***Direct Identification of Three Crystalline Phases in PEO-b-PCL-b-PLLA
Triblock Terpolymer by In Situ Hot-Stage Atomic Force Microscopy***

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ABSTRACT

In this work, we provide a detailed description of the tri-lamellar nanoscale morphology of a triple crystalline PEO-*b*-PCL-*b*-PLLA triblock terpolymer obtained by Hot-Stage Atomic Force microscopy (AFM) imaging and Wide Angle X-ray scattering (WAXS) analysis for the first time. The precursor PCL-*b*-PLLA diblock copolymer has also been included in the study for comparison purposes. A two-step crystallization protocol has been applied to create a distinct lamellar morphology. Both WAXS and AFM revealed the double crystalline nature of the diblock copolymer. However, the identification of multiple crystalline phases in the triblock terpolymer by AFM and WAXS at room temperature is not straightforward. The advantages of hot-stage AFM allowed following the evolution of the lamellar morphology and the successive melting of the tricrystalline PEO-*b*-PCL-*b*-PLLA sample during heating. Taking into account the melting temperature of each crystalline block, the existing lamellar populations were clearly identified. At 45 °C, the thinnest lamellae disappeared, due to the melting of PEO crystals. The medium size lamellae disappeared at 60 °C when PCL crystals melt. At that temperature, the only remaining crystals are those of the PLLA block. AFM mechanical modulus images provide further evidence of the lamellar self-assembly of the triblock terpolymer. The nanoscale arrangement includes lamellae of PCL, PEO, or both in between the PLLA lamellae. Hot-Stage AFM is a valuable technique to elucidate the morphological features of complex multi-crystalline systems.

Keywords: PEO-*b*-PCL-*b*-PLLA triblock terpolymer; tri-lamellar morphology; Hot-Stage AFM; WAXS

INTRODUCTION

The morphology of block copolymers has attracted broad interest in the polymer scientific community in the last decade. The micro and nanostructural features are influenced by several factors, such as melt miscibility, crystallinity, composition, and thermal conditioning (i.e., crystallization, annealing, and thermal history in general). A wide range of different morphologies can be developed (such as lamellae, spheres, cylinders, 2D and 3D aggregates) that depend on whether the block copolymer is miscible or melt segregated, or if it is amorphous, semicrystalline or combined (double crystalline, double amorphous, crystalline-amorphous, etc.).¹⁻⁷

To study the morphology of block copolymers in real space, a series of techniques can be used, such as polarized light optical microscopy (PLOM), transmission electron microscopy (TEM), and atomic force microscopy (AFM). Among them, AFM is an imaging technique of high-resolution that not only substantially complements the interpretation of TEM and PLOM observations but also of other structural characterization techniques in reciprocal space, such as X-ray diffraction. AFM allows direct visualization of the nanoscale structure. Besides, one of the advantages of AFM is sample preparation. Unlike TEM, AFM does not require thin sectioning, etching, or staining. However, it should be kept in mind that AFM is a surface technique, and it might not represent bulk structural behavior. AFM applications in polymers include the study of polymer morphologies at micro and nanoscale level, the identification of molecular order in single crystals and oriented samples, individual polymer chains and branches, filled polymer systems and composites, polymer blends and block copolymers.⁸⁻¹⁰

AFM has been used to detect microphase-separated morphologies of block copolymers. Block copolymers display different morphologies depending on three main factors: copolymer composition and architecture, melt segregation strength, and thermal transitions (e.g., order-disorder, crystallization, and glass transition temperatures).^{3, 5} The ordered superstructure sizes range from micro to nanoscale level. For instance, AFM has been useful to detect several types of microdomain patterns in strongly segregated systems, such as spheres, “worm”-like, lamellae, etc., as well as, lamellar nanodomains in weakly segregated or melt miscible systems. Particularly, melt miscible (or weakly segregated) block copolymers with crystallizable blocks, the final morphology is a consequence of the crystallization conditions and the microphase segregation driven by the crystallization event. Employing suitable crystallization conditions, AFM examinations at room temperature reveal the self-assembly of the polymer chains into mixed axialitic or spherulitic-type superstructures composed of lamellar arrangements.^{2, 6, 7, 11-28}

A group of well-investigated melt miscible (or weakly segregated) double crystalline systems are the diblock and triblock copolymers composed of poly(L-lactide) (PLLA), poly(caprolactone) (PCL) and poly(ethylene oxide) (PEO). These diblock copolymers are of high interest because of their good physical properties and biodegradability. Extensive research has been published regarding the microscale morphology of these diblock copolymers and their corresponding homopolymers. Spherulites, banded or concentric spherulites, axialities, 2D aggregates, among others crystalline textures, have been reported.^{24, 27, 29-33}

For a few years, we have been investigating the complexity of the morphology and crystallization of unique ABC triblock terpolymers, in which the three blocks are able to

crystallize when the length of the blocks and the crystallization conditions are adjusted properly. To that purpose, model triblock terpolymers of PLLA, PCL, and PEO blocks (PEO-*b*-PCL-*b*-PLLA) have been exhaustively studied.^{4, 33-36} Particularly, the morphology of these melt miscible and triple crystalline triblock terpolymers have been reported by Chiang et al.³⁷ and Palacios et al.^{33, 35, 36} Wide-angle X-Ray scattering (WAXS) measurements, carried out during cooling, confirmed that the PLLA block is the one that crystallizes first, followed by the PCL and lastly, the PEO. PLOM observations indicated that the microscale structure is templated by the PLLA block as a result of its crystallization. The successive crystallization of the PCL and PEO blocks does not change the microscale superstructure templated by the PLLA block. The evidence of the crystallization of the other two blocks is that the magnitude of the birefringence varies.³³ Linear and cyclic diblock copolymers including PCL, PLLA and PEO also exhibit this behavior.^{11, 31, 32, 38-43} Chiang et al.³⁷ presented single crystals of PEO-*b*-PCL-*b*-PLLA triblock terpolymers crystallized from solution. A sequential layer-by-layer crystallization was observed by modifying the crystallization conditions (e.g., polymer-solvent interaction, fuming time).

Some features of the triple crystalline nanoscale morphology of PEO-*b*-PCL-*b*-PLLA triblock terpolymers have been reported by some of us in a previous publication.³⁵ Polymer sample films were crystallized from melt, and the final morphology was observed by AFM at room temperature. A tri-lamellar self-assembly that included lamellae of the three phases was indirectly elucidated from AFM observations complemented with SAXS experiments and theoretical simulations. From our observations, we proposed a lamellar arrangement that includes the alternation of only one lamella of either PCL or PEO in between two lamellae of PLLA. However, a clear identification of such peculiar variation of the lamellar crystalline

phases was not possible by only room temperature AFM observations. Therefore, the next step would be to apply a thermal scan to the lamellar morphology observed at room temperature to get further insight into the tri-lamellar structure and long-range order.

AFM advantages include that the measurements can be extended from room to higher temperatures. Prilliman et al.⁴⁴ reported in 1998 the development of a Hot-Stage AFM in tapping mode. A coupled cooling/heating device allows *in situ* visualization of the morphological changes resulting from thermal transitions. The possibility of imaging phase thermal transitions is a valuable analysis that complements other thermal characterization techniques such as differential scanning calorimetry (DSC) and dynamic mechanical analysis (DMA). To study phase transitions, images at different temperatures are recorded after the sample has been heated or cooled, or the AFM probe is heated and is kept in constant contact with the sample during the heating scan or during the phase transition. As the probe temperature exceeds the thermal transition, the polymeric material under it can be recognized.^{8,9}

More recently, Zhang et al.⁴⁵ reported a very innovative approach for an AFM heating/cooling device. The concept is highly useful to *in situ* apply a thermal treatment to a sample, and then, observe it with the AFM. The authors coupled a sensor for fast scanning chip calorimetry (FSC) to the scanner head of a non-contact mode AFM. The sensor includes the sample, and it is placed under the AFM probe tip for scanning. This sensor is simultaneously connected to the electronic of an FSC device. The FSC technique can perform DSC scans at extremely fast cooling and heating rates (up to 1.000.000 K/s) and apply isothermal treatments to a sample. In this way, a particular morphology can be induced under specific conditions. Then, the morphology created can be observed by AFM at that

temperature, or it can be “frozen” by quenching the sample rapidly to room temperature (or lower) and then observed it. The novel approach represents a Fast Hot-Stage for AFM that simultaneously provides DSC information of the sample (crystallinity, thermal transitions, etc.).

AFM has already been used to image the melting and crystalline morphology during crystallization at high temperatures for some semicrystalline polymers. Some examples are the reports published on polyethylene (PE),^{8, 46-48} PCL,⁴⁹⁻⁵¹ and PEO.⁵²⁻⁵⁵ Additionally, hot-stage AFM has been used to examine polymer blends and block copolymers. Early studies were performed in PEO/poly(methyl methacrylate) blends by Pearce et al. in 1998. The authors followed the crystallization of the PEO and observed the lamellar growth and the impingement of adjacent spherulites. Wang et al.⁵⁶ also reported the morphological changes during crystallization in blends of PEO and poly(butylene succinate) (PBS) with different compositions. The authors employed hot-stage AFM to examine the crystallizability of the blends and observed that the isothermal crystallization temperature is chosen for the PBS phase and the composition, both had an important effect on PEO crystallization and on the local distribution of the PEO crystals. The PEO crystalline phase can locate at the interlamellar spaces of the PBS crystals or can grow on the edges of the PBS lamellae.

Imaging semicrystalline diblock copolymers at high temperatures, employing hot-stage AFM, should provide more insight into the lamellar arrangement at the nanoscale. However, only a few reports on single and double crystalline diblock copolymers have been published, and most of the AFM polymer crystal observations deal with samples crystallized from solution and only a few with samples crystallized from the melt. In diblock copolymers of PEO containing a tablet-like block of poly(2,5-bis[(4-

methoxyphenyl)oxycarbonyl]styrene) (PMPCS) (PEO-*b*-PMPCS),⁵⁷ hot-stage AFM has been used to follow the PEO block crystallization. Crystallizing ultrathin films from the melt usually produces flat-on lamellae with chain direction perpendicular to the substrates. Under isothermal conditions, the PEO crystallized into dendritic structures if the crystallization temperature is lower than 44 °C, but the morphology changed to square-shaped crystals at temperatures higher than 48 °C. The crystals were completely melted at 56 °C or temperatures above this one. By AFM, the authors were also able to determine that the lamellar thickness increased as the crystallization temperature was higher.⁵⁷ Hot-stage AFM has also been used by Zhan et al.⁵⁸ to examine the relief structure of PS-*b*-PCL diblock copolymers. In these copolymers, the PCL block only crystallized, and the AFM technique was employed to analyze the coupling and complex competition between dewetting and microphase separation.

To our knowledge, only Cui et al.⁵⁹ have followed the structural changes upon heating and cooling in double crystalline PCL-*b*-PLLA diblock copolymers employing hot-stage AFM. The authors studied the PLLA block crystallization behavior upon cooling from melt and upon heating from room temperature with AFM. Different crystalline morphologies were observed depending on the crystallization conditions. However, there was not a clear distinction between the PCL and PLLA lamellae.

In this paper, we take advantage of the Hot-Stage AFM approach to provide a clear elucidation of the trilayered lamellar morphology of an ABC-type triple crystalline triblock terpolymer. To our knowledge, this is the first time that Hot-Stage AFM is employed to identify three different crystalline phases successfully in the same polymeric sample. The PEO-*b*-PCL-*b*-PLLA is a triblock terpolymer with an alternating superstructure of three

crystalline phases, as we had reported in previous publications.^{4, 33-36} The PLLA block crystallizes first and produces a spherulitic template where the PCL and PEO blocks have to crystallize. Here, we report the *in situ* hot-stage AFM observations of the sequential melting of isothermally crystallized model PEO-*b*-PCL-*b*-PLLA triblock terpolymer films. Complementary WAXS analysis is provided to support the AFM evidence. Assessing the distribution of discrete crystals provides a deeper understanding of the sequential crystallization and melting in PEO-*b*-PCL-*b*-PLLA triblock terpolymers.

EXPERIMENTAL PART

Materials

A diblock copolymer precursor (PCL-*b*-PLLA) and a PEO-*b*-PCL-*b*-PLLA triblock terpolymer were selected for the morphological characterization. These materials were synthesized as previously reported by a one-pot sequential organocatalytic ring-opening sequential polymerization of ethylene oxide, ϵ -caprolactone, and L-lactide. A phosphazene base, 1-tert-butyl-2,2,4,4,4-pentakis-(dimethylamino)-2 λ 5,4 λ 5-catenadi(phosphazene) (t-BuP2) was employed as a single catalyst. For more details on the synthesis procedure and characterization, refer to^{60, 61}, and the references in them. The PLLA and PCL block length in both terpolymer and diblock copolymer was very similar (see Table 1) to avoid any influence of molecular weight on crystallization. We obtained molecular weight distributions ($\mathcal{D} < 1.20$) that can be considered relatively narrow. Nuclear magnetic resonance spectra (¹H NMR) and size exclusion chromatography (SEC) were employed to determine chemical structure and number-average molecular weights (M_n) of the materials and their block

components. The samples under study are listed in Table 1, with composition of the blocks as subscript numbers and molecular weights of the entire diblock copolymer and terpolymer as superscript numbers. The melting points of each component determined by DSC previously³³⁻³⁶ are also given in Table 1.

Table 1. Molecular weight, composition and melting point data of the two samples employed in this work.

Sample code	M_n	T_m (°C)	M_n	T_m (°C)	M_n	T_m (°C)	$\bar{D}$
	(gmol ⁻¹)	PEO	(gmol ⁻¹)	PCL	(gmol ⁻¹)	PLLA	(M_w/M_n)
	PEO	block	PCL	block	PLLA	block	
	block		block		block		
PCL ₄₃ PLLA ₅₇ ^{15.4}	-		6600	52-55	8800	134-139	1.16
PEO ₂₃ PCL ₃₄ PLLA ₄₃ ^{19.9}	4600	41-46*	6800	52-57*	8500	120-127*	1.18

*The melting point values reported here are a range because they depend on previous thermal history (controlled cooling or isothermal crystallization) and scanning rates employed during their determination (1, 5 or 20 °C/min)³³⁻³⁶

Films Preparation.

Film samples were prepared by spin coating. The polymers were dissolved in chloroform at room temperature to make 0.1%, 0.2% and 0.5% w/w solutions. The polymer solution was dropped onto a 1 cm² Si wafer, which was previously cleaned and oxidized with UV light, and then, it was spin-coated.

Thermal Treatment to Induce Microphase Separation.

All blocks within the diblock copolymer and triblock terpolymer under study were previously crystallized, employing a two-step crystallization method. To erase thermal history, the sample was first melted at 160 °C for 3 minutes. Then, to crystallize the PLLA block, it was cooled from the melt to 0 °C at 20 °C min⁻¹ and finally heated up to 81 °C at 60 °C min⁻¹. It was held at this temperature for 1 h crystallize the PLLA block to saturation. To crystallize the PCL block, the sample was then cooled to 49.5 °C at 60 °C min⁻¹ and held at this temperature for 1 h. Finally, the sample was quenched to 25 °C at 100 °C min⁻¹ (during this cooling, the PEO block can crystallize³⁵, forming the thinnest lamellae of the three components). After the thermal treatment, the samples were examined by AFM and WAXS.

Morphological Observations.

The as-crystallized films were measured with a Dimension Icon microscope (Bruker, USA) equipped with heating accessories. The measurements were performed by ScanAsyst mode with Bruker probes (SCANASYST-AIR mode; 12 nm tip radius; 0.4N/m spring constant and 70kHz resonant frequency) to simultaneously image the microstructure topography and the mechanical properties (i.e., modulus).

To evaluate the microphase separation by atomic force microscopy (AFM) with an *in situ* heating stage, two particular *in situ* thermal protocols were used:

- (i) One step heating method. The as-crystallized PCL₄₃PLLA₅₇^{15,4} sample was heated from room temperature to 70 °C (a temperature above the melting point of the PCL block crystals, see Table 1) at 1 °C min⁻¹ and held at this temperature for *in situ* characterization by AFM.
- (ii) Two steps heating method. The as-crystallized PEO₂₃PCL₃₄PLLA₄₃^{19,9} sample was

heated from room temperature to 49 °C (to melt the PEO block crystals, see Table 1) at a heating rate of 1 °C min⁻¹ and kept at this temperature for *in situ* AFM observations. Subsequently, it was heated up to 60 °C (to melt the PCL block crystals, see Table 1) also at 1 °C.min⁻¹, and *in situ* AFM characterization was again performed to image the PLLA block crystals.

Wide Angle X-ray Scattering (WAXS) experiments.

WAXS experiments were carried out on films of the samples that were previously subjected to the crystallization protocol described earlier. The WAXS scattering patterns were acquired *in situ* to follow the evolution of the microphase separation as the samples were heated. The *in situ* WAXS measurements were conducted in the ALBA Synchrotron Radiation Facility (Cerdanyola del Valles, Barcelona, Spain) (beamline BL11-NCD). A Rayonix LX255-HS detector with a resolution of 1920 × 5760 pixels (pixel size: 40 μm²) was employed to record the WAXS patterns on heating. Silver behenate and Cr₂O₃ standards were used to perform the calibration. The physical parameters were the following: tilt angle, 30°; effective scattering vector q range, 8–22 nm⁻¹; and sample-to-detector distance; 126.8 mm. The radiation source had a wavelength (λ) of 0.9999 Å. The DAWN software was used to process the data and to produce intensity plots as a function of the scattering vector, q ($q = 2\pi/d = 4\pi \sin \theta/\lambda$). The temperature was controlled employing a Linkam Scientific Instruments THMS600 hot-stage with a liquid nitrogen cooling system. WAXS patterns were recorded on heating between 25 and 160 °C. The heating rate was set at 5 °C min⁻¹. An acquisition time of 6 s was used for each pattern. Thus, the temperature resolution was 0.5 °C. The WAXS patterns are presented between 1 and 2 Å⁻¹. In this range, most crystallographic reflections of the blocks species under study are observed.

RESULTS AND DISCUSSION

Two samples were selected in this work, a PEO₂₃PCL₃₄PLLA₄₃^{19,9} triblock terpolymer and its precursor, a PCL₄₃PLLA₅₇^{15,4} diblock copolymer, as they can be regarded as model di and triblock copolymer and terpolymer samples with crystallizable blocks. Both samples are most probably melt miscible. SAXS observations in the melt showed no scattering,^{33, 35} although the electron density between these blocks is quite similar.²⁶ Upon cooling from the melt, both DSC and WAXS analysis showed that the two phases in the diblock and the three phases in the triblock crystallize sequentially, being the PLLA block the one that crystallizes first. Therefore, the crystallization of the PLLA block templates the morphology of both diblock and triblock. As a consequence, the successive crystallization of the other blocks takes place inside the interlamellar zones of the spherulites of PLLA. The result is a special spherulitic-type superstructure with intercalated lamellae of PEO and/or PCL self-assembled in a random fashion. After PLLA crystallization, the WAXS analysis demonstrated in the triblock terpolymer that PCL block is the second to crystallize and then, the PEO block.³³

An image of this tri-lamellar morphology at room temperature observed by AFM has been published by us before.³⁵ However, we could not observe the morphology by AFM as the sample was heated. In this work, on the other hand, by employing *in situ* hot-stage AFM, we are able to show the presence of the three clear, distinct phases and corroborate the DSC and WAXS observations. Samples were prepared by spin coating employing different solution concentrations to determine the best sample preparation conditions. The samples were subjected to a thermal protocol that includes two isothermal steps to induce each block crystallization until saturation. The crystallization time of each isothermal step was 1 h,

which is sufficient time to induce the thickest lamellar thickness at the conditions chosen and a saturated crystallization process (see Figure 1).

In the case of the triblock terpolymer (see Figure 1), first, the PLLA block was crystallized by cooling down the sample from 160 °C (in melt state) to 0 °C and then heating up until 81 °C. The PLLA block was isothermally crystallized at 81 °C during 1 h, while the other two blocks are in the molten state. Afterwards the sample was rapidly quenched until 49.5 °C, which is a temperature that induces the PCL block crystallization only. At that temperature, the PCL block was crystallized until saturation during 1 h. Finally, the sample was rapidly quenched again down to 25 °C and, during this last cooling ramp, the PEO block (present only in the triblock terpolymer) can crystallize (please see reference 35 for further details). The same protocol described in Figure 1 was used for the precursor diblock copolymer for comparison purposes.

Figure 1. Thermal crystallization protocol with two isothermal steps.

The as-crystallized samples (following the protocol of Figure 1) were observed by AFM at 25 °C and during a subsequent heating scan employing a hot-stage. Two thermal protocols were employed (Figure 2): one step heating scan, in which AFM observations were made at 25 and 70 °C, and a two-step heating scan, in which the observations were made at 25, 45, and 60 °C. Figure 2 shows the thermal protocols applied during the *in situ* heating scans performed and the temperatures chosen.

Figure 2. Thermal protocols applied to the crystallized samples (according to Figure 1) during heating: (a) One step heating protocol and (b) Two steps heating protocol.

The alternated morphology of the PCL-*b*-PLLA diblock copolymer

The nanoscale morphology of the PCL₄₃PLLA₅₇^{15.4} diblock copolymer was examined first. Figure 3 shows AFM height images of a sample prepared initially by spin-coating from solution but then subjected to the thermal crystallization protocol indicated in Figure 1.

Figure 3a and b show the lamellar structure at 25 °C before applying the one-step heating scan described in Figure 2a. Most of the lamellae have grown in an edge-on fashion, allowing lamellar thickness measurements.

Figure 3a shows for the PCL₄₃PLLA₅₇^{15,4} diblock copolymer sample, several packed lamellae that include alternated PCL and PLLA blocks lamellae. After careful observation and measurements of the AFM micrographs, two populations of different lamellar thickness were identified. One of them has an average thickness of 15 nm, indicated with red dotted lines and the other, of 10 nm, indicated with green dotted lines (Figure 3b). These values agree well with our previous report on the lamellar structure of these copolymers.³⁵ In that report,³⁵ the AFM observations were made by Multimode Scanning Probe Microscope (tapping mode) and using microfabricated silicon tips/cantilevers. We established that the thickest lamellae (15 nm, red) should belong to the PLLA block because this block is the one that crystallizes first, while the thinnest lamellae (10 nm, green) should belong to the PCL block.

A WAXS experiment of the as-crystallized sample taken at 25 °C confirmed the presence of both types of crystals (see Figure 4a). The characteristic crystallographic planes of the α -form of the PLLA crystals (110/200 and 113/203) can be observed, as well as, the 110, 111 and 200 reflections of the PCL crystals. However, and until now, it was not possible to assign without doubts to which block correspond each lamellar thickness.

Based on the different melting temperatures of each block (the PCL block melts at around 55 °C and PLLA block at around 120 °C, see Table 1 and our previous work³⁵), the premise is that if the sample is heated until a temperature above the melting point of the PCL

lamellae but sufficiently low to keep the PLLA phase crystallized, we should be able to see the disappearance of the PCL phase, while the PLLA lamellae will remain intact.

Figure 3. AFM height images of a $\text{PCL}_{43}\text{PLLA}_{57}^{15.4}$ sample prepared initially by spin-coating from a 0.2 wt% solution and then subjected to the thermal crystallization protocol indicated in Figure 1. Images are given at 25 °C (a, b) and at 70 °C (c, d) after the heating protocol described in Figure 2a. The dotted lines indicate PCL lamellae (green), PLLA lamellae (red), and molten PCL regions (yellow).

Thus, to confirm the melting transitions of the $\text{PCL}_{43}\text{PLLA}_{57}^{15.4}$ diblock copolymer, the as-crystallized sample (according to the protocol of Figure 1) was heated up, and simultaneous WAXS measurements were taken during heating.

Figure 4. WAXS patterns of $\text{PCL}_{43}\text{PLLA}_{57}^{15.4}$ diblock copolymer taken at 25 °C, after crystallizing the sample in two isothermal steps (a). WAXS patterns evolution of $\text{PCL}_{43}\text{PLLA}_{57}^{15.4}$ during subsequent heating between 25 and 92 °C (b) and between 92 and 160 °C (c).

It can be seen in Figure 4b that at 25 °C, both PCL, and PLLA phases are present. But beyond 60 °C, the reflections of the crystallographic planes of the PCL disappeared due to the melting of the crystals while the PLLA crystals remained unmolten. The PLLA phase melts completely at 160 °C (see Figure 4c).

The as-crystallized sample was carefully heated ($1\text{ }^{\circ}\text{C}\cdot\text{min}^{-1}$) up to 70 °C (protocol is shown in Figure 2a), and AFM observations at that temperature were performed. Unlike our previous report³⁵, the AFM used on this occasion consists of a Dimension Icon microscope equipped with a hot-stage device that allows heating the sample *in situ* at a very low heating rate.

Figure 3c and d show the lamellar structure of the $\text{PCL}_{43}\text{PLLA}_{57}^{15.4}$ diblock copolymer after heating the sample to 70 °C (a temperature above the melting point of the PCL block crystals). Comparing Figure 3b (at 25 °C) and Figure 3d (at 70 °C), it is apparent that the smaller size lamellae (10 nm, green) disappeared as a consequence of the melting of the PCL lamellae. Darker regions (indicated with yellow dot lines) can now be observed in between the bigger size lamellae that remained unchanged. These darker interlamellar regions correspond to the amorphous PCL phase that at 70 °C is molten. In fact, at this temperature, only PLLA reflections are observed in the WAXS spectrum (see Figure 4b). Therefore, the lamellar structure observed at 70 °C corresponds to PLLA lamellae, which are 15 nm in thickness. Thus, the PCL-*b*-PLLA diblock copolymer indeed exhibited a double crystalline morphology that includes lamellae of both PCL and PLLA at room temperature.

Cui et al.⁵⁹ have previously reported the morphology of PCL-*b*-PLLA diblock copolymers employing a hot-stage AFM. However, the authors did not report a lamellar

structure. On the contrary, at 25 °C, a wormlike co-continuous morphology of the blocks was proposed. However, the WAXS analysis demonstrated that only PCL crystals were present at room temperature. As the sample was heated, the PCL crystals melted, and the PLLA crystals started to grow, as confirmed by WAXS. The authors claimed that the wormlike structure was deformed due to the emergence of PLLA lamellae. However, a truly lamellar arrangement is not clear from the AFM images presented. Cooling from the melt to 80 °C, a spherulitic-type texture seemed to emerge as a consequence of PLLA crystallization. Subsequent cooling to 27 °C induced the PCL block crystallization, and the morphology was slightly changed. However, the crystalline structure is not clear⁵⁹. These observations demonstrate the importance of a suitable sample preparation method and crystallization protocol to promote a clear lamellar crystalline morphology. Therefore, to our knowledge, this is the first time that *in situ* heating AFM experiments are employed to elucidate microphase separation and lamellar structure of different crystalline blocks in block copolymers.

Besides AFM techniques, the nanoscale lamellar morphology of PLLA-*b*-PCL diblock copolymers has also been observed in the literature by other microscopic techniques. In a previous report from Ho et al.⁶², the authors observed by TEM the morphology of crystals that have been crystallized from melt and grown epitaxially. Different crystallization temperatures and substrates were tested. At the crystallization temperatures chosen, only the PLLA block could crystallize, forming a branched crystalline lamellar structure. Flat-on PLLA lamellae were induced by the epitaxial crystallization on different substrates, but the authors neglected the possible PCL crystallization at room temperature. Therefore, the

authors presented a lamellar phase-separated structure that included both crystalline flat-on PLLA lamellae and alternating amorphous layers containing both PCL and PLLA chains.

Casas et al.⁶³, on the other hand, reported solution grown single crystals of PCL-*b*-PLLA diblock copolymers that were observed by TEM. The PLLA block was crystallized first, forming truncated lozenge shape single crystals. Later, the PCL block crystallized forming fringed and small PCL crystals adjacent to the PLLA lamellae. Depending on the crystallization conditions, very complex crystalline morphologies were developed.

Further evidence of the alternated lamellar structure and phase assignment can be provided by the Dimension Icon Microscope employed in this work. This AFM device gives an image of the microstructural topography and at the same time determines the mechanical properties of the sample surface. Thus, properties such as adhesion and modulus can be scanned and mapped in the sample area.

The as-crystallized sample (crystallized as explained in Figure 1) consists of different phases that include PCL and PLLA crystalline phases and a mixed amorphous phase (containing both PCL and PLLA blocks chains). These three phases should exhibit a different mechanical response. For instance, the amorphous phase is softer than the crystalline one. And between PCL and PLLA, the mechanical features are also different. In general, tensile modulus values between 3000 and 4000 MPa have been reported for PLA,⁶⁴ while PCL has been described as a softer material with modulus around 300-400 MPa.⁶⁵ Hence PLA is a more rigid material than PCL. Taking all this into consideration, the mechanical performance of the sample was determined employing the Dimension Icon Microscope AFM. We expected that some differences could be distinguished between the phases. Figure 5 shows

the AFM modulus images of the $\text{PCL}_{43}\text{PLLA}_{57}^{15.4}$ sample, initially prepared by spin-coating from solution, and then subjected to the thermal crystallization protocol indicated in Figure 1.

Figure 5. AFM modulus images of a $\text{PCL}_{43}\text{PLLA}_{57}^{15.4}$ sample, originally prepared by spin-coating from a 0.2 wt% solution, and then subjected to the thermal crystallization protocol indicated in Figure 1. Images are given at 25 °C (a, b), and at 70 °C (c, d) after the heating protocol described in Figure 2a. The dotted lines indicate PCL lamellae (green), PLLA lamellae (red) and molten PCL regions (yellow).

At 25 °C (see Figure 5a and b), it could be ³⁵ to elucidate the phases present, taking into consideration the modulus values only. If relative comparisons are made without taking into account the absolute values, a tentative assignment can be made as the brighter lamellae (of higher modulus) should correspond to the PLLA block (indicated with a red dotted lines) while less bright regions in between PLLA lamellae should be assigned to the PCL lamellae (green dotted line). However, this assignment is confirmed when the sample is heated to 70 °C (see Figure 5d). At that temperature, only the PLLA crystals remain, and the PCL lamellae have melted. Therefore, the very bright lamellae of high modulus are characteristic of the more rigid PLLA crystals. The PLLA lamellae remained unchanged and at the exact position as they were marked with red dotted lines in Figure 5b. On the contrary, the interlamellar PCL block regions appeared completely dark, indicating much lower values of elastic modulus than those in the PLLA block region. Being the amorphous phase a softer one, that observation is an indication of the melting of PCL lamellae and a clear evidence of the alternated lamellar structure, in which the PCL lamellae locates in an interdigitated fashion between the PLLA lamellae when it crystallizes within the previously formed PLLA spherulitic or axialitic templates.

The interlamellar self-assembly of the PEO-*b*-PCL-*b*-PLLA triblock terpolymer

Following the same approach employed for the diblock copolymer, the particular interlamellar arrangement of a tri-crystalline PEO-*b*-PCL-*b*-PLLA triblock terpolymer was studied. The sample selected, the PEO₂₃PCL₃₄PLLA₄₃^{19,9}, is a triblock terpolymer with the same PLLA and PCL block lengths as in the previously discussed diblock copolymer. The

length of the PEO block is the smallest one (see Table 1). Thus, comparisons between the triblock terpolymer and the diblock copolymer (which is the precursor of the triblock terpolymer) can be made without any influence of molecular weights of the blocks under consideration.

The complexity of the nanoscale morphology of triblock terpolymers is high when the three blocks are able to crystallize. In our previous reports,^{33,35} we demonstrated by DSC and WAXS that the three blocks in PEO₂₃PCL₃₄PLLA₄₃ are able to crystallize, and the SAXS analysis proved the melt miscibility. Upon cooling from melt, PLOM and WAXS experiments confirmed that PLLA crystallizes first, creating a spherulitic superstructural template. Then, the next block to crystallize is the PCL block, which must crystallize inside the interlamellar spaces of the PLLA spherulites (or axialites). The last block to crystallize is the PEO block, and it has no other option but to grow its lamellar crystals inside the available interlamellar spaces aside PCL and PLLA lamellae, as it is covalently bonded to the PCL block. The templated morphology did not change during the successive crystallization of the other PEO and PCL blocks, and only a slight change in the magnitude of the birefringence was observed. Therefore, after crystallization, the lamellae of the three blocks should coexist together in an alternated fashion. To confirm this hypothesis, we applied the crystallization protocol of Figure 1 to the PEO₂₃PCL₃₄PLLA₄₃^{19,9} sample. The crystallization temperatures were chosen to be sufficiently high to induce the crystallization of each block separately and avoid the crystallization of the other two blocks. Hence, at 81 °C, the PEO and PCL blocks are molten, and only the PLLA block has the ability to crystallize. Then, at 49.5 °C, only the PCL block can crystallize (surrounded by the previously formed PLLA crystals), and the

PEO block remains molten. Finally, the PEO block will crystallize during the last final quench treatment applied by cooling the sample to 25 °C at 100 °C.min⁻¹.³⁵

In our previous report ³⁵, we were able to identify three different lamellar thicknesses by AFM (15, 10, and 7 nm), and we hypothesized about their origin. The thickest lamellae of 15 nm should belong to the PLLA block because this value was similar to that of the PLLA block in the analogous diblock copolymer, and both blocks have the same molecular weight and were crystallized under identical conditions (i.e., at 81 °C for 1 h). Then, the 10 nm lamellae were assigned to the PCL block due to the same reasons mentioned above: the PCL lamellae in the diblock copolymer were also around 10 nm thickness. Finally, the smallest lamellae should belong to the PEO block. But, to prove that, we had to run some WAXS measurements at room temperature and on heating to confirm the presence of PEO crystals. Specifically, the crystallographic planes in both the diblock copolymer and the triblock terpolymer were compared. Both PLLA and PEO reflections were observed. However, some reflections of PLLA and PEO crystals overlap (more details are in³⁵). Therefore, after the WAXS experiment, the presence of the PEO crystals could only be indirectly confirmed.

In the present work, we take advantage of the AFM experiments employing a Dimension Icon microscope equipped with a hot-stage. The PEO₂₃PCL₃₄PLLA₄₃^{19,9} sample was crystallized, as explained in Figure 1, and then it was *in situ* slowly heated following the thermal protocol described in Figure 2b. Then, AFM images were registered at different temperatures on heating.

Figure 6. AFM height images of a $\text{PEO}_{23}\text{PCL}_{34}\text{PLLA}_{43}^{19,9}$ sample, initially prepared by spin-coating from a 0.2 wt% solution and then subjected to the thermal crystallization protocol indicated in Figure 1. Images are given at 25 °C (a, b), and at 70 °C (c, d) after the heating protocol described in Figure 2a. The dotted lines indicate PCL/PEO lamellae (white), PLLA lamellae (red) and molten PCL/PEO regions (yellow).

Figure 6 shows AFM height images of the triblock terpolymer sample prepared first by spin-coating, and then thermally treated according to Figure 1. The aim is to identify the tri-lamellar structure of this multiphasic terpolymer. At 25 °C, it can be seen that some

lamellae grew edge-on, while others are slightly tilted. This lamellar arrangement should include lamellae of the PLLA, PCL and PEO crystals. The WAXS experiment at 25 °C revealed the crystallographic planes of the three blocks (see Figure 7a).

Detailed measurements of the lamellar thickness at 25 °C (see Figure 6a and b) revealed two populations of different lamellar thickness. The thickest one is 17 nm (red dotted line), and between these lamellae, another phase measures 11 nm (white dotted lines). Comparing these values with those of the diblock copolymer, the thickness values of the bigger size lamellae are close. Since the molecular weight of the PLLA blocks in both samples is similar and the crystallization conditions are the same, it is reasonable to assume that the 17 nm lamellae correspond to the PLLA block in the terpolymer. This value is also close to the 15 nm thickness that it was measured in the AFM images observed through the Multimode Scanning Probe Microscope that we previously reported.³⁵ The small difference obeyed the fact that determining lamellar thickness by AFM is more sensitive to errors because it is a technique that scans a rather small surface area of the sample. Some limitations of determining lamellar thickness by AFM have been discussed before.^{66, 67}

Besides the 17 nm phase, the other one measured 11 nm (white dotted line). It is challenging to elucidate to which of the other two blocks correspond this phase. Increasing the temperature should melt one phase while keeping the other crystalline. It can be seen in Figure 7b and c that beyond 60 °C, only PLLA crystals remain. The effect of slowly increasing the temperature can be seen in the AFM images taken at 70 °C. At this temperature, both PEO and PCL crystals are molten. It is clear that the phase marked with the white dotted line in Figure 6a and b disappeared in Figure 6c and d (signaled with a yellow dotted line).

Figure 7. WAXS patterns of PEO₂₃PCL₃₄PLLA₄₃^{19.9} diblock copolymer taken at 25 °C, after crystallizing the sample in two isothermal steps (a). WAXS patterns evolution of PEO₂₃PCL₃₄PLLA₄₃^{19.9} during subsequent heating between 25 and 92 °C (b) and between 92 and 160 °C (c).

Figure 8. AFM modulus images of a $\text{PEO}_{23}\text{PCL}_{34}\text{PLLA}_{43}^{19.9}$ sample, initially prepared by spin-coating from a 0.2 wt% solution, and then subjected to the thermal crystallization protocol indicated in Figure 1. Images are given at 25 °C (a, b), and at 70 °C (c, d) after the heating protocol described in Figure 2a. The dotted lines indicate PCL/PEO lamellae (white), PLLA lamellae (red) and molten PCL/PEO regions (yellow).

Therefore, the AFM images allow confirming that the thickest lamellae (17 nm, red dotted line) belong to the PLLA crystals since these are the only lamellae remaining at 70 °C (a temperature above the melting point of both PEO and PCL blocks). The AFM modulus

images also confirmed this observation (see Figure 8). The darker interlamellar region in-between the PLLA lamellae account for the softer amorphous phase resulting mainly from the molten PEO/PCL phase.

Figure 9. AFM height images of a $\text{PEO}_{23}\text{PCL}_{34}\text{PLLA}_{43}^{19.9}$ sample, initially prepared by spin-coating from a 0.1 wt% solution, and then subjected to the thermal crystallization protocol indicated in Figure 1. Images are given at 25 °C (a, b), 45 °C (b, c) and 60 °C (d, e). The dotted lines indicate PEO lamellae (blue), PCL lamellae (green), PLLA lamellae (red), and the PEO/PCL melt region (yellow).

Nevertheless, trying to differentiate the PEO lamellae from the PCL lamellae from the images shown in Figure 8 for the $\text{PEO}_{23}\text{PCL}_{34}\text{PLLA}_{43}^{19.9}$ triblock terpolymer is more

difficult than in the case of the diblock copolymer (Figure 6). Thus, to improve the quality of the AFM images, another sample solution of 0.5 wt% was prepared by spin-coating, and the film obtained melt crystallized as in Figure 1. Even though the sample solution was more concentrated, the multiphasic lamellar structure of the 0.5 wt% sample was not sufficiently clear in the AFM images taken with the Dimension Icon microscope (see Supplementary Information), even after melting the samples. Therefore, the solution concentration was diluted down to 0.1 wt%, and the sample film was again crystallized following the same protocol described in Figure 1. After crystallization, the sample was slowly *in situ* heated, and AFM images were taken at 25, 45, and 60 °C, as described in Figure 2b. The sequence of AFM images on heating is presented in Figure 9 and Figure 10.

After employing a more diluted sample solution, the Dimension Icon AFM microscope confirms the multiphasic lamellar morphology that the authors hypothesized earlier.³⁵ An extraordinary view of the tri-lamellar arrangement is given by the AFM height images taken at 25 °C (see Figure 9a). Some of the lamellae are edge-on, and other lamellae are slightly tilted. After a closer observation and exhaustive measurements, three populations of different lamellar thickness were identified: 18, 14, and 10 nm (see Figure 9d). These values are slightly higher than those that were previously reported: 15, 10, and 7 nm.³⁵ As was aforementioned, the small differences between AFM techniques obey their inherent sensitiveness to measurement errors.

What is relevant is the fact that a clear trilayered morphology is observed at 25 °C. In Figure 9d, the thickest lamellae (i.e., 18 nm) are signaled with a red dotted line. While the intermediate size lamellae, with a green dotted line (i.e., 14 nm), and the thinnest lamellae (i.e., 10 nm) with a blue dotted. Thus, the premise is that each lamellae population might

correspond to a different crystalline phase: whether PLLA, PCL, or PEO. This hypothesis was only indirectly proved through WAXS analysis measurements in a previous publication.³⁵ In this report, we took advantage of the hot-stage AFM to determine to which phase belongs each lamella. Since each phase melts at different temperatures, we should be able to observe their disappearance as the sample is *in situ* heated.

Under standard crystallization conditions, the PEO and PCL blocks in the PEO₂₃PCL₃₄PLLA₄₃^{19,9} triblock terpolymer melt at approximately 45 and 54 °C, respectively, while the PLLA block does it at a much higher temperature, around 112-122 °C (see also Table 1).³³ Figure 9b shows the AFM height image of the sample after heating to 45 °C. A closer observation (see Figure 9e) revealed the disappearance of the thinnest lamellae that were first marked with blue dotted lines. Since the PEO block melts at this temperature, the AFM height image proves undoubtedly that the thinnest lamellae belong to the PEO crystals. A yellow dotted line marks the position where the PEO lamellae were.

Further heating the sample up to 60 °C causes the melting of the PCL phase. The medium size lamellae, indicated with a green dotted line in Figure 9, have now disappeared while the thickest lamellae marked with the red dotted line remain (see Figures 9c and f). At that temperature, only the PLLA block is crystalline, while the other two are completely molten.

The AFM images upon sequential heating confirm that the 14 nm lamellae are PCL block lamellar crystals, and the 18 nm lamellae correspond to the PLLA block. The exact position of where PCL lamellae were has been marked with yellow dotted lines in Figure 9f, while the lamellae that remain correspond to the PLLA block. The trilayered morphology has

been confirmed, and the PEO₂₃PCL₃₄PLLA₄₃^{19,9} triblock terpolymer can be prepared as a truly multi-crystalline system, provided suitable crystallization conditions are applied.

Although less clear, the multiphasic nanoscale morphology was also observed through AFM modulus images. Figure 10 shows the lamellar structure of the PEO₂₃PCL₃₄PLLA₄₃ triblock terpolymer at different temperatures. It could be difficult to elucidate each phase by taking into account the mechanical modulus only.

Figure 10. AFM modulus images of a PEO₂₃PCL₃₄PLLA₄₃^{19,9} sample, originally prepared by spin-coating from a 0.1 wt% solution, and then subjected to the thermal crystallization protocol indicated in Figure 1. Images are given at 25 °C (a, b), 45 °C (b, c) and 60 °C (d, e). The dotted lines indicate PEO lamellae (blue), PCL lamellae (green), PLLA lamellae (red), and the PEO/PCL melt region (yellow).

Even though the PLLA is a more rigid material, while the PCL and PEO are softer polymers, it is complicated to differentiate them in the AFM modulus images at 25 °C (see Figure 10a and d). The reason is probably that PCL and PEO have comparable mechanical properties.^{65, 68} However, as the sample is heated to 45 and 60 °C, some lamellae disappeared while others remained brighter. Since the amorphous phase is a softer one, the mechanical modulus is lower as a result of the melting of the PCL and PEO crystals. Therefore, at 60 °C, only the rigid-higher modulus crystalline phase of the PLLA block is observed, surrounded by a darker area (signaled with yellow dotted lines) that correspond to the melt mixed PCL/PEO phase.

Besides elucidating the nanoscale morphology, the hot-stage AFM technique could also be useful to provide some clarity in the fashion the lamellae arrange. In our previous publication,³⁵ an exhaustive theoretical analysis of SAXS characterization upon heating suggested that a lamella of either PEO or PCL randomly inserts between two adjacent PLLA lamellae. This premise was proposed after modeling SAXS theoretical curves considering a one-dimensional structural model. At temperatures beyond the melting of the PCL and PEO blocks, the system can be described as a two-phase model. After several simulations, the best fit to the experimental SAXS curve was when only one lamella (of PEO or PCL) fits into the interlamellar spaces of the PLLA crystals. However, the nanoscale structure observed in Figure 9a and d seems contrary to this statement. In Figure 9d, it can be observed that some lamellae of both PCL and PEO (green and blue dotted lines) intercalate between two lamellae of PLLA (red dotted lines). But, it is also possible to observe only one lamella of PEO (blue dotted line) in between two lamellae of PLLA.

Despite this observation, it is essential to understand that the SAXS analysis is a broader range technique that can assess the structural features of the system in bulk. On the contrary, the AFM technique is a surface characterization tool that scans a very particular area of the sample surface and, therefore, might not represent the entire sample. What is significant is that the Dimension Icon AFM microscope not only showed to some extent the random lamellar arrangement proposed earlier,³⁵ but in addition, it gives clear evidence of the multi-crystalline morphology of triple crystalline PEO-*b*-PCL-*b*-PLLA triblock terpolymers. To our knowledge, this is the first time that hot-stage AFM is used to provide an accurate description of the nanoscale morphology of ABC type triblock terpolymer with three crystalline phases.

CONCLUSIONS

The hot-stage atomic force microscopy (AFM) has been used to accurately elucidate the tri-crystalline tri-layered morphology of a PEO-*b*-PCL-*b*-PLLA triblock terpolymer for the first time. This ABC type triblock terpolymer is melt miscible, and the three blocks can crystallize sequentially upon cooling from the melt. A two-step crystallization protocol was applied to the PEO-*b*-PCL-*b*-PLLA triblock terpolymer and to an analogous PCL-*b*-PLLA diblock copolymer for comparison purposes. Clear lamellar self-assembly, including lamellae of different lamellar thickness, was observed by AFM at room temperature. The WAXS analysis confirmed the presence of lamellae of each block. However, identifying each lamella in the terpolymer is not as straightforward. Considering the differences between the melting temperatures of the blocks, the sequential melting of the PEO-*b*-PCL-*b*-PLLA

sample in the hot-stage allowed undoubtedly assigning the three crystalline lamellar thickness populations observed in the AFM images. The thickest lamellae belong to the PLLA block, the medium size lamellae to the PCL block, and the thinnest lamellae to the PEO block. The lamellar self-assembly includes lamellae of PLLA that intercalates randomly with lamellae of PCL, PEO, or both. The hot-stage AFM is a valuable technique to elucidate the nanoscale lamellar morphology of complex multi-crystalline systems.

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PEO-b-PCL-b-PLLA Melting sequence