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Tailoring the wettability and mechanical properties of electrospun poly(L-lactic acid)-poly(glycerol sebacate) core-shell membranes for biomedical applications

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Tailoring the wettability and mechanical properties of electrospun poly(L-lactic acid)-poly(glycerol sebacate) core-shell membranes for biomedical applications

Abstract

Tissue and biomedical engineering fields are in constant mutation and in searching for innovative processing techniques capable to tailor the material properties. In this work, poly(L-lactic acid) (PLLA) and elastomeric poly(glycerol sebacate) (PGS) were dissolved in the same solvents and electrospun together, in a single needle system. A core-shell structure where the hydrophilic PGS was placed onto the surface of the hydrophobic PLLA fibre was obtained for elastomeric concentrations up to 25 wt%. It was found that the PLLA:PGS blends are immiscible and the blends present the melting temperatures of the individual polymers. Moreover, their surface properties were deeply influenced by the presence of the PGS, and a superhydrophilic membrane was obtained, after PGS curing at 120 °C for 48 h. When the concentration of PGS is up to 25 wt%, the blend's Young modulus decreases from $\sim 35.9 \pm 7.1$ to 7.5 ± 1.4 MPa and a twofold improvement in the sample stretchability was observed, compared with the pristine PLLA electrospun samples. Finally, *in vitro* hypothalamus A59 nerve cell culture shows that the core-shell electrospun samples enhanced cell adhesion and proliferation, suggesting that these developed materials have great potentials for nerve regeneration and biomedical engineering applications.

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Tailoring the wettability and mechanical properties of electrospun poly(L-lactic acid)-poly(glycerol sebacate) core-shell membranes for biomedical applications

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Abstract: Tissue and biomedical engineering fields are in constant mutation and in searching for innovative processing techniques capable to tailor the material properties. In this work,

poly(L-lactic acid) (PLLA) and elastomeric poly(glycerol sebacate) (PGS) were dissolved in the same solvents and electrospun together, in a single needle system. A core-shell structure where the hydrophilic PGS was placed onto the surface of the hydrophobic PLLA fibre was obtained for elastomeric concentrations up to 25 wt%. It was found that the PLLA:PGS blends are immiscible and the blends present the melting temperatures of the individual polymers. Moreover, their surface properties were deeply influenced by the presence of the PGS, and a superhydrophilic membrane was obtained, after PGS curing at 120 °C for 48 h. When the concentration of PGS is up to 25 wt%, the blend's Young modulus decreases from $\sim 35.9 \pm 7.1$ to 7.5 ± 1.4 MPa and a two-fold improvement in the sample stretchability was observed, compared with the pristine PLLA electrospun samples. Finally, *in vitro* hypothalamus A59 nerve cell culture shows that the core-shell samples produced enhanced cell adhesion and proliferation, suggesting that these developed materials have great potentials for nerve regeneration and biomedical engineering.

Keywords: Electrospun membranes, core-shell electrospun materials, superhydrophilic, nerve tissue engineering

1. Introduction

Polymer membranes and scaffolds are widely used in tissue engineering, drug delivery, wound dressing and other biomedical applications [1]. Different techniques are used to produce fibrous membranes, e.g. drawing, template synthesis, phase separation and self-assembly [2, 3]. Nevertheless, electrospinning seems to be the ideal processing method since it is cost-effective and allows the production of one-by-one continuous micro and nanofibers from various polymers, and it can be scaled to industrial production [2, 3].

Poly(glycerol sebacate) (PGS), is a ductile and biodegradable elastomer usually obtained by poly-condensation of glycerol and sebacic acid [4, 5]. The mechanical properties and biodegradation kinetics are deeply influenced by the temperature and time of the synthesis and curing process used. It was reported that the PGS Young modulus increases with the temperature and/or time of curing [6]. Previous studies reported that the curing of the PGS pre-polymer was performed at temperatures in the range of 90 – 150 °C, and at low pressure, for 24 up to 96 h [6].

Electrospinning PGS pre-polymer does not lead to the production of polymeric fibres due to their low molecular weight [6, 7], and has a glass transition temperature below room temperature, which promotes flow to the polymer chains, ultimately giving origin to a polymer film [8]. Moreover, cross-linked PGS is insoluble in organic solvents [6, 8], and is not possible to produce a suitable solution for electrospinning.

Core-shell electrospinning can use an electrospinnable polymer as a carrier to produce electrospun fibres of non-electrospinnable materials. Due to the high temperatures used for the PGS curing process, the carrier material needs to be thermally stable and with a melting transition above the temperature used for the PGS curing.

Different strategies are being developed to produce PGS electrospun membranes, based in the blend of the PGS pre-polymer blend with a suitable carrier, that can form fibres and facilitates the fibre formation. Jeffries et al. [8] blended PGS pre-polymer with poly(vinyl alcohol) (PVA) in a common 1,1,1,3,3,3-hexafluoroisopropanol (HFIP) solvent, and the electrospun sample was submitted to a cross-linking procedure, followed by removal of the carrier polymer. PVA:PGS overall fibre diameter was around 2.8 µm, with high extensions of fibre fusion, especially between their contact points.

Poly(methyl methacrylate) (PMMA) [9] and gelatin [10] were also used as carrier materials to form electrospun PGS fibres membranes. When PMMA was used, the average fibre

diameter of the electrospun membranes was ~ 200 nm, with a strong hydrophobic behaviour, which was probably due to the presence of higher amounts of PMMA on the surface of the fibres [9]. When gelatin was used as a carrier polymer for the PGS pre-polymer, an increase of the average diameter of electrospun fibre and hydrophilicity was observed [10].

Poly (L-lactic acid) (PLLA) was also used as a carrier polymer to produce the PGS electrospun fibre membranes. Xu et al. [11] demonstrated that core-shell electrospinning was a feasible technique to immobilise the PGS pre-polymer on the surface of the PLLA fibres. In their work, they electrospun the PLLA in the outer layer of the needle and the PGS prepolymer was fed onto the shell of the needle, immobilising the PGS prepolymer in the core of the produced PLLA fibres. After cross-linking at 130°C for three days, fibres with an average diameter of $10\text{ }\mu\text{m}$ were obtained, and the membranes produced presented high extensions of fibre fusion.

The objective of this work is to provide a novel, reliable and reproducible method to produce electrospun PLLA:PGS membranes, where the PGS elastomer is immobilised on the surface of the PLLA fibre. The PGS prepolymer was blended with the PLLA solution and electrospun together in a single needle system, followed by PGS cross-linking. The effect on fibre average diameter of the PGS pre-polymer concentration in the electrospinning solution was systematically assessed. Moreover, the influences of the PGS layer on the fibre membranes wettability, thermal and mechanical properties were studied. Finally, biological assays demonstrated that the core-shell samples produced have great potentials for nerve regeneration and biomedical engineering.

2. Experimental

2.1. Materials: Medical degree poly(L-lactic acid) (PLLA, Purasorb PL18, $M_w = 217 - 225$ kDa, from Corbion Netherlands), glycerol, sebacic acid, N,N-dimethylformamide (DMF),

dichloromethane (DMC), hexane and formamide (from Sigma-Aldrich Australia) were used as received.

2.2. PGS synthesis: For PGS prepolymer preparation, equimolar glycerol and sebacic acid were mixed in the flask at 120 °C under argon for 24 h, and used without further purification during the materials processing. PGS prepolymer was cured in the oven (Shel Lab, Model 1401D) at 120 °C under vacuum, for 48 h to prepare cross-linked PGS films.

2.3. PLLA and PGS:PLLA electrospun membranes: PLLA pellets were dissolved in a mixed solvent of DMF and DMC (3/7 v/v) to achieve a solution with a final polymer concentration of 10 wt%. PGS:PLLA solutions were prepared by adding the desired amounts of PGS prepolymer (0, 25, 40 and 50 wt%) to the PLLA solution prepared according to the method described above. The PLLA and PGS:PLLA were dissolved at room temperature with the help of a magnetic stirrer (Velp, Model MST) until complete dissolution. After, the solution was transferred to a commercial 10 mL glass syringe fitted with a steel needle (inner diameter 500 µm). Electrospinning system was set at 1.67 kV/cm with a high voltage power supply (Gamma High Voltage). A syringe pump (KDScientific) fed the polymer solutions into the needle tip at the rate of 0.5 mL/h. Randomly aligned PGS:PLLA fibres were collected in ground collecting plate 15 cm away from the needle tip. All experiments were conducted at 21 ± 2 °C and a relative humidity of $43 \pm 5\%$.

PGS cross-linking was performed as per the method described above for the PGS film. Briefly, the samples were placed inside a vacuum oven at 120 °C, for 48 h. The same procedure was also performed to a neat PLLA to understand the effect of the curing temperature.

2.4. Materials characterisations: Surface morphology of the samples was analysed by scanning electron microscopy (JEOL-6490LV), after deposition of a thin layer of platinum (10 nm) on the sample surface (Dynamac Sputter Coater). For sample cross-section analysis, the material was immersed in the liquid nitrogen for around 10 min for thorough cooling, followed by breaking material, and then attached to the cross-section holder for platinum coating, followed by morphology observation using JEOL JSM-7500.

Fibres diameters were measured using ImageJ [12] based on the surface morphology SEM images with at least 100 fibres included for each sample.

Thermal gravimetric analysis (TGA) was carried out using TA instruments TGA (Q500). Samples of around 10 mg were prepared and heated from room temperature to 600 °C at 20 °C/min. Differential scanning calorimetry (DSC) experiments were conducted by TA instruments (Q100). Samples of around 5 mg were placed inside 50 µL aluminium pans, and heated between – 50 and 200 °C at a heating rate of 10 °C/min. All experiments (TGA and DSC) were performed under a nitrogen atmosphere.

The PGS:PLLA electrospun samples and PGS film, were cut into dog-bone shape of 2 mm wide and 18 mm for the gauge length. Quasi-static tensile tests were conducted by Shimadzu EZ-L mechanical tester using the 2 N load cell (for PGS) and 10 N load cell (for fibres) with the cross-head speed of 1 mm/min at room temperature. At least three measurements were conducted for each sample.

2.5. Samples wettability: Water contact angle was measured using goniometer (DataPhysics OCA 20 contact angle system from Germany) to study the surface properties of the fibrous samples. At least five measurements were done for each sample.

2.6. *In vitro* biocompatibility: All the fibrous samples were cut into round shape with a diameter of 6.4 mm. To remove the possibly left unreacted monomers, the samples were immersed in ethanol for around 2 h, followed by washing twice with phosphate-buffered saline (PBS, pH = 7.4) and then mechanically stirred in PBS for 12 h. Samples were sterilised with ultra violet light ($\lambda = 254\text{ nm}$) for 20 min each side before cell seeding. Adult mouse hypothalamus neurons A59 cell line (mHypoA-59, Cellutions biosystems) was used to examine the *in vitro* biocompatibility. The samples were put on the bottom of the wells of 96 well plate and 1000 cells were seeded for each well. Cells were incubated in 100 μL Dulbecco's modified Eagle medium (DMEM) (D5796, Sigma-Aldrich) at 37 °C and 5% CO_2 for 5 days with the medium refreshed on day 2 and day 4. The cell culture wells with cells only were used as control.

Methotrexate (MTX) assay was performed on day 1, day 3 and day 5. 20 μL MTX solution was added into each well and the culture plate was incubated at the same condition for 2 h. Supernatants were collected to detect absorbance value via using Spectromax plus 384 plate reader. Cell viability of the samples after 1, 3 and 5 days was obtained by comparing the optical density values of the sample groups to control group.

The outgrowth and morphology of the cells were observed by JEOL-6490LV. On day 1, day 3 and day 5, cells were fixed with 4% paraformaldehyde in Dulbecco's PBS for 20 min at room temperature and then the samples were dehydrated by immersion in a series of ethanol solutions (50, 75, 90, 95 and 100%), dried at room temperature and coated with a platinum thin film of 10 nm on the surface for SEM observation.

2.7. *Statistics and Data Analysis:* Statistical analysis was performed by a Kruskal-Wallis test with a Dunn's post hoc test, using GraphPad Prism 6.01 (GraphPad Software, Inc.).

Statistical differences were assigned to the experimental groups with $p < 0.05$.

3. Results and discussion

3.1. Morphology and structural analysis

In this work, PLLA polymer and PGS prepolymer were mixed in the same solution and electrospun together. PLLA electrospun fibre mats showed a random distribution and cylindrical, smooth fibres, in the absence of bead defects, and the thermal annealing at 120 °C, and during 48h (simulating the PGS curing procedure) does not influence the fibre morphology (Figure 1a). Moreover, when 25 wt% PGS prepolymer is added to the polymer solution and electrospun together, smooth fibres without the presence of bead defects are obtained (Figure 1b). However, when the prepolymer concentration increases above 25 wt%, spots of prepolymer randomly distributed within the fibre membrane were observed, increasing their size with the increase of the amount of prepolymer added to the electrospun solution (Figure 1c and d).

The PGS curing procedure at 120 °C didn't alter the fibre morphology, or removed the PGS prepolymer spots from the membrane. On the other hand, for the sample with 25 wt% PGS, it was observed that the PGS polymer was dragged by the PLLA polymer during electrospinning, and after curing, the elastomeric material was placed on outside, and the PLLA was on the core of the electrospun fibre, creating a core-shell structure, in a single electrospinning step (Figure 1e). Usually, core-shell fibre area obtained by feeding two independent solutions through a core-shell needle, where one polymer is fed in the core of the needle and the other is fed on the outside chamber of the needle, and both solutions, only met on the edge of the needle [13, 14].

The processing method developed to electrospun the PGS:PLLA polymer, creates a core-shell structure in a continuous manner without clogging the needle tip and avoids the search for solvent compatible for electrospinning two different solutions [14].

The influence of the amount of PGS prepolymer in the final electrospun fibre diameter was measured over 100 fibres from different SEM images with the help of Image J. It was observed that the pristine PLLA electrospun fibres had an average diameter of 746 ± 274 nm, and with the incorporation of PGS prepolymer, a reduction in the fibre average diameter down to 332 ± 103 nm was observed for the sample with 50 wt% PGS (Figure 1f). This reduction in the fibre diameter shows that the PGS prepolymer probably affects charge density of the solutions. PGS prepolymer can be ionised and carry more charges, stretching the polymer droplet even further, resulting in a decrease of the average fibre diameter of the core-shell structure. Similar effect was observed for mixture of polymers with ceramic fillers, e.g. PLLA with glass reinforced hydroxyapatite [15], or for PLLA zeolite nanocomposite samples [16]. In comparison with other works, the average diameter of the electrospun core-shell fibres obtained in this work, was remarkably thinner. Previous works reported a fibre average diameter between ~ 2 and $8 \mu\text{m}$ for PGS electrospun membranes produced with PCL [17] or PVA [8] as carrier polymers.

Figure 1:

The effect of the PLLA:PGS electrospinning processing parameters can be monitored by Fourier transform infrared (FTIR) spectroscopy in Attenuated Total Reflectance (ATR). PLLA absorption band at 1757 cm^{-1} can be assigned to the $\text{C}=\text{O}$, attributed to the amorphous regions of the polymer chains, while the characteristic absorption band for the amorphous α and α' was observed at 1183 cm^{-1} ($\nu_{as}(\text{C}-\text{O}-\text{C}) + \nu_s(\text{CH}_3)$) and 1092 cm^{-1} ($\nu_s(\text{C}-\text{O}-\text{C})$), respectively [18]. PGS elastomer presents a characteristic absorption band at 1736 cm^{-1} , associated to the stretching of the carbonyl ester group ($\text{C}=\text{O}$) and the absorption band at 1185 cm^{-1} is due to the $\text{C}-\text{O}$ in-plane bending vibration [19]. Moreover,

there is also a broad absorption band between 3300 and 3800 cm^{-1} for hydroxyl bond stretch vibration [20].

The electrospun fibre membranes present the characteristic absorption bands of the core PLLA and the shell PGS. No new absorption band was totally suppressed or new ones appeared in the core-shell PLLA:PGS electrospun mats, suggesting that the blends between these polymers are difficult to occur. Moreover, for the PLLA:PGS, two shoulders were observed between the 1757 cm^{-1} (C = O) of PLLA and the carbonyl ester absorption band of PGS (1736 cm^{-1}), which is in line with the spectra of the pure polymers (Figure 2).

Figure 2:

3.2. Thermal properties

The evolution of the thermal degradation of the PLLA, PGS and PLLA:PGS was assessed by thermogravimetric analysis (TGA). PLLA presents a major weight loss in the temperature range of 250 - 350 °C, and no water adsorption was observed around 100 °C (Figure 3a). Moreover, PGS polymer presented a single degradation process between 350 - 500 °C, and no significant dehydration process was observed at lower temperatures (≤ 100 °C, Figure 3a) [21]. PLLA:PGS core-shell fibres presented two thermal degradation steps; the first one between 200 and 350 °C, associated to the degradation process of the PLLA polymer core [6, 17], and the second one between 350 - 500 °C, due to the thermal degradation of the PGS shell layer. It was also noticed that for the core-shell PLLA:PGS samples, the T_{onset} , calculated by extending the pre-degradation portion of the curve to the point of the interception with a line drawn as a tangent to the steepest portion of the mass curve occurring during degradation, started at lower temperatures for the PLLA:PGS fibres, when compared to

the pristine materials (Table 1), suggesting that the thermal stability of the core-shell membranes is lower when compared to the neat polymer constituents, which is probably due to interaction between the PLLA core and PGS filler at the interface of the core-shell fibres (Figure 3a).

Figure 3:

Differential scanning calorimetry was performed to the different samples to assess the evolution of the glass transition (T_g), cold crystallisation and melting behaviour of the pristine materials and core-shell fibre samples (Figure 3b). PLLA pristine fibres present a glass transition around 55 °C, followed by the melting of the crystalline lamellas around 150 °C. The absence of the cold-crystallisation region in the electrospun PLLA area is due to the annealing procedure performed to the samples at 120 °C for 48 h (Figure 3b) [18, 22]. Furthermore, pristine PGS samples show a melting temperature (T_m) around 5 °C (Figure 3b) with an associated enthalpy of fusion of 27.1 J.g⁻¹, which is in the range of the values found in literature [23]. The PLLA:PGS fibre membranes present the characteristic T_m of the PGS and PLLA polymers, and no significant shift in the T_g of the PLLA core material is noticed in Figure 3b. Our results suggest that the PLLA and PGS polymers do not interact between each other, which corroborate the SEM results (Figure 1), where a clear PGS hollow fibre was observed.

The degree of crystallinity (ΔX) of the pristine PLLA and PLLA:PGS electrospun samples, after cross-linking under vacuum for 48 h and at 120 °C was calculated with the following equation:

$$\Delta X = \frac{\Delta H_m}{\Delta H_m^0} \quad (1)$$

where, ΔH_m is the sample melting enthalpy, and ΔH_m^0 the enthalpy for a 100 % crystalline PLLA sample (93 J.g^{-1}) [21, 22]. After cross-linking, the PLLA polymer in the PLLA:PGS electrospun samples presented a crystallinity degree of 44 %, independently of the amount of PGS in the shell of the core-shell fibre structure. The value calculated through Equation 1 is similar to the value reported for the PLLA electrospun samples annealed for 24 h at 140°C [24].

Table 1: Onset temperatures and weight residue of PLLA and PLLA:PGS samples.

Amount of PGS (wt%)	T_{onset} ($^\circ\text{C}$)	Residues at 400°C (%)
0	305.2	2
25	255.4	19
40	248.6	30
50	254.5	36
100	398.6	83

3.2. Mechanical properties

The mechanical behaviour of scaffolds is a key parameter to evaluate the suitability of the developed material for biomedical applications. In this work, quasi-static mechanical analysis was performed to the PLLA, PGS and to the PLLA:PGS core-shell electrospun membranes. Table 2 lists the values for the Young's modulus (E), the stress at break (σ_{break}) and strain at break (ε_{break}) for all cured membranes. Our results suggest that when an amount of PGS up

to 40 wt% is added to the electrospinning solution, the ductility of the polymeric blend is improved compared to the pristine PLLA electrospun fibres. For instance, the cured PLLA:PGS membranes with 25 and 40 wt% show an approximately 2-fold increase of the strain at break when compared to the pristine PLLA. Moreover, the incorporation of the PGS into the PLLA:PGS membranes leads to a decrease of the σ_{break} when 25 wt% PGS is incorporated into the polymer solution, however, for higher PGS concentrations, there is an increase of the stress at break, probably due to the reduction of the pore size and the presence of the PGS agglomerates, present not only on the surface of the material but also through the bulk of the membrane (Figure 1). Frydrych et al. [25] found that the amount of PGS added to the PLLA:PGS scaffold influences the polymeric blend mechanical behaviour. In their work, they found that the incorporation of higher amounts of PGS, led to a reduction of the pore diameter, and consequently to an increase of the mechanical stiffness and reduction of the strain at break of the prepared scaffolds.

Table 2: Mechanical properties of electrospun fibres and PGS.

Amount of PGS (wt%)	σ_{break} (MPa)	ϵ_{break} (%)	E (MPa)
0	2.6 ± 0.3	29 ± 6	35.9 ± 7.1
25	1.1 ± 0.2	66 ± 8	7.2 ± 1.4
40	6.8 ± 0.2	52 ± 9	379.0 ± 29.9
50	6.7 ± 1.1	13 ± 5	336.2 ± 25.8
100	0.18 ± 0.01	219 ± 8	0.08 ± 0.01

3.4. Wettability properties

Membrane surface properties and wettability are important features to assess the potential of the developed membranes for biomedical applications, because they play a vital role in cell proliferation and adhesion [26]. The average water contact angle (WCA) measured for the PLLA electrospun fibres was $125 \pm 2^\circ$, revealing a strong hydrophobic behaviour, probably due to the overall membrane roughness [15, 22]. Moreover, the PGS polymer film showed to have a hydrophilic behaviour, with a WCA of $42 \pm 3^\circ$. Interestingly, for all PLLA:PGS electrospun samples, the water drop placed on top of the membrane was not stable and the membrane was capable of uptake of almost instantaneously the liquid. The results reveal that the incorporation of PGS onto the shell of the PLLA:PGS fibres improves the wettability of the electrospun membranes significantly, and no WCA measurements and drop penetration test are possible to perform on this superhydrophilic membranes. Similar results were reported for poly(caprolactone)-PGS electrospun fibres, and the superhydrophilic behaviour was attributed to the presence of the hydroxyl groups attached to the backbone of the PGS polymer, and due to the penetration and capillary effect of the porous membrane [20].

3.5. *In vitro* biocompatibility of PLLA:PGS fibrous blends

Biomaterial-cell interaction is affected by material roughness, surface free energy, hydrophilicity, among other material properties [27]. Thus, for tissue engineering and biomedical applications, it is highly desirable to have hydrophilic surfaces [22, 27]. In the present work, it was observed that the immobilisation of the PGS polymer onto the surface of the PLLA fibre gives origin to superhydrophilic membranes, capable to almost instantaneously uptake water.

A59 neuronal cell viability in PLLA and PLLA:PGS fibrous membranes was evaluated *in vitro* by using MTX assay, which gives an indication of cell metabolic activity. Cells were

seeded on the surface of the fibrous samples, and grown for one, three and five days, after which proliferation was assessed by measuring the metabolic activity of the cells with an MTT assay (Figure 4). After one and three days of incubation, the A59 neuronal cells cultured on the PLLA and PLLA:PGS fibrous mats showed a proliferation profile to the one obtained with the control, where the cells were cultured in a standard polystyrene (TCPS) wells. After five days of incubation, proliferation of cells grown on PLLA and PLLA:PGS electrospun membranes was slightly lower when compared to the control (Figure 4). Nevertheless, no significant differences were detected between the different PLLA and PLLA:PGS samples, which suggests that the presence of the hydrophilic PGS shell is suitable material for biomedical applications.

Figure 4:

The micrographs recorded with SEM, showed spread cells on the membrane surface (Figure 5), displaying the characteristic flat phenotype, with extended cytoplasm, and appearing to interact and associate with surrounding fibres (Figure 5) [28, 29]. Even at day 1, the morphology of the cells was dominated by cells in advanced stage of adhesion (Figure 5). It was possible to observe that cells were spread on the surface of the fibres, adopting a polygonal morphology, and adhered to the surface with lamellipodia and filopodia extensions. It was observed that in the case of the PLLA:PGS fibres, the cells spread out and elongated to form long processes of neurite varicosities, tried to interact with the electrospun fibres and communicate with neighbour cells through the nanofibers (Figure 5), suggesting that the developed membranes have the ability to induce the differentiation of A59 cells into neuron-like cells, even in the absence of growth factor or chemical treatments.

In the first few hours after culture, cells often attempt to explore the surroundings by dynamically extruding and retracting thin filopodia protrusions in arbitrary directions. As the cell begins to flatten, on the surface of the fibrous membranes, wider lamellipodia protrusions at the leading edge of the cell starts to appear and actively propel the cell front forward [30]. After 5 days, cells actively proliferated, and the establishment of intercellular contacts were identified within PLLA and PLLA:PGS electrospun membranes (Figure 5).

Figure 5:

4. Conclusions

PLLA and PLLA:PGS fibre structure was obtained by a single needle electrospinning system, which is versatile, reproducible and can be readily upscale, without compromising the membrane characteristics. It was found that the incorporation of PGS amounts up to 25 wt%, individual and defect free fibres were obtained, where the PGS shell wraps the PLLA core, while for higher amounts of PGS pre-polymer present in the electrospun solution, randomly dispersed agglomerates of PGS film was observed in the membrane samples. Further, the blends between the PGS pre-polymer and the PLLA are immiscible and a melting transition can be observed at $\sim 5^\circ\text{C}$ for the PGS shell, and at $\sim 150^\circ\text{C}$ corresponding to the PLLA fibre core melting. When PGS is added with a concentration up to 25 wt%, the blend's Young modulus decrease from $\sim 35.9 \pm 7.1$ MPa (pristine PLLA) down to 7.5 ± 1.4 MPa (core-shell membrane), and a two-fold improvement in the sample stretchability was observed, when compared with the pristine PLLA electrospun samples. Moreover, by incorporating the PGS polymer on the surface of the core-shell PLLA:PGS fibres, the wettability of the materials is enhanced, and a superhydrophilic material was achieved for the sample with

small amount of PGS. Finally, *in vitro* hypothalamus A59 nerve cell culture shows that the core-shell samples produced enhanced cell adhesion and proliferation, suggesting that these materials have great potentials for nerve regeneration and biomedical engineering.

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Figure 1: Electrospun sample surface morphology: a) PLLA annealed at 120 °C for 48 h, b) cured sample with 25 wt% PGS, c) cured sample with 40 wt% PGS, d) cured sample with 50 wt% PGS, (e) cross-section detail of the cured sample with 25 wt% PGS, and h) average fibre diameter $\pm$ standard deviation, measured with image J. Insets of a), b), c) and d) show a higher magnification of the electrospun samples.

Figure 2: Infrared spectroscopy measurements recorded for the PLLA, PGS and PLLA:PGS samples.

Figure 3: a) Thermogravimetric behavior, and b) differential scanning calorimetry recorded for the PLLA, PGS and PLLA:PGS core-shell fibres.

Figure 4: Proliferation of A59 neuronal cells cultured on PLLA, and PLLA:PGS electrospun membranes for 1, 3 and 5 days. Statistical analysis: * $p < 0.05$. Results are presented as the mean $\pm$ sd of three independent assays. Symbols (*) denote statistical differences between the experimental groups and their respective controls (C) obtained by Kruskal-Wallis test with Dunn`s multiple comparison post-hoc test ($p < 0.05$).

Figure 5: SEM micrographs of A59 neurons culture: 1 day cell culture on PLLA membrane (a); PLLA:PGS (25 wt% PGS) membrane (b); 5 days PLLA cell culture (c) and 5 days cell culture on PLLA:PGS (25 wt% PGS) membrane (d).