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Additional Information

Electrospinning of Biodegradable Polylactide/Hydroxyapatite Nanofibers: Study on the Morphology, Crystallinity Structure and Thermal Stability

Agueda Sonseca^{1*}, Laura Peponi², Oscar Sahuquillo¹, José M. Kenny², Enrique Giménez¹

¹Instituto de Tecnología de Materiales. Universidad Politécnica de Valencia (UPV), 46022 Valencia, (SPAIN). E-mail address; agsonol@posgrado.upv.es. Phone number; +34 963879625.

²Instituto de Ciencia y Tecnología de Polímeros (ICTP - CSIC), 28006 Madrid, (SPAIN).

Abstract

Aligned mats of poly(lactic acid) (PLA) nanofibers containing nano-sized hydroxyapatite filler have been obtained via electrospinning onto a rotating mandrel. Their structure and morphology have been characterized as a function of the production parameters, by SEM, DSC, DMA and WAXS, with emphasis in the effects of the take-up velocity. SEM, DSC and X-ray diffraction studies confirmed a reduction in fiber diameter, an increase of fiber orientation and a highly crystalline structure of the mats collected at higher speed due to the stretching produced during the process. Double melting behavior was observed, suggesting the presence of two crystalline forms. Moreover the higher storage modulus (E' modulus), and glass transition temperatures of the higher speed collected mats was correlated with an enhanced in the thermal stability and nanofiller distribution.

Keywords: Polylactide; Hydroxyapatite; Electrospinning; Morphology; Crystallinity; Thermal stability.

*Author to whom the correspondence should be addressed

Introduction

Aliphatic polyesters as poly(lactic acid) (PLA), poly(glycolides) (PGA) and poly(ϵ -caprolactone) (PCL) have attracted wide attention for their biodegradability and biocompatibility in the human body. In order to obtain new biomaterials with enhanced properties, there is a trend towards the introduction of organic and inorganic nanofillers into biodegradable polymers to produce materials containing hydroxyapatite, metal nanoparticles or carbon nanotubes [1].

Electrospinning is a straightforward technique to produce non-woven micro or nanofibrous mats, based on the application of high voltage to a polymeric solution, in order to create an electrically charged jet randomly collected onto a grounded target [2]. The current interest on nano and micro fibers has produced a particular relevance for developing this process. This technique has been recognized as an efficient, simple and versatile method for the fabrication of ultrathin fibers with diameters between 50-500nm and has been successfully used with a wide range of polymers, some of them biodegradable [3]. Due to biodegradable materials are often easy to dissolve, they are suitable for the formation of mats by electrospinning, so until date, this process has been widely used in the field of biomedical engineering for the fabrication of nanofibrous scaffolds for tissue engineering, cardiac grafts, wound dressings, blood vessel substitutes, carriers for delivery of drugs and other therapeutic agents from numerous biodegradable polymers such as poly(ϵ - caprolactone) (PCL), poly(lactic acid) (PLA), poly(glycolic acid) (PGA), some polyurethanes (PU) and their copolymers [4-6].

The morphology of obtained nanofibers can be controlled to a certain extent changing the voltage, feed rate, collector shape and distance values, but basic electrospinning can only yield randomly aligned nanofibers. This will limit the areas which electrospun fiber mesh can be used in. Thus, to fully explore the potential of electrospinning, it is necessary to have some control on the deposition of the fibers [3]. It is well known that solution parameters as viscosity, surface tension, concentration and dielectric properties, and process parameters such as the feed rate and the acceleration voltage have a great influence on the nanofibers morphology. Recently it was determined that the nature of the collector plays a significantly role in the morphological and the physical characteristics of the spun fibers [7, 8]. In fact, it has been seen that nanofibers can be aligned introducing a specially designed collectors, among them a cylinder collector capable of rotating at high speeds and taking up the fibers on the surface of the cylinder tightly

in a circumferential manner [9, 10]. At rotational speeds slower or higher than the optimum fiber take-up speed, randomly or non-continuous fibers are collected respectively. Ideally, the linear rate of the rotating drum should match the evaporation rate of the solvent, such that solvent free fibers are deposited and engaged on the surface of the drum [11]. However, precise control of the alignment of spun fibers remains a challenge.

The electrospun fibers have large surface area to volume ratio and should also have good mechanical properties combining mechanical strength with flexibility and lightness [12]. Incorporating determinate fillers is a way to increase these mechanical properties, as well as diversifying and exploring potential fields of application offering new electrical [13-16], magnetic [17-22], optical [23-26] and biological [27-30] functionalities. Nowadays the use of nanofillers instead of microfillers inside of a polymer matrix, has raised a huge interest in the scientific community. In the biomedical field, the nanocomposites based on biodegradable polymers filled with hydroxyapatite (HA) particles have received special attention due to their good osteoconductivity, osteointegration, biodegradability and enhanced mechanical properties [1]. Due to its biodegradability and biocompatibility, poly(lactic acid) (PLA) is one of the polymers where micro/nanocrystals of HA have been dispersed. Despite numerous reports on the effect of processing conditions on the PLA/HA fibers morphology, degradation properties and biological efficacy of these bioresorbable polymer mats, limited studies have been devoted to investigate the influence of this nanofiller on the morphology and structure of electrospun PLA fibers [31-33]. In this work, the morphology, thermal behaviour and crystalline structure of PLA/HA nanofibers obtained by electrospinning process under both low and high take-up velocities have been investigated. Special emphasis on crystal modifications and orientations has been made, and their effect on mechanical properties and the dimensional stability has been determined. Moreover the work involves the use of a cylindrical rotating collector as a key component because it is generally used to produce high collecting areas, as well as a scalable-up process potentially suitable for future mass production combined with other processing strategies as multiple needle nozzles [34].

1. Experimental

1.1 Materials

Biodegradable Poly(lactic acid) was supplied by NatureWorks® (USA) under the trade name PLA3051D, with 3% of D-lactic acid monomer. Its molecular weight (M_n) was ca. 142×10^4 g/mol, and its density was 1.24 g/cm³. Dichloromethane (DCM) and N-N dimethylformamide (DMF) from Sigma Aldrich were used as solvents. Hydroxyapatite nano filler (HA) was a non-commercial grade synthesized by chemical precipitation method employing calcium and phosphorus precursors in solutions and 30g/L concentration of commercial gelatine (Dr Oetker). To avoid the formation of hydroxyapatite aggregates in the polymer solution, sodium dodecyl sulphate was used as an anionic surfactant.

1.2 Synthesis of hydroxyapatite

For the nanosized hydroxyapatite synthesis, the gelatin was dissolved in 250 mL of deionized water at 40°C, calcium hydroxide ($\text{Ca}(\text{OH})_2$) (2.0 mol/L) was then added to this solution and the mixture was added drop by drop to 250 mL of an aqueous suspension of ortho-phosphoric acid (H_3PO_4 , 85%) (1.2 mol/L) in order to obtain a stoichiometric hydroxyapatite with a ratio of calcium (Ca) and phosphorous (P) moles of 1.67 (Ca/P). Ammonium hydroxide was added to obtain a final pH of 10. The precipitate was left to age for 24 h. The resulting particle size and surface area measured by BET (Brunauer, Emmett and Teller) were 35.74 nm and 54.2038 m²/g respectively, and crystallite size measured by WAXD was 47.98.

1.3 Preparation of electrospun nanofibers

Non-woven and aligned PLA/HA nanofibers were prepared by electrospinning method. In our case, we worked with a standard horizontal electrospinning setup with a rotating mandrel as a collector, connected directly to a high voltage power supply, and a syringe infusion pump. Viscous polymer solutions with a concentration of 7% wt were prepared by dissolving PLA3051D in dichloromethane:N,N-dimethylformamide (70:30). Different contents of nano-hydroxyapatite (HA) were added to the polymer solutions and thoroughly mixed by sonication. Each solution was loaded into the syringe and an electrode was clipped onto the needle. The whole apparatus was enclosed with a methacrylate cover in order to reduce the effect of air currents on the trajectory of the electrospun jet. All air bubbles were purged prior to electrospinning and the solutions were electrospun at 14kV and a flow rate of 0.6 ml h⁻¹. The ground

mandrel was at a distance of 13 cm from the charged capillary tip and electrospun mats were collected at two different take-up velocities; randomly oriented fibers were produced on a stationary shaft (low collecting rate fibers; **LCR**), whereas highly oriented fibers were produced when rotation speed was increased around 1100 rpm (high collecting rate fibers; **HCR**). Series of samples were made from pure polymer and different weight ratios polymer/HA (9:1, 5:1 and 3:1) and all of them were obtained from 10mL of solution.

1.4 Characterization techniques

The morphology of the electrospun fibers was examined using scanning and transmission electron microscopy (SEM, JEOL JSM6030 and TEM, PHILIPS CM10). Samples were gold-coated to minimize the charging effect during SEM observation, an accelerating voltage of 10 kV and a working distance of 10-15 mm were used for viewing the samples. For TEM, a few fibers were collected on carbon covered mesh copper grids and observed at 100kV. SEM images at x5000 magnifications and the ImageJ software (National Institute of Health in USA) were used to evaluate the diameters and the alignment of the nano-fibers. Diameters and fiber orientation angle were calculated as an average of 30 randomly measures. The angle was measured between the long axes of the fibers and the vertical axis of the image.

The thermal behavior was studied by using a differential scanning calorimetry (DSC, Mettler-Toledo DSC 800). Samples were heated from 30 to 220°C at a heating rate of 10°C/min under a nitrogen atmosphere. The calibration of the DSC was carried out with a standard sample of indium. The glass transition temperature (T_g), cold-crystallization temperature (T_{cc}), melting temperature (T_m), cold-crystallization enthalpy (ΔH_c), melting enthalpy (ΔH_m) and degree of crystallinity ($X_c\%$) of the electrospun fibers mats were evaluated from first heating curves. The glass transition was calculated as the midpoint of the jump in heat capacity (ISO 11357-2). The crystallization (exothermal) and melting (endothermal) were characterized by the onset and peak temperatures, as well as by the area under the corresponding peaks. The crystallinity content ($X_c\%$) was evaluated according to the following equation:

$$X_c\% = 100(\Delta H_m - \Delta H_c) / \Delta H_m^0 \quad (1)$$

where ΔH_m and ΔH_c are the melting and the crystallization enthalpies respectively, and ΔH_m^0 is the reference ΔH_m (93.6J/g) of 100% crystalline PLA.

The thermal gravimetric analysis (TGA) was conducted on a TA Instrument Q50 under N₂ flow from 25 to 650 °C at 10 °C/min. Dynamic mechanical analysis (DMA) was carried out on a Mettler-Toledo DMA/SDT A 861 at the frequency of 1 Hz and at the heating rate of 10°C/min from 30 to 130 °C at which the sample lost its dimensional stability.

The crystalline structure of the samples was monitored by wide-angle X-ray diffraction (WAXS, RX Siemens D5000) with a Cu K α source. The scanning range was 2°-40°, step-size and count time per step were 0.02° and 8 seconds respectively. The crystalline structure of the samples was monitored by wide-angle X-ray scattering (WAXS; RX Siemens D5000) with a Cu K α source. The scanning range was 2°-40°, step-size and count time per step were 0.02° and 8 seconds, respectively. The percentages of crystallinity were calculated by a fitting process using a deconvolution method of the peaks. A peak fitting program (**PeakFit**; www.systat.com) was used. In all cases, samples were cut from the middle regions of electrospun mats, with a thickness about 80-150 μ m.

2. Results and Discussion

2.1 . Nanofibers morphology

Figure 1 shows SEM images of the microstructure of pure PLA and PLA/HA nanofibers mats produced on a stationary shaft (LCR) and at rotation speed nearly 1100 rpm (HCR) with neat polymer and different polymer/HA weight ratios: 9:1, 5:1 and 3:1. It is possible to note that through optimized solvent system and electrospinning parameters, uniform PLA and PLA/HA fibers mats with a random or more directional distribution were obtained. The surface of PLA/HA nanofibers was much coarser than the neat PLA fibers due to the dispersion of HA particles. Agglomerates gradually appeared on the fiber surface along with increase of the nano-hydroxyapatite concentration and the deformation of the fiber. For the same electrospinning conditions and the stationary collector, the neat PLA fiber average diameter was nearly to 400nm, as shown in **Table 1**. Increasing collector rotating speed to 1100 rpm, the neat PLA fiber diameter was reduced by nearly 20%. For all mats, the fiber average diameter collected at higher speed was smaller than those of collected at lower speed. Likewise, the fiber diameter increased with filler content. It was found that the dynamic fluctuations of Taylor cone, electric field strength and charge density are the main important process variation factors which determine the resulting fiber diameter

distributions [35-37]. According to the related literature, most obtained electrospinning mats with diameters in the nanometer range, showed Gaussian even Bimodal diameter distributions with standard deviations between 20-60% [36, 38-39] depending on the material and processing conditions. In this regard, the standard deviation values showed in **Table 1** were within the normal range due to the instabilities in the jet during the electrospinning process.

Figure 2 shows the frequency distribution of the fiber orientation angle based on SEM images. When the speed increased to 1100 rpm, the fiber angle decreased and the amount of fiber in the same direction increased to approach to collector rotating direction, which implied that nanofiber orientation was improved. So the alignment degree of the electrospun nanofibers was increased with higher rotation speed of the collector.

Furthermore, the morphology of the hydroxyapatite embedded in the nanofibers was analyzed using TEM. As shown in the **Figure 3**, HA nanoparticles were dispersed in the matrix of PLA for low and high collecting rates. The fiber collected with the highest speed, seems to have low amount of aggregates for the same content of filler. In this regard, the smaller diameter, smoother surface and lower deformation of the fiber can be observed specially in the 3:1 sample collected at high speed, probably due to the high stretching force over the electrospinning jet induced by the faster rotation of the collector, which avoids the formation of the agglomerates and improves the dispersion of the filler inside the fiber.

2.2 Thermal and crystalline behaviour nanofibers

The thermal behavior of PLA/HA nanofibers mats was characterized by means of DSC and the data were summarized in **Table 2**. **Figure 4** shows thermograms obtained from 1st heating run curves of pure PLA and PLA/HA (3:1) mats at high and low collecting rates (HCR and LCR). This heating run is characterized by a change in the heat capacity of the mats associated with the glass transition of PLA (between 55-60°C), followed by an exothermic peak resulting from a partial crystallization of the material, attributed to the re-arrange in amorphous molecular chains into a more ordered crystalline phase. The observed T_g for the casting PLA was 54.79°C and it showed a single melting peak at 152.31°C. As can be seen, DSC curves of mats collected at high and low rates, experiment the glass transition and cold crystallization process.

After the rapid crystallization process, two well differentiate melting peaks (at about 150-166 °C and 195°C respectively) appear only for PLA/HA samples collected at higher speeds, instead of the typical single melting peak that occurs on stationary mats. These melting peaks are separated by several degrees (28°C for the PLA/HA (3:1) mat, and about 44°C for the rest of mats). The double melting phenomenon is generally ascribed to several factors as reorganization processes of imperfect crystals leading to melt-recrystallization that occurs during the DSC heating scan, and that may result from different crystal morphologies formed during electrospinning [40-42].

Poly(lactic acid) can exhibit up to three possible crystal modifications (α , β and γ), depending upon the crystallization conditions [43]. The crystal structure of α and β forms have been widely investigated by several research groups, the first one was studied by De Santis and Kovacs [44] while the second one was reported by Eling [45]. Hoogsteen [46], who found that the β structure melts at temperature about 10° below the melting temperature of the α structure. From this observation they concluded that the PLA β structure is some-what less stable than the α structure. In our case, the LCR and HCR electrospinning mats conserved the single melting peak that occurs in the casting PLA, but the second melting peak developed in the high collecting mats occurs about 40°C above the first, that is, it has a higher thermal stability than the first one. So, the double melting peak phenomenon can be attributed here to the cold crystallization during the heating scan probably due to orientated crystals formed at a higher collection speed. As can be seen in **Table 2** a faster speed of the rotating collector not only produced two melting peaks in DSC results, but also a slightly increase in the degree of crystallinity comparing the results of LCR with HCR mats.

Figure 5a), shows the wide angle diffraction pattern for neat hydroxyapatite and PLA/HA (3:1) mat obtained at high collecting speed. All peaks observed in the WAXS pattern of hydroxyapatite (HA) correspond to the hexagonal crystal system and are identified according to ICDD (International Centre for Diffraction Data) standard (PDF Card no. 9-432) [33, 47]. These peaks appears for all PLA/HA mats around $2\theta=25^\circ$, revealing the presence of the filler as can be previously seen in SEM images (**Fig.1**). The peaks intensity increases with the filler concentration.

Figure 5b) shows the diffraction patterns of low and high collecting rates for neat PLA mats, which exhibited notable differences in the crystalline peaks between them. LCR diffraction pattern exhibited six diffraction peaks with different intensities; two strong at $2\theta = 17.1^\circ$ and 21.4° , and four weaker peaks at $2\theta = 8.5^\circ, 22.6^\circ, 23^\circ$ and 28° . The HCR diffraction pattern shows at least eight peaks; three strong at $2\theta = 4.57^\circ, 6.83^\circ$ and 21.4° and five weaker at $2\theta = 14.9^\circ, 16.8^\circ, 20.6^\circ, 22.8^\circ$ and 23.8° . **Figure 6** shows the resulting WAXS pattern for neat PLA mat after the fitting process.

Generally, authors have identified four PLA representative crystalline peaks in samples obtained by precipitations methods (casting) around $2\theta = 15^\circ, 16^\circ, 18.5^\circ$ and 22.5° which are associated with the pure PLA crystallizing in a pseudo-orthorhombic α -form crystal with a 10_3 helical chain conformation and unit cell dimensions of $a=1.07$ nm, $b=0.595$ nm, and $c=2.78$ nm [48]. In the scientific literature regarding PLA electrospun mats, the diffraction peaks are partly or totally detected in many cases for annealed samples, depending on the treatment conditions. Zong and Fundador produced PLA electrospinning mats, and only after annealing processes the as-spun material showed at least the two main PLA diffraction peaks at around 16° and 18.5° [49, 50] which were in agreement with the values indexed as 110 and 131 reflections by Ikada for the α form of the PLA crystal [48]. Fundador reported two other PLA characteristic peaks at 21.8° and 28.38° [50]. With respect to electrospun of poly(lactic acid)/Hydroxyapatite mats, Jeong and Sui [51], reported peaks at around $2\theta=26^\circ$ and 32° which correspond to the typical diffraction angles of crystalline hydroxyapatite, indexed as 002 and 211 respectively [52].

Liao [53] used an additional centrifugal field to introduce a stronger stretching force in the preparation of aligned polylactic acid nanofibers. The diffraction patterns of their material exhibited nine diffraction peaks, demonstrating that this additional centrifugal field induces crystalline features in the spun nanofibers. In our case, the LCR mats present the main peaks at about 17° and 21° which are in agreement with previous reports as characteristic peaks for the pseudo-orthorhombic α -form crystal with a 10_3 helical chain conformation form of the PLA crystal. The development of new peaks in the case of HCR mats demonstrate that an increase in collector speed not only increases the degree of alignment of the nanofibers, but can also induce crystalline features in PLA nanofibers, which is supported by WAXS and DSC crystallinity results (**Table 2**). Both methods show different values but the same general trend. The differences in the results between both crystallinity measuring methods is habitual in the published

literature, and may be explained because of the X-ray diffraction measures the intramolecular order as a result of chain packaging and DSC measures the “meltable” crystalline portion which numerical values are not as exactly (real) as X-ray measurements. If we focus the attention on the X-ray crystallinity measurements, the results show evidence of the possible nucleating effect of the hydroxyapatite on the PLA as a consequence of the linear increase in crystallinity in the low volume fraction region. This effect decreased for high filler contents probably due to the formation of agglomerates, being more prominent in randomly oriented mats, as can be seen in the SEM images (**Fig.1**).

The T_g values obtained in second DSC heating scans are shown in **Table 2**. The T_g of neat PLA and PLA/HA at a weight ratio of 9:1, 5:1, 3:1 were in case of LCR mats: 59.7 °C, 60.4 °C, 60.1 °C and 60.6 °C and in case of HCR mats: 51.4 °C, 54.9 °C, 55.1 °C and 60.9 °C respectively. So, as expected, glass transition temperatures slightly increased with the addition of HA with an evident higher effect in the case of stretched HCR nanofibers.

Finally, regarding the thermal properties, **Figure 7** shows the TGA curves for PLA and PLA/HA nanofibers mats with different hydroxyapatite content. The % of filler was found to be reasonably close to the values used in the initial formulations. HCR mats start their thermal decomposition at temperatures slightly higher than LCR mats, and the higher alignment produced less difference in the drop between neat and filled mats; but in all cases, an increase in the thermal stability of the filled samples confirms the stiffening effect of the nanoparticles. Moreover, thermal results measured as a maximum in a derivative curve, demonstrated a loss weight at about 230°C in HCR samples probably due to the solvent remaining because of the high collecting speed which prevents its full evaporation.

2.3 Dynamic mechanical properties;

DMA thermograms of neat PLA and PLA/HA (3:1) nanofibers mats for LCR and HCR are reported in **Figure 8**. The storage modulus (E') of HCR mats was higher than LCR probably due to a high degree of alignment and more packing structure. Moreover the E' values of the electrospun nanofibers mats of PLA/HA were slightly higher, and the drops were more gradual than that of the unfilled samples. Also in this case, the higher alignment of the mats produced less difference in the drop between neat and filled mat. So it can be concluded that while the main effect of the filler in the LCR mats is the stiffness

increase produced, in case of HCR mats, the synergic effect of the alignment with the filler content is more noticeable.

3. Conclusions

Nanofibers mats of PLA/HA have been obtained via electrospinning onto a rotating mandrel, and their morphology has been characterized as a function of the production parameters. Electrospun mats were collected at two take-up velocities; randomly oriented fibers were produced on a stationary shaft, whereas highly oriented fibers were produced when rotation speed was increased to 1100 rpm. On one hand, the added HA particles into the nanofibers produced an efficient nucleating effect, and as a result of it, an enhanced in thermal and mechanical properties was observed. On the other hand, thanks to the high collection speed, the HCR resulting mats showed higher alignment and crystallinity degree until a certain value above which, the properties of the mats did not further improve probably due to the formation of filler aggregates. Crystallinity behavior was studied by DSC and supported by WAXS, both methods showed different numerical values due to they measured different kinds of crystallinity, but the general trend was the same. Finally, TGA results confirmed a higher thermal stability for aligned mats, as well as for mats with higher filler content.

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Table 1: Electrospinning conditions for PLA and PLA/HA nanofibers mats and their corresponding average fiber diameter.

Sample code	Electrospinning conditions						²⁾ Average fiber diameter (nm)
	¹⁾ PLA/HA	Rotation speed	Collector distance	Flow rate	Voltage	mL/mat	
HCR PLA/HA (3:1)	3:1	1100	130	0.6	14	10	439±147
HCR PLA/HA (5:1)	5:1						357±92
HCR PLA/HA (9:1)	9:1						308±88
HCR PLA	1:0						323±117
LCR PLA/HA (3:1)	3:1	300					600±148
LCR PLA/HA (5:1)	5:1						523±120
LCR PLA/HA (9:1)	9:1						335±124
LCR PLA	1:0						409±163

Experimental results are expressed as mean ± standard deviation. ¹⁾ Concentration of 7 wt.-% PLA solutions in DCM/ DMF (70/30); ²⁾ Average diameter was calculated by measuring the size of 30 fibers from SEM images at x5000 magnifications.

Table 2: The melting and crystallization behaviors of PLA/HA nanofibers mats.

Sample	DSC 1 st heating								DSC 2 nd heating	Crystallinity (X _c %)	
	T _g (°C)	ΔC _p J/(g°C)	T _{cc} (°C)	ΔH _c (J/g)	T _{m1} (°C)	ΔH _{m1} (J/g)	T _{m2} (°C)	ΔH _{m2} (J/g)	T _{g2} (°C)	DSC 1 st h.	WAXS
HCR PLA/HA (3:1)	60.9	0.5	89	10.0	166.7	24.7	195.6	1.7	60.9	17.5	17.2
HCR PLA/HA (5:1)	58.2	0.5	85	11.4	151.4	24.7	195.6	2.3	55.1	16.7	13.5
HCR PLA/HA (9:1)	57.1	0.6	84.5	11.8	152.1	32.0	195.6	3.4	54.9	25.3	16.3
HCR PLA	60.3	0.7	86.8	15.4	150.9	27.1	195.6	4.3	51.4	17.2	14.2
LCR PLA/HA (3:1)	63.5	0.2	96.7	15.5	164.3	23.1	--	--	60.6	8.1	9.3
LCR PLA/HA (5:1)	62.3	0.6	90.5	14.1	150.2	26.6	--	--	60.1	13.3	11.8
LCR PLA/HA (9:1)	61.3	0.8	87.4	15.9	153.3	35.1	--	--	60.4	20.5	11.5
LCR PLA	61.2	0.7	89.9	17.3	152.6	32.7	--	--	59.7	16.4	4.7
BULK PLA	54.8	0.4	--	--	152.3	1.1	--	--	61.0	1.2	0

*Glass transition temperature (T_g), cold crystallization temperature (T_{cc}) melting temperatures (T_{m1} and T_{m2}), crystallization and melting enthalpies (ΔH_c and ΔH_m) calculated from the first DSC heating run. T_{g2} was calculated from the second DSC heating run. DSC crystallinity values for LCR mats were calculated since formula (1), note that for HCR the ΔH_m value results are the sum of ΔH_{m1} + ΔH_{m2}.

Figure Captions

Figure 1. SEM images of electrospun PLA/HA mats obtained with different hydroxyapatite (HA) content at low and high collecting speeds (LCR and HCR). All the images are x5000 magnifications: a, b) neat PLA, c, d) PLA/HA 9:1, e, f) PLA/HA 5:1, g, h) PLA/HA 3:1

Figure 2. Fiber aligned angle distributions of PLA/HA mats obtained with different hydroxyapatite (HA) content at low and high collecting speeds (LCR and HCR): a, b) neat PLA, c, d) PLA/HA 9:1, e, f) PLA/HA 5:1, g, h) PLA/HA 3:1

Figure 3. TEM images of electrospun fiber prepared with PLA/HA (3:1) at low and high collecting speeds (LCR and HCR): a) LCR b) HCR.

Figure 4. First heating run DSC thermograms of electrospun mats prepared from neat poly-lactic acid and PLA/HA (3:1) obtained at low and high collecting speeds (LCR and HCR).

Figure 5. a) WAXS patterns for neat hydroxyapatite (HA) and PLA/HA (3:1) mat obtained at high collecting speed (HCR) b) WAXS patterns for neat poly-lactic acid (PLA) mats at low and high collecting speeds (LCR and HCR).

Figure 6. Gaussian peak fitting for neat PLA mat at low collecting speed (LCR PLA).

Figure 7. TGA curves of electrospun PLA and PLA/HA nanofibers mats. a) Low collecting speed mats (LCR) b) High collecting speed mats (HCR).

Figure 8. DMA thermograms of neat PLA and PLA/HA (3:1) nanofibers mats obtained at low and high collecting speeds (LCR and HCR).