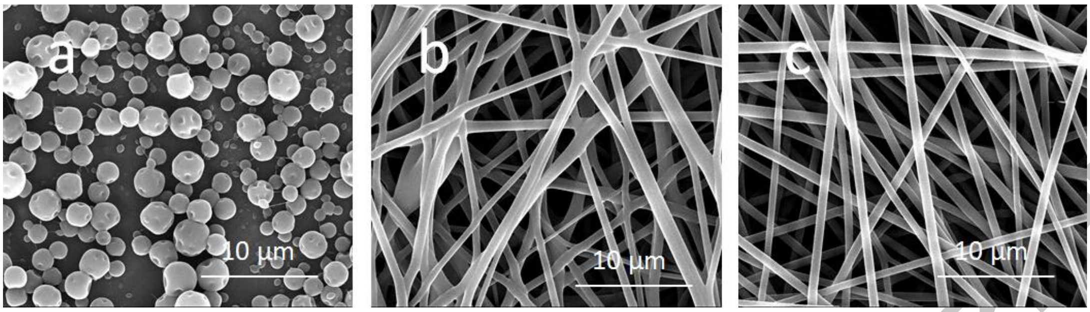


Figure 4. SEM images of pre-crosslinked nanofibers electrospun from 15 wt% PEtOx-BP solution: (a) 15 wt% PEtOx-BP untreated reference sample, (b) 15 wt% PEtOx-BP, exposed to UV-irradiation for 3 hours and (c) 15 wt% PEtOx-BP, exposed to UV-irradiation for 6 hours.

To test if crosslinking of the nanofibers by UV-irradiation was successful and sufficient to obtain stability in aqueous environments, the photo-irradiated membranes were immersed in water for several periods of time. In contrast to non-crosslinked PEtOx-BP nanofibers that immediately dissolve when added into water, the nanofibers electrospun from UV-irradiated polymer solutions did not dissolve instantaneously. The pre-crosslinked nanofibers electrospun from the 15 wt% polymer solutions dissolved slowly over a few hours while the pre-crosslinked nanofibers electrospun from 25 wt% and 30 wt% PEtOx-BP solutions even remained stable for two weeks. This increase in water stability can be ascribed to increased chain entanglements resulting from the higher molar mass of the partially crosslinked polymers and potentially from enhanced chain entanglements resulting from branching of the polymer structures (Figure S3). The extent of crosslinking is, however, limited by the respective increase in viscosity which eventually inhibits a stable electrospinning process. Although these results clearly indicate that pre-crosslinking the polymer solutions by UV-irradiation allows to increase the water stability of the produced nanofibers, complete insolubility cannot be achieved. On the other hand, this pre-crosslinking strategy enables to tune the crosslinking density easily by adapting the polymer concentration and UV-irradiation time to the requirements of the foreseen application. Such control over the dissolution time of the nanofibers may be beneficial for tuning drug release rates from such materials, which is the focus of ongoing work.

3.2.2 Crosslinking during electrospinning

Instead of crosslinking the polymer solutions prior to electrospinning, the nanofibers can also be crosslinked by so-called UV-irradiation on the fly. In this approach, a higher amount of crosslinks might be achieved without compromising the electrospinnability of the solution because of too high viscosities. For this strategy, the UV-lamp is placed close to the electrospinning jet with the distance between the UV-lamp and the needle being an important parameter of the process as also the UV-lamp can be seen as a grounded collector. Although this strategy sounds promising, the electric field was highly disturbed by the presence of the UV-lamp resulting in an unstable electrospinning process. Although this crosslinking strategy resulted in some interesting fiber morphologies such as very short fibers, the sample reproducibility was too low (Figure S4) to enable future upscaling and was therefore not further investigated. Further the water stability tests showed the obtained membranes dissolved within a few seconds after immersion in deionized water. As such the strategy of crosslinking the nanofibers during electrospinning did not lead to uniform nor water-stable nanofibers.

3.2.3 Post-crosslinking of electrospun nanofibers

Because electrospun nanofibers are characterized by a high specific surface area, UV-irradiation of the membranes after electrospinning may be anticipated to result in a high degree of crosslinking that may not be achievable by the previous strategies. For post-crosslinking the diameter of PEtOx-BP nanofibers was found to be an important parameter, which was regulated by controlling the concentration of the PEtOx-BP solutions in this study. Meanwhile, the relative humidity during photo-crosslinking was found to be another key parameter and the relative humidity was set from 30% to 90% as shown in Table 1. Crosslinking did not occur when the relative humidity was below 60%, presumably due to limited chain mobility at lower relative humidity. A high relative humidity showed to be critical to allow for crosslinking and PEtOx-BP nanofibers with a larger diameter could be successfully crosslinked at a relative humidity between 65% and 75%. The thinner PEtOx-BP nanofibers were more difficult to crosslink, ascribed to a too small number of polymer chains to get a homogeneous crosslinking of the PEtOx-PEI with rather low BP functionalization degree. When the relative humidity during crosslinking was further increased above 80%, the PEtOx nanofibers disappeared in a few minutes due to the fast water-uptake and subsequent dissolution of the nanofibers, already before the crosslinking reaction could occur. In this study, the optimal conditions for photo-crosslinking of the electrospun PEtOx-BP nanofibers were found to be 23°C and 70% relative humidity as controlled in a climate chamber. Under these conditions, the morphology of the nanofibers electrospun from 25 wt% and 30 wt% PEtOx-BP solutions was well-preserved during a post-crosslinking treatment of 10 min (Figure 5). However, longer irradiation times of 30 min led to loss of nanofiber integrity due to exposure to the increased temperature produced by UV-irradiation, again posing a limit to the possible amount of crosslinking (Figure S5). The loss of fiber integrity could be prevented by producing thicker nanofibrous membranes. This even allowed for an improved crosslinking of the smaller diameter nanofibers, electrospun from 20 wt% PEtOx-BP solutions, by exposing them for a prolonged time (Figure S6a). Where the thinner nanofibrous membranes already lost their integrity after 10 min of UV-irradiation, the morphology of the thicker membranes (with a similar fiber diameter) was not altered by the post-crosslinking treatment (Figure S6b).

Table 1. Overview of the results obtained for post-electrospinning photo-irradiation of the electrospun PEtOx-BP nanofibers(15-30 wt%) at 23°C and different relative humidity.

Samples	Below 60%	Between 65% and 75%	Above 80%
15 wt% PEtOx-BP	non-crosslinked	non-crosslinked	dissolved
20 wt% PEtOx-BP	non-crosslinked	poor-crosslinked	dissolved
25 wt% PEtOx-BP	non-crosslinked	crosslinked	dissolved

30 wt% PEtOx-BP	non-crosslinked	crosslinked	dissolved

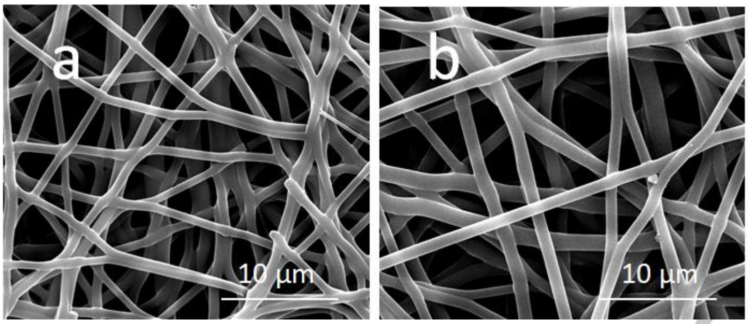


Figure 5. SEM images of the post-crosslinked nanofibers electrospun from 25 wt% and 30 wt% PEtOx-BP solution with a 10 min UV-irradiation: (a) 25 wt% PEtOx-BP nanofibers and (b) 30 wt% PEtOx-BP nanofibers.

Where the post-crosslinked membranes electrospun from 15 wt% PEtOx-BP solutions dissolved instantaneous on contact with water, the samples based on 20 wt% PEtOx-BP solutions partially dissolved in water after 6 hours of immersion, probably because the nanofibers were not yet perfectly uniform and the crosslinking density was not high enough to provide sufficient water stability. However, thicker electrospun membranes, based on the 20 wt% solutions, were stable in water for at least 24 hours without loss of fiber integrity (Figure 6a). This again indicates that thicker nanofibrous membranes are less prone to solubilization as was observed for the post-UV-treatment at 70% relative humidity. Further increasing the fiber diameter resulted in a further improved water stability. For both the 25 wt% and 30 wt% polymer solutions the nanofiber integrity was preserved and these membranes remained stable even after submerging in deionized water for one month (Figure S7). Where a minor swelling is still noticeable for the post-crosslinked nanofibers electrospun from the 25 wt% polymer solutions (Figure 6b) the 30 wt% polymer solution based nanofibers did not show this swelling behavior at all, possibly because of their larger diameter and more efficient crosslinking (Figure 6c). It can thus be concluded that, for nanofibers with appropriate diameters, the post-UV-irradiation strategy enables sufficient crosslinking to provide complete long term water stability.

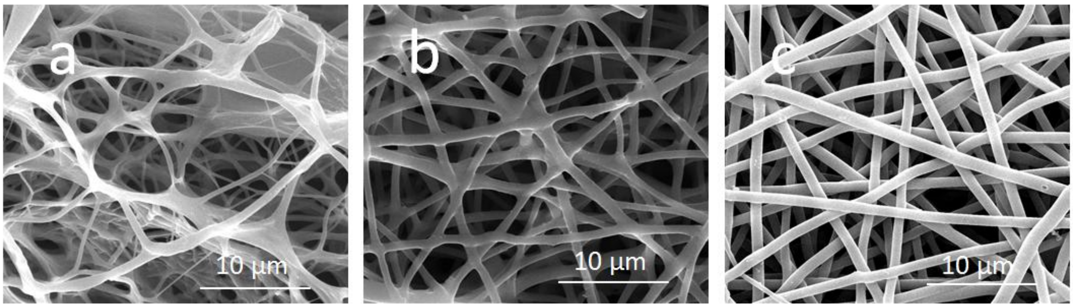


Figure 6. SEM images of the post-crosslinked nanofibers immersed in water for 24 h: (a) electrospun 20 wt% PEtOx-BP nanofibers (5 g/m^2), (b) electrospun 25 wt% PEtOx-BP nanofibers, and (c) electrospun 30 wt% PEtOx-BP nanofibers.

4. Conclusions

This work aimed to broaden the scope of PEtOx nanofibers by making them stable in aqueous environments through three different crosslinking strategies. First, crosslinking sites were introduced on the polymer backbone by partially hydrolyzing PEtOx, forming PEtOx-PEI statistical copolymers. Benzophenone was subsequently linked to these sites, enabling crosslinking of the polymer by UV-irradiation. UV-irradiation was then performed either before, during or after electrospinning from aqueous solutions, whereby it was found that no stable electrospinning process could be obtained for the approach where photo-crosslinking is performed during the electrospinning. Uniform, bead-free nanofibers were successfully obtained with both the pre-crosslinking and post-crosslinking strategy yielding water-stable nanofibers. The results indicated that the polymer concentration, UV-irradiation time and thickness of the membranes played an important role in the water-stability of the membranes. It was shown that the viscosity of the polymer solutions increased drastically during the pre-crosslinking treatment, which could enhance the electrospinnability of low-viscosity solutions. The pre-crosslinking strategy could not provide sufficient crosslinking density for complete insolubility due to viscosity limitations prior to the electrospinning process, yet enabled simple tunability of the crosslinking density and thus water solubility by altering polymer concentration or UV-irradiation times. With the post-crosslinking strategy on the other hand completely water-stable nanofibers were obtained. Thanks to these straightforward crosslinking strategies, water-insoluble PEtOx nanofibers can easily be produced, which greatly enhances their applicability in biomedical applications such as drug delivery and tissue engineering.

ASSOCIATED CONTENT

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Acknowledgments

YL acknowledges the financial support of the China Scholarship Council (201308410056). ES thanks FWO Strategic Basic Research grant 1S05517N. RH and KDC thank FWO Flanders and Ghent University for continued support of their research.

Data Availability

The raw/processed data required to reproduce these findings cannot be shared at this time due to technical or time limitations.

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Supporting information

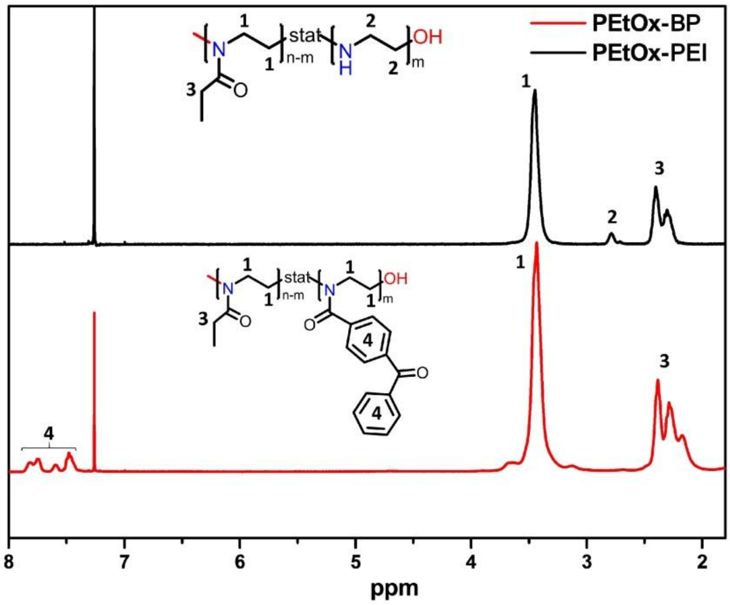

Figure S1. ^{1}H NMR spectra of PEtOx-PEI and PEtOx-BP.

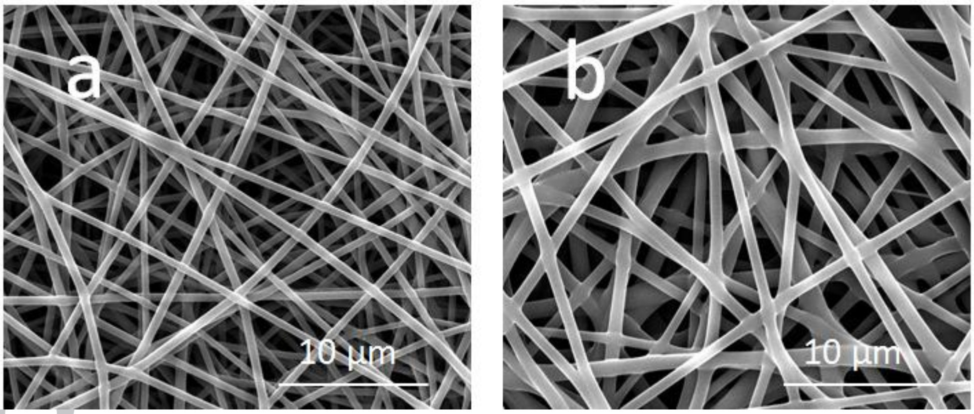

Figure S2. SEM images of pre-crosslinked nanofibers electrospun from 25 wt% and 30 wt% PEtOx-BP solutions, exposed to UV-irradiation for 6 hours: (a) 25 wt% PEtOx-BP and (b) 30 wt% PEtOx-BP.

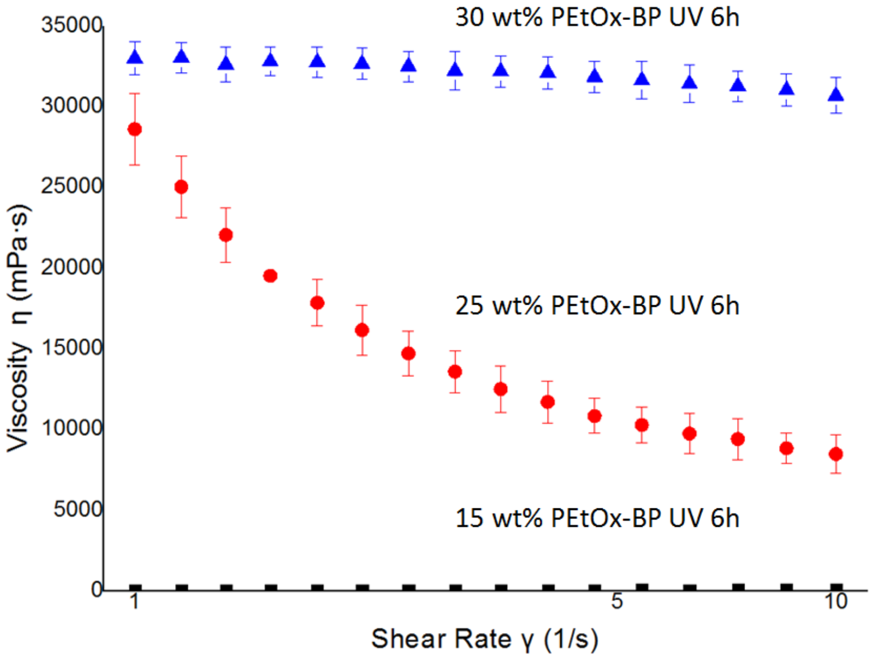

Figure S3. Viscosity versus shear rate plots of pre-crosslinked 15, 25 and 30 wt% PEtOx-BP solution of 6 hours UV-irradiation.

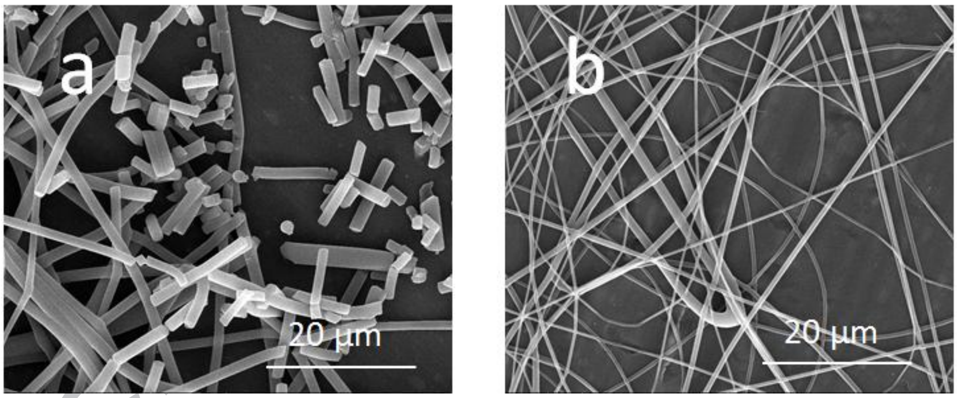

Figure S4. SEM images of 25 wt% electrospun PEtOx-BP nanofibers of UV-irradiation during electrospinning: (a) short nanofibers and (b) non-uniform nanofibers.

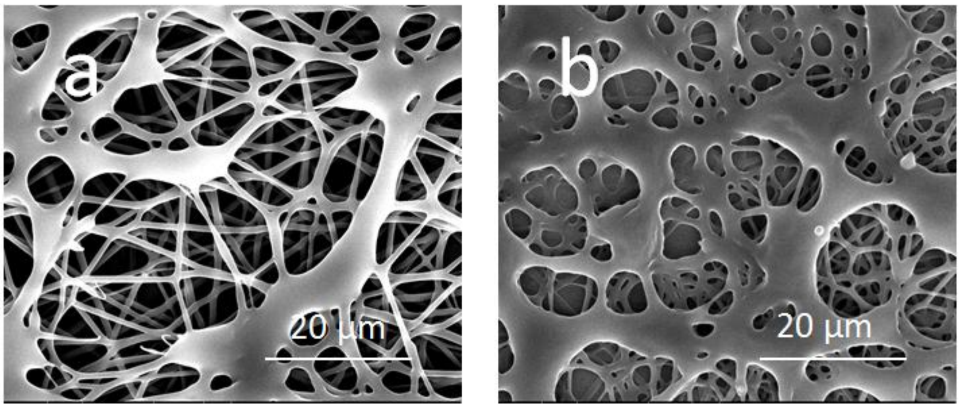

Figure S5. SEM images of the post-crosslinked nanofibers electrospun from 25 wt% and 30 wt% PEtOx-BP solution with a 30 min UV-irradiation: (a) 25 wt% PEtOx-BP nanofibers and (b) 30 wt% PEtOx-BP nanofibers.

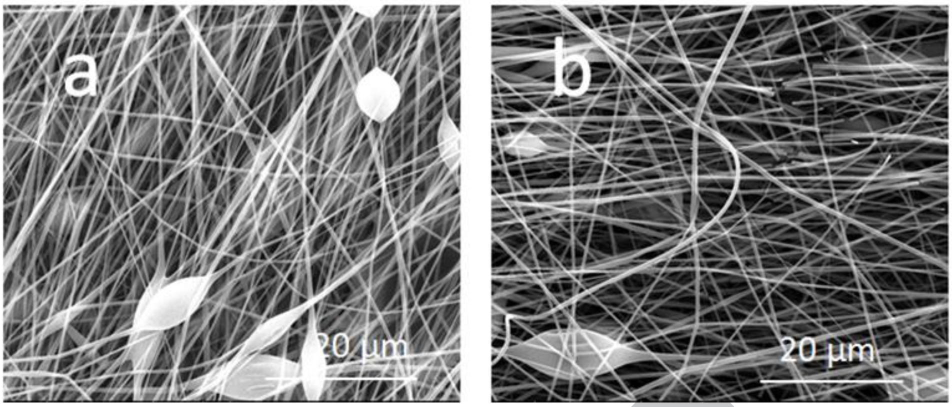

Figure S6. SEM images of 20 wt% PEtOx-BP nanofibers electrospun on a rotating drum: (a) 20 wt% PEtOx-BP nanofibers and (b) post-crosslinked nanofibers electrospun from 20 wt% PEtOx-BP solution with a 10 min UV-irradiation.

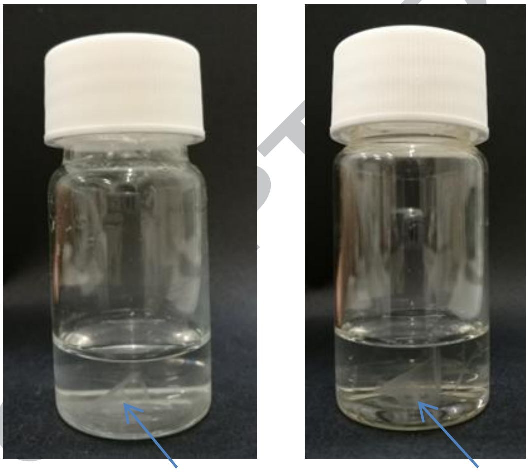

Figure S7. 25 wt% and 30 wt% post-crosslinked PEtOx-BP membranes immersed in deionized water for a month.

Highlights

- Functionalization of poly(2-ethyl-2-oxazline) with benzophenone to enable crosslinking
- Green electrospinning of poly(2-ethyl-2-oxazoline) nanofibers from water
- Study of the effect of pre-, in situ, and post-crosslinking by UV-irradiation respectively before, during or after the electrospinning process
- Different crosslinking stages provide variable crosslinking densities resulting in tunable water-stability of nanofibrous membranes, of great interest to biomedical applications