Fibrous Materials Made of Poly(ε-caprolactone)/Poly(ethylene oxide)-b-Poly(ε-caprolactone) Blends Support Neural Stem Cells Differentiation

1. Block Copolymers Characterization

1.1. Structure and Molecular Weight by 1H-NMR and GPC

The block copolymers have been characterized by proton nuclear magnetic resonance (1H-NMR, Bruker, 600 MHz) and gel permeation chromatography (GPC, Jasco). Samples for 1H-NMR characterization were dissolved in deuterated chloroform, and samples for GPC were dissolved in THF. Figure S1 shows the corresponding 1H-NMR spectra of the PEOₘ macroinitiator and the resulting PEOₘ-b-PCLₙ block copolymers. For the macroinitiator, the protons corresponding to the repeating unit of ethylene oxide moiety (Hₐ`) are observed at 3.67 ppm as a singlet, meanwhile the methoxy terminal protons (Hₐ`) appear at 3.41 ppm as a singlet, and the terminal hydroxyl proton (Hₐ`) appear at 2.16 ppm also as a singlet. The chemical shift corresponding to Hₐ` protons in the synthesized block copolymers are coincident with those of the macroinitiator; however, the corresponding resonance peaks of Hₐ` and Hₐ` are lost in the block copolymer spectrum, due to the low intensity of these resonance peaks. Protons Hₐ, Hₐ, Hₐ-t, and Hₐ of ε-caprolactone appear at 4.06 (t), 2.31 (t), 1.65 (m), and 1.38 (m) ppm, respectively.

Figure S1. Proton nuclear magnetic resonance (1H-NMR) spectra corresponding to the macroinitiators PEOₘ, and the corresponding of diblock copolymers.
The degree of polymerization of PEO (averaged number of repeating units, \( N_{\text{PEO}} \)) can be calculated comparing the intensities of the \(^1\)H-NMR resonance peaks of methyl end-group and the methylene groups. The results show good agreement with those reported by the supplier. Our own results are used in this work. Once \( N_{\text{PEO}} \) is known, similar magnitude for PCL (\( N_{\text{PCL}} \)) can be calculated using an integral method of \(^1\)H-NMR resonance peaks by comparing proton peak intensities of the PEO methylene repeating units (Hb) and that corresponding to, for example, proton Hc of PCL. The area or intensity of a proton resonance peak of a given species is proportional to the amount of these species present in the sample, and thus:

\[
A_{\text{Hb}} = k4N_{\text{PEO}} \\
A_{\text{Hc}} = k2N_{\text{PCL}}
\]

Rearranging,

\[
N_{\text{PCL}} = \frac{2N_{\text{PEO}}A_{\text{Hc}}}{A_{\text{Hb}}}
\]

\( M_n \) of the copolymers can be calculated as:

\[
M_n = N_{\text{PEO}}M_{EO} + N_{\text{PCL}}M_{CL} + M_e
\]

where \( M_{EO} \) is the molecular weight of ethylene oxide, \( M_{CL} \) is the molecular weight of \( \varepsilon \)-caprolactone, and \( M_e \) corresponds to the molecular weight of the PEO methyl end-groups. Data corresponding to the analysis of molecular weight of the block copolymers and polydispersity index (PDI) are shown in Table S1. For comparison, molecular weight measurements by GPC (THF as solvent), using narrow molecular weight PEO as standards, are also shown in Table S1.

**Table S1.** Molecular weight estimation of PEO\(_n\) and PEO\(_n\)-b-PCL\(_n\) block copolymers by integral \(^1\)H-NMR calculations and by gel permeation chromatography (GPC) measurements.

| Sample     | M / I | \( N_{\text{PEO}} \) | \( N_{\text{PCL}} \) | \( M_n \text{ 1H-NMR} \) | \( M_n \text{ GPC} \) | \( M_w \text{ GPC} \) | PDI  |
|------------|-------|-----------------------|-----------------------|---------------------------|------------------------|------------------------|------|
| PEO\(_{45}\) | -     | 45                    | -                     | 2,027                     | 1,607                  | 2,175                  | 1.35 |
| PEO\(_{45}\)-b-PCL\(_{11}\) | 10.84 | 45                    | 11                    | 3,415                     | 1,918                  | 3,007                  | 1.57 |
| PEO\(_{148}\) | -     | 148                   | -                     | 6,531                     | 3,585                  | 5,789                  | 1.61 |
| PEO\(_{148}\)-b-PCL\(_{13}\) | 10.84 | 148                   | 13                    | 8,259                     | 3,035                  | 6,366                  | 2.10 |
| PEO\(_{230}\) | -     | 230                   | -                     | 10,165                    | 5,954                  | 10,362                 | 1.74 |
| PEO\(_{230}\)-b-PCL\(_{184}\) | 180   | 230                   | 184                   | 34,031                    | 14,261                 | 23,460                 | 1.64 |

2. Internal Structure of PCL Fibers.

2.1. X-Ray Diffraction (XRD) and Small-Angle X-Ray Scattering (SAXS)

The internal crystalline structure of PCL fibrous materials has been characterized by XRD and SAXS. Figure S2 shows the x-ray diffractogram of the fibrous materials. Two peaks appearing at 20 of 21.98 and 24.22°, related with the (110) and (200) crystallographic planes of PCL are observed, characteristics of the orthorhombic lattice structure of PCL.
Figure S2. XRD diffractogram of fibers prepared using PCL394 (a), PCL394/PEO45-b-PCL11 (b), PCL394/PEO148-b-PCL13 (c), and PCL394/PEO230-b-PCL184 (d).

The Scherrer equation was used to estimate the thickness of the crystallite perpendicular to the lattice plane (L), according to:

\[ L = \frac{K\lambda}{\beta\cos\theta} \]  

where \( K \) correspond to a shape factor with a value of 1, \( \lambda \) represent the X-ray wavelength from monochromatic Cu K-\( \alpha \) radiation (0.154184 Å), \( \beta \) corresponds to the half at the maximum intensity (FWHM) of peaks observed in diffractogram, and \( \theta \) is the Bragg angle. Pristine PCL394 fibers present crystalline domains of 17 nm, meanwhile in those prepared using block copolymers of PEO45-b-PCL11 and PEO230-b-PCL184, the ordered domain increases from 19–21 nm to 22–26 nm, respectively, and a decrease to 9 nm in the presence of PEO45-b-PCL13 is observed.

2.2 Small-Angle X-Ray Scattering (SAXS)

Complementary to XRD, SAXS measurements were done in order to study the changes in the crystalline/amorphous domains of PCL394 fibers in the presence of block copolymers. In the Figure S3 it is shown the one-dimensional SAXS plots (1D-SAXS) of fibrous materials, where a clear peak centered around 0.50 nm\(^{-1}\) (\( q_{\text{max}} \)) is observed, characteristic of a lamellar morphology [7–9]. This peak is a result of the electron density differences between the crystalline and amorphous domains of PCL in the fibers [8]. The long period (\( L' \)) of semicrystalline PCL structure, corresponding to the periodicity of lamellar stacks, can be estimated from SAXS curves using the equation:

\[ L' = \frac{2\pi}{q_{\text{max}}} \]  

In the fibrous materials prepared using PCL394, the long period is approximately 12–13 nm. The peak observed in 1D-SAXS plots does not shift at higher or lower \( q \) in the presence of the block copolymers. However, an increase on the intensity in the low-\( q \) range is observed, due to fluctuations
on the electronic density, indicating a relative loss of crystallinity. The absence of high order peaks in the 1D-SAXS is an indicative of the lack of other lamellar superstructures.

**Figure S3.** Kratky small-angle X-ray scattering (SAXS) plot (represented as $I(q)q^2$ versus $q$) for PCL$_{394}$ (a), PCL$_{394}$/PEO$_{45}$-$b$-PCL$_{11}$ (b), PCL$_{394}$/PEO$_{48}$-$b$-PCL$_{13}$ (c), and PCL$_{394}$/PEO$_{230}$-$b$-PCL$_{184}$ (d).

In order to obtain a detailed description about changes in the lamellar packing of PCL$_{394}$ in the long-range order, in the absence and in the presence of the block copolymers, the one-dimensional correlation functions were computed using the cosine transformation from scattering intensity curves using:

$$K(r) = \frac{1}{Q} \int_0^\infty I(q)q^2 \cos(rq) dq$$  \hspace{1cm} (7)

Where $r$ represents the distance in real space, and $Q$ correspond to the invariant function, defined as:

$$Q = \int_0^\infty I(Q)Q^2 dQ$$  \hspace{1cm} (8)

As observed in Figure S4, the structural parameters such as the long period ($L_o$) and the lamellar thickness ($L_c$) are obtained from the first local minima ($K(r) < 0$) and the first local maxima (with $K(r) > 1$).
Figure S4. Normalized one-dimensional correlation functions, $K(r)$, for PCL$_{394}$ (a), PCL$_{394}$/PEO$_{45}$-b-PCL$_{11}$ (b), PCL$_{394}$/PEO$_{148}$-b-PCL$_{13}$ (c), and PCL$_{394}$/PEO$_{230}$-b-PCL$_{184}$ (d).

3. Thermal Analysis

3.1. Differential Scanning Calorimetry (DSC)

DSC measurements were done to finely characterize the melting and crystallization temperature of PCL$_{394}$ in the absence and in the presence of the block copolymers, allowing calculation of the crystallinity ratio from enthalpies of melting state, by comparing with the enthalpy of melting of 100% crystalline PCL (139.5 J/g). DSC heating and cooling cycles are shown in the Figure S5. The presence of the block copolymers in the fibers does not produce significant changes in the position of the melting and the crystallization temperatures. However, differences in the enthalpy of melting of bulk PCL$_{394}$ and PCL$_{394}$ fibrous materials are observed, suggesting that the processing of bulk PCL$_{394}$ into fibrous materials or in the presence of the block copolymers changes the crystallinity ratio. The blending of PCL with the block copolymers produces a reduction in the crystallinity ratio of the main component, achieving a 45% of crystallinity in materials prepared with the block copolymer PEO$_{45}$-b-PCL$_{11}$. Similar crystallinity ratios were obtained in PCL$_{394}$ blended with PEO$_{148}$-b-PCL$_{13}$ and PEO$_{230}$-b-PCL$_{184}$.
3.2. Dynamical Mechanical Analysis (DMA)

PCL is a semicrystalline polymer with characteristic transition temperatures such as the glass transition \( T_g \) and melting point \( T_m \). Although DSC is a powerful technique to characterize \( T_m \), it loses sensibility to characterize \( T_g \) \([10,11]\). We performed DMA measurement to characterize the glass transition of PCL in order to evaluate the effect of the block copolymers in these phase transitions. The results are shown in Figure S6 as storage modulus and \( \tan \delta \) as a function of temperature. Although high values of storage modulus are observed, these are related with the use of the metallic material pocket where the polymer samples were loaded for DMA measurement. The storage modulus of the studied samples decreased at around \(-50^\circ C\), associated to the glass transition of PCL. As long as the temperature increases, a second pronounced decrease on the storage modulus is observed around 50 \( ^\circ C \), assigned to the melting of PCL. Small shifts of the maximum of \( \tan \delta \) to lower and higher temperatures are observed when pristine PCL fibers are compared with those prepared using block copolymers.

**Figure S5.** DSC curves corresponding to bulk PCL (a), and fibers composed by pristine PCL (b), and those blended with the block copolymers PEO45-b-PCL11 (c), PEO148-b-PCL13 (d), and PEO230-b-PCL184 (e), respectively.
Figure S6. Storage modulus and \( \tan \delta \) as function of temperature of PCL\(_{394}\) (a), PCL\(_{394}\)/PEO\(_{45}\)-b-PCL\(_{11}\) (b), PCL\(_{394}\)/PEO\(_{148}\)-b-PCL\(_{13}\) (c), and PCL\(_{394}\)/PEO\(_{230}\)-b-PCL\(_{184}\) (d).

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