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Biocompatibility Studies of Electrospun Nanofibrous Membrane of PLLA-PVA Blend

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ABSTRACT: Electrospinning, self-assembly, and phase separation are some of the techniques available for the synthesis of nanofibers. Of these techniques, electrospinning is a simple and versatile method for generating ultrafine fibers from a wide variety of polymers and polymer blends. Poly L-lactide (PLLA) and Poly (vinyl alcohol) (PVA) are biodegradable and biocompatible polymers which are mainly used for biomedical applications. Nanofibrous membranes with 1:9 ratio of PLLA to PVA (8 to 10 wt % and 10 wt %) were fabricated by electrospinning. The percentage porosity and contact angle of PVA in the PLLA-PVA nanofibrous mat increased from 80 to 83% and from 39 ± 3° to 55 ± 3°, respectively. The water uptake percentage of PVA nanofibers decreased from 190 to 125% on the addition of PLLA to PVA in the PLLA-PVA nanofibrous mat. The nanofiber morphology, structure and crystallinity were studied by Scanning electron microscopy (SEM), Transmission electron microscopy (TEM), Fourier transform infrared spectroscopy (FT-IR), and X-ray diffraction (XRD), respectively. The thermal properties were studied by thermogravimetric analysis (TGA) and differential scanning calorimetery (DSC). The biocompatibility studies of PLLA-PVA blend were performed using fibroblast cells (NIH 3T3) by MTT assay method. The release of Curcumin (0.5, 1.0, and 1.5 wt %) from PLLA-PVA blend was found to be ~ 78, 80, and 80%, respectively, in 4 days. © 2012 Wiley Periodicals, Inc. J. Appl. Polym. Sci. 000: 000–000, 2012

KEYWORDS: nanofiber; biodegradable; biocompatibility; biofibers; blending

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INTRODUCTION

Nanotechnology is an interdisciplinary area of research, to develop nanoscale building blocks to meet the demand for new materials and devices with improved properties and functionalities.[1,2] Nanofibers exhibit unique properties due to their extremely small diameter and very high aspect ratio.[3–5] Nanofibrous blends can be either natural or synthetic and with morphologies similar to natural tissues. The general criteria for selecting a polymer blend for use as biomaterial in tissue engineering applications, apart from being biocompatible and biodegradable is to match the mechanical properties to suit its desired application. An important class of synthetic degradable polymers include poly (α-hydroxyl ester) such as poly L-Lactide (PLLA). PLLA has been attracting the attention of researchers in tissue engineering applications because of its biocompatibility, biodegradability, and FDA approval. However, one of the major limitations in the use of PLLA for tissue engineering is its hydrophobicity, resulting in low hydraulic permeability and limited cell adhesion. As the mechanical properties of PLLA are often too stiff for many soft–tissue applications,[6] a blend of PLLA with a soft hydrogel such as PVA which is also biocompatible and biodegradable is seen as a better option. The blending of PLLA with PVA shows the properties of both polymers and has controlled hydrophilicity and high flexibility which is useful for tissue engineering. The hydrophilic polymers such as PEG,[7] PVP,[8] and PVA[9–11] have been already reported to improve the permeability of the scaffolds. Of these PVA is one of the best polymers to blend with PLLA because of its low solubility at room temperature. The blended scaffold is used as a carrier of drugs and proteins because of its good tissue compatibility and ease of manipulation under swelling conditions, solute permeability, and particularly excellent electrospinnability. PVA is also cost effective compared with polyester polymers.

The nanofibers produced by electrospinning have gained popularity due to morphological characteristics similar to extracellular matrix. During electrospinning, due to large static electric forces in the polymer solution, the polymers are spun into nanosized nonwoven mesh composed of fibers with high surface area and porosity.[12]The electrospun nanomesh is capable of supporting cellular attachment.[13] The morphology and

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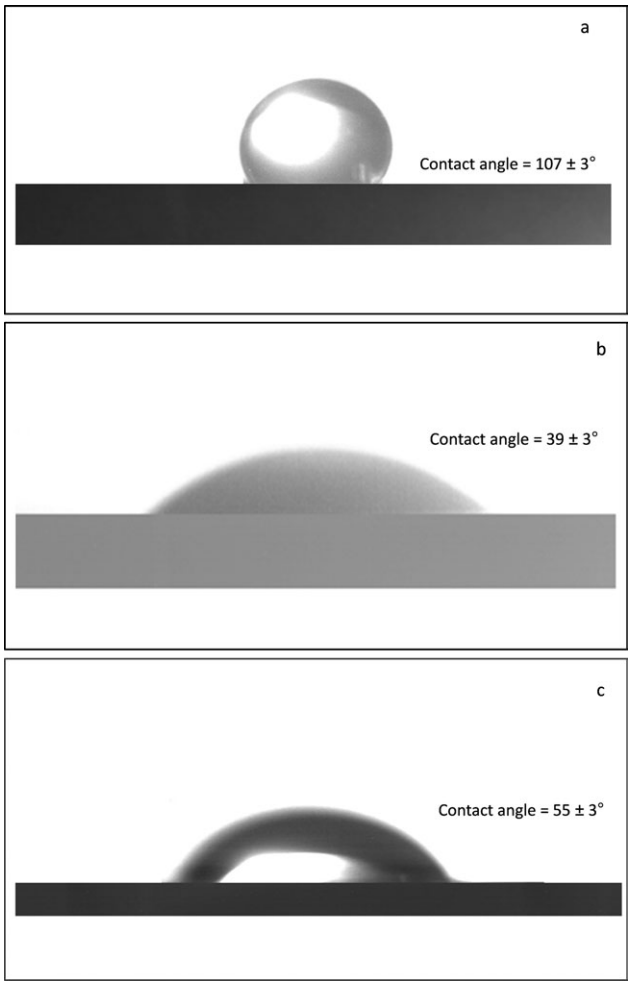

Figure 1. Wettability studies of electrospun nanofibrous mat (a) PLLA (10 wt %), (b) PVA PVA (10 wt %), and (c) PLLA-PVA (8 wt %).

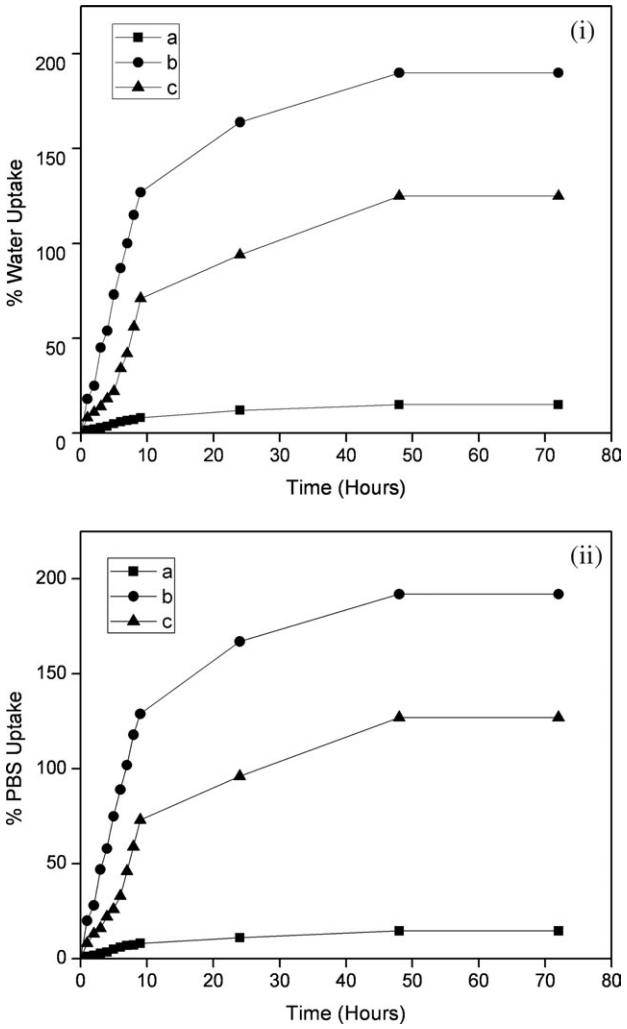

Figure 2. (i) Water uptake and (ii) PBS uptake test of electrospun nanofibrous mat (a) PLLA (10 wt %), (b) PVA PVA (10 wt %), and (c) PLLA-PVA (8 wt %).

properties of the electrospun fibers depend on a number of parameters such as solution concentration, solution conductivity, applied electric field, collection distance, and collection time.

PLLA and PVA blend copolymers are able to form micelles and hydrogels in aqueous solution.[14] Blend compatibility studies on PLLA and PVA have shown that PLLA and PVA exhibit widely varying miscibility/immiscibility trends depending upon their compositions.[15] In recent years, molecular modeling simulations have been advanced to such a level to predict the blend compatibility of polymers.[16]

The present study is an attempt to electrospin a synthetic blend of PLLA and PVA by optimizing the composition of the blend, concentration of polymer solution, spinning voltage, the distance between the tip to target and the flow rate of the polymer solution. The biocompatability studies of PLLA-PVA blend were performed using fibroblast cells (NIH 3T3) by MTT assay method. The PLLA-PVA blend was loaded with curcumin (0.5, 1.0, and 1.5 wt %) and the release of curcumin at the end of 4th day was found to be approximately 78, 80, and 80%, respectively.

Table I. Apparent Density and Porosity of PLLA, PVA, PLLA-PVA Nanofibers

Sample	Weight of polymer nanofibrous mat (W_t) (g)	Length × width of polymer nanofibrous mat ($L \times W$) (cm^2)	Thickness of polymer nanofibrous mat (T_h) (cm)	Apparent density (g/cm^3) ($\rho = W_t/L \times W \times T_h$)	Calculated average porosity (%) $P = (1 - \rho/\rho_0) \times 100$
PLLA	0.008	2 × 2 = 4	0.017	0.11765	91
PVA	0.029	2 × 2 = 4	0.029	0.25	80
PLLA-PVA	0.032	3 × 2 = 6	0.025	0.21333	83

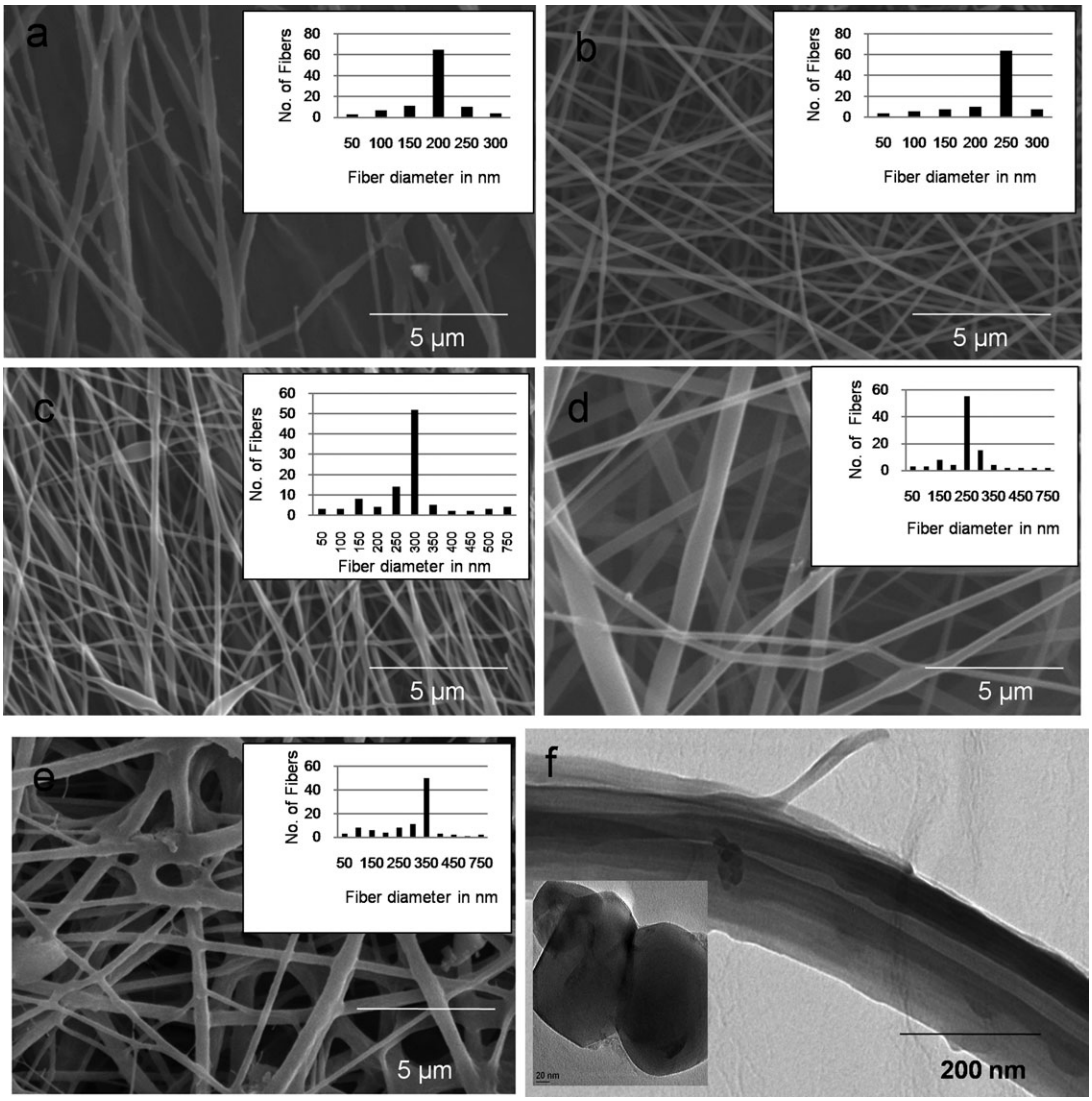

Figure 3. SEM pictures and the histogram graph (inset) of electrospun (a) PLLA (10 wt %), (b) PVA (10 wt %), (c) PLLA-PVA (8 wt %), (d) PLLA-PVA (10 wt %), (e) C-PLLA-PVA, and (f) TEM pictures of C-PLLA-PVA. The diameters of the fibers are in the range of a few nanometers to few micrometers. Voltage applied was 15 kV and the distance between electrodes was 12 cm.

MATERIALS AND METHODS

Materials

Poly-L-Lactide (PLLA) (M_w = 1, 00,000–1, 50,000) and Curcumin from Curcuma longa (turmeric) was purchased from Sigma - Aldrich, India. Poly (vinyl alcohol) (PVA) (Medium average molecular weight), Dimethyl Sulfoxide (DMSO) and Hexafluoroisopropanol (HFIP) was purchased from Sisco Research Laboratories Private Limited, India. All chemicals were used as received without further treatment or purifications.

Electrospinning Process

The electrospinning unit used in this experiment has been designed and developed in the Conducting polymer lab, IITM, Chennai. PLLA (8–10 wt %) and PVA (10 wt %) were dissolved in HFIP and gently stirred continuously for 2–4 h separately. Then the two solution was blended in the ratio of 1:9 (PLLA: PVA) ratio. The polymer solution was taken in a 2-mL syringe to which a needle tip of 0.56 mm inner diameter was attached. The positive electrode of the high voltage power supply was connected to the needle and the negative terminal to the collector which is covered with aluminum foil as shown in Figure 1. Electric voltage was optimized at 15 KV. The polymer solutions were electrospun at a flow rate of 0.35 mL/h and the tip to collector distance was kept at 12 cm.

CHARACTERIZATION OF NANOFIBERS

Physico–Chemical Characterization

Porosity Measurements. Percentage Porosity (P) of the nanofibrous mat was calculated by comparing its apparent density (ρ) to the bulk density.[17]

Contact Angle Measurements. Euromex Optical Microscope equipped with a CCD camera[18] was used to measure the hydrophilicity of the polymer nanofibers.

Water Uptake Test. The water uptake characteristics of polymer nanofibrous mat in deionsed water (DW) and phosphate

Table II. The Distribution of Diameter of Polymer Nanofibers

Polymer	Average diameter	Uniform distribution
PLLA	50–300 nm	150–200 nm
PVA	50–300 nm	200–250 nm
PLLA-PVA (8 wt %)	30–700 nm	250–300 nm
PLLA-PVA (10 wt %)	30–700 nm	250–300 nm
Curcumin (1 wt %) loaded PLLA – PVA	50–750 nm	300–350 nm

buffered solution (PBS) (pH = 7.4) were measured using already reported procedure.[19]

Surface Morphological Studies

The morphologies of the electrospun fibers were observed using SEM (FEI Quanta FEG 200 - HRSEM) at 15 kV and TEM (TF 20: Tecnai G2) at 200 kV, respectively. The diameters of the fibers were measured using UTHSCSA image tool.

Fourier Transform Infrared Spectroscopy

The functional groups were characterized by using Perkins-Elmer spectrum RX 100 in the range of 400 cm^{-1} to 4000 cm^{-1}.

X-Ray Diffraction Studies

The crystallinity of the polymer nanofibers was examined on XRD–X'pert pro PANalytical Instrument using Cu Kα radiation (λ = 1.5418 Å).

Thermal Analysis

The TGA of the polymer nanofibers was performed on TGA/DTA model SDT 2600 at a heating rate of 10°C/min from 37°C to 800°C under constant nitrogen flow of 20 cm^3/min. The DSC spectra of the blended polymer nanofiber were obtained on NETZSCH-Geratebu model DSC 200PC.

In-Vitro Release of Curcumin

The *in-vitro* release of curcumin from PLLA-PVA blend nanofibers were studied by incorporating 0.5, 1, and 1.5 wt % curcumin into the blend. The *in-vitro* curcumin release studies were performed using High Performance Liquid Chromatography (HPLC) equipped with Ultra-violet (UV) detector at 450 nm.

Biocompatibility Study

The biocompatibility study of PLLA-PVA blend was performed using mouse embryonic fibroblast (NIH 3T3). The cell line was grown and maintained in DMEM with 10% fetal calf serum (FCS) supplemented with penicillin (120 units/ml), streptomycin (75 mg/ml), gentamycin (160 mg/ml) and amphotericin B (3 mg/ml) at 37°C in a incubator humidified with 5% CO_2. To observe the degree of cellular viability 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium (MTT) assay[20] was employed and compared with tissue culture treated polystyrene (control).

Experimental Design for Biocompatability Tests on PLLA-PVA Matrix

The biocompatability tests of PLLA-PVA blend nanofibers were carried out using UV sterilized PLLA-PVA nanofibrous blend. The blend was placed in wells of culture plate and was neutralized with phosphate buffer saline (pH = 7). The polystyrene surface (wells without PLLA-PVA blend nanofibers) was treated as control. The cells were seeded at the density of 2×10^4 cells per well and incubated for 72 h at 37°C in a humidified atmosphere containing 5% CO_2. The medium was changed every day. After 24, 48, and 72 h of incubation, the supernatant of each well was replaced with MTT diluted in serum-free medium and the plates were incubated at 37°C for 2 h. After aspirating the MTT solution, acid isopropanol (0.04 *N* HCl in isopropanol) was added to each well to dissolve the dark blue crystals and then left at room temperature for a few minutes to ensure all crystals are dissolved. The absorbance was measured at 630 nm using UV spectrophotometer. Each experiment was performed at least three times to check for consistency. The sets of three wells for the MTT assay were used for each experimental variant. The results are expressed as mean standard error of the absorbance. Data were analyzed by student's *t*-test and differences at the 95% confidence level were considered to be significant.

RESULTS AND DISCUSSIONS

Physico-Chemical Characterization

The percentage porosity and the apparent density of PLLA, PVA, and PLLA-PVA nanofibrous mat are shown in Table I. The percentage porosity of PLLA, PVA, and PLLA-PVA nanofibrous mat was found to be 91, 80, and 83%, respectively. This shows that the porosity of PVA increased on the addition of PLLA to PVA in the blend. The hydrophilicity test of PLLA, PVA, and PLLA-PVA nanofibrous mat is shown in Figure 1. The contact angle of PLLA and PVA was found to be 107 ± 3° and 39 ± 3°, respectively. The addition of PLLA to PVA in the blend increases the contact angle of PVA from 39 ± 3° to 55 ± 3° as shown in Figure 1. These results are in close agreement with Ya et al.[21] who have reported the contact angles of PLLA-PVA composite nonwovens to be 51.17°. The water uptake percentage of PLLA, PVA, and PLLA-PVA was found to be approximately 15, 190, and 125% in both DW and PBS media as shown in Figure 2.

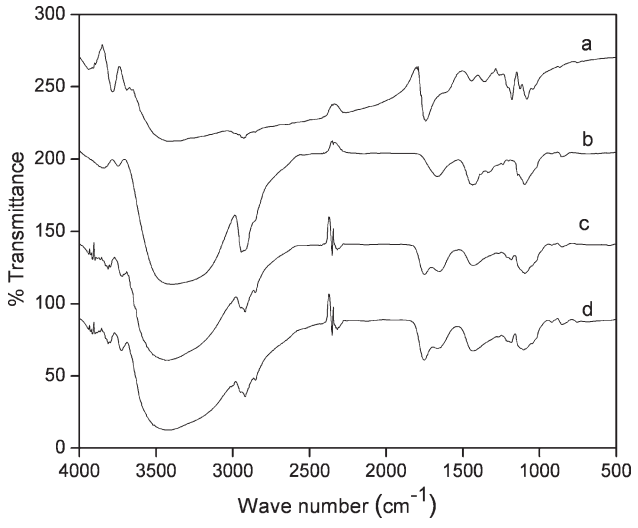

Figure 4. FT-IR Spectrum of electrospun mats (a) PLLA (10 wt %), (b) PVA PVA (10 wt %), (c) PLLA-PVA (8 wt %), and (d) PLLA-PVA (10 wt %).

Table III. The Assigned Bands of the Polymer Nanofibers

Electrospun polymer wavenumbers (cm^{-1})				
PLLA	PVA	PLLA-PVA (8 wt %)	PLLA-PVA (10 wt %)	Mode of vibration
1759	-	1749	1751	C=O bond Stretching
2995	-	2920	2918	C—H Stretching of CH_3 group
1184	-	1183	1183	Stretching of C—O—C bond of the ester group
-	3747	3720	3725	Hydroxyl bonds for free alcohol (non-bonded O—H Stretching bond)
-	3397	3441	3417	O—H Stretching Vibration
1097	1097	1097	1097	Carboxyl Stretching band (C—O)

Surface Morphological Studies

The morphology of the electrospun nanofibers depends on several parameters such as viscosity, applied voltage, diameter of the needle, flow rate, and the distance between the needle tip and collector. A 1:1 ratio of PLLA:PVA blend did not show good miscibility. Hence different blend ratios were tried of which 1:9 ratio of 10 wt % PLLA:PVA showed good miscibility which resulted in bead free nanomesh. The SEM images of PLLA, PVA, PLLA-PVA blend (8 and 10 wt %) and Curcumin loaded PLLA-PVA blend are shown in Figure 3(a–e), respectively. The TEM image of Curcumin loaded PLLA-PVA blend are given in Figure 3(f) which clearly show the uniform dispersion of Curcumin in the PLLA-PVA nanofibrous mat. The mean diameter of the PLLA, PVA, and PLLA-PVA nanofibrous mat were measured using UTHSCSA image tool and tabulated in Table II. The FT-IR spectra of electrospun PLLA, PVA, and PLLA-PVA (8 and 10 wt %) nanofibrous mat are shown in Figure 4. PVA is known to form inter and intramolecular hydrogen bonding in the pure state. Hence, it would be interesting to observe whether there is any interaction between PLLA and PVA through hydrogen bonding which might be detected through FT-IR spectra from the peaks corresponding to the line shape and vibrational frequency of the PVA hydroxyl bands. The presence of peaks at 3397 cm^{-1} corresponds to O—H bond stretching of PVA. This band is shifted slightly to higher frequency of 3441 cm^{-1} and 3417 cm^{-1} in the case of 8 and 10 wt % PLLA-PVA blends. The peak at 1759 cm^{-1} corresponds to the C=O bond stretching, which is a characteristic peak of PLLA. This band is shifted slightly to lower frequency of 1749 cm^{-1} and 1751 cm^{-1} in the case of 8 and 10 wt % PLLA-PVA blends. This clearly brings out the existence of strong interpolymer hydrogen bonding in the blends. Shuai et al.[15] did not observe any change in the hydroxyl band but they observed the shift in carbonyl bands in the PLLA-PVA blends. Table III shows the assigned bands of PLLA, PVA, and PLLA-PVA (8 and 10 wt %) blend. XRD profiles of PLLA, PVA, and PLLA-PVA blend (8 and 10 wt %) are shown in Figure 5(a–d). The characteristic peak of PLLA and PVA was found to be at $2\theta = 16.9°$ and 20° respectively as reported by many authors.[22–25] Figure 5(c, d) shows strong interaction between PLLA and PVA which leads to the amorphous nature of the blend.[24,26]

Thermal Properties

Figure 6 shows the TGA curves of PLLA, PVA, and PLLA-PVA blend. There is a slight weight loss below 100°C due to the evaporation of solvent. The decomposition of PVA began around 280°C but in the case of 8 and 10 wt % PLLA-PVA blends, it began around 293 and 294°C. The loss of half of its mass occurred at 350°C for PVA and for PLLA-PVA blends of 8 and 10 wt % it occurred at 395 and 360°C. The residue of 8 and 10 wt % PLLA-PVA blends was found to be 0.74% and 0.23% at 700°C. On comparing all the thermograms, a shift in the decomposition temperature towards the higher side was observed in the blends compared to pure PLLA and PVA. These results further confirm the strong hydrogen bonding between PLLA and PVA. The DSC graphs in Figure 7(a) shows a first endothermic peak at 58°C which corresponds to glass transition temperature T_g of PLLA. The next important endothermic peak at 171°C is due to the melting temperature T_m of PLLA.[27] In Figure 7(b) the first endothermic peak at below 100°C is due to

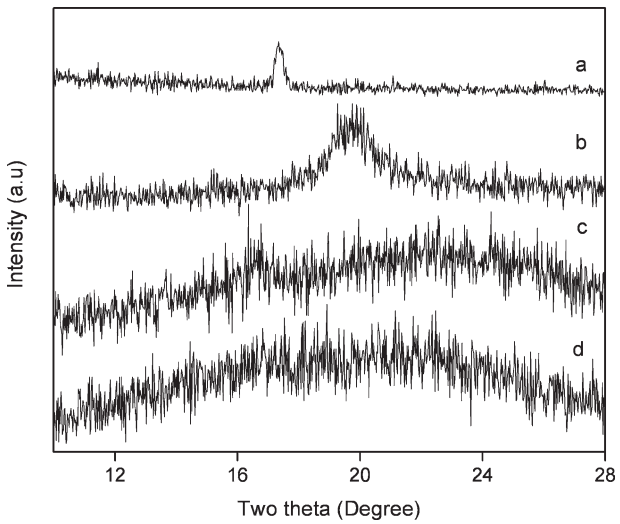

Figure 5. XRD Spectrum of electrospun PLLA and PVA is compared with the blend PLLA and PVA at two different compositions. This XRD results clearly reveals that both are semi-crystalline polymer. A 2θ value of PLLA is 16.9° and PVA is 19.7°. (a) PLLA (10 wt %), (b) PVA (10 wt %), (c) PLLA-PVA (8 wt %), and (d) PLLA-PVA (10 wt %).

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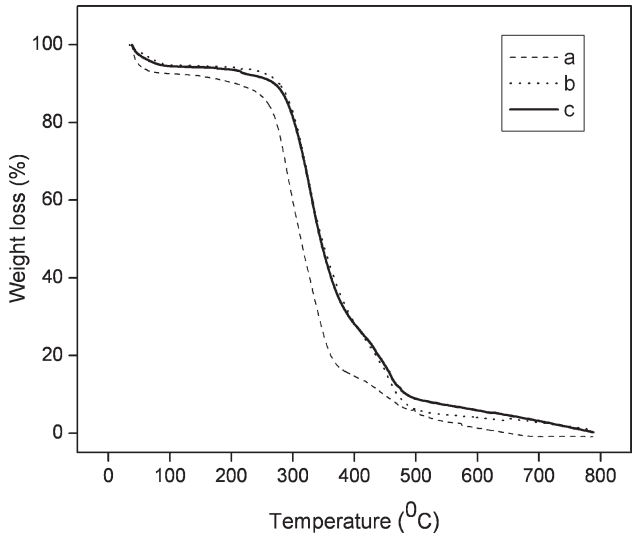

Figure 6. TGA graph clearly shows that the PLLA-PVA blend stability is more compared to either PLLA or PVA. (a) PVA (10 wt %), (b) PLLA-PVA (8 wt %), and (c) PLLA-PVA (10 wt %).

loss of solvent and the peak at 210°C is the melting temperature (T_m) of PVA. In the PLLA-PVA blend, the glass transition temperature and the melting temperature of PLLA is shifted to 61 and 174°C, respectively. On the other hand, the melting temperature of PVA is shifted to 212°C. The shift in T_g to higher temperature possibly indicates better chain entanglement of PLLA with PVA in the blend which further confirms the results of FT-IR and TGA.

Biocompatibility Study and In Vitro Curcumin Release Studies

To observe the biocompatibility of 10 wt % PLLA-PVA nanofibrous mat, the MTT assay was performed using NIH 3T3 fibroblast for 24, 48, and 72 h, respectively. The results are shown in Figure 8 which show good cell viability and in all the incubation periods. The cellular viability as shown in Figure 8(a) was found to be more than 100% in comparison to the control. This in turn confirms that the nutrition to the cells was good in all parts of the nanofibrous mat which could be attributed to the comfortable porosity present in the scaffolds. Similar patterns have been observed from the SEM analysis supporting the

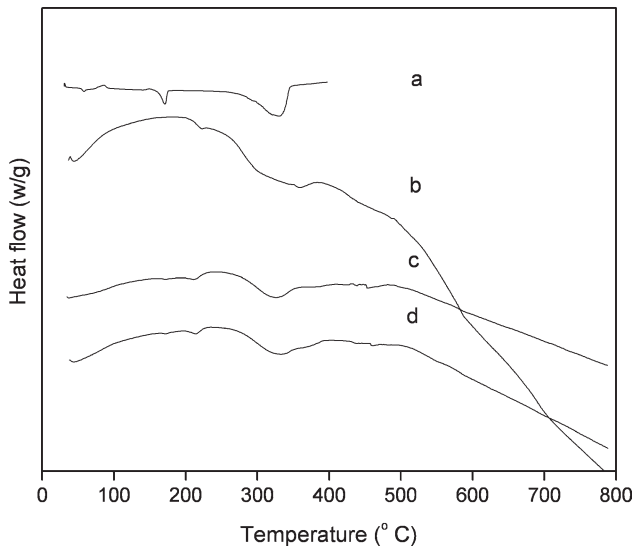

Figure 7. DSC graph of electrospun mats (a) PLLA (10 wt %), (b) PVA PVA (10 wt %), (c) PLLA-PVA (8 wt %), and (d) PLLA-PVA (10 wt %).

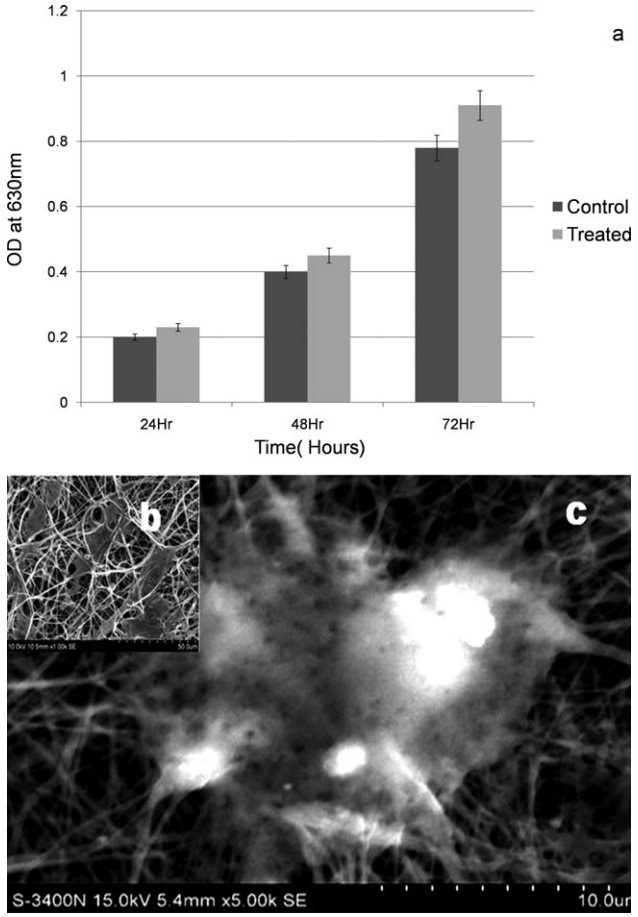

Figure 8. MTT assay for PLLA-PVA nanofibrous mat using NIH3T3 fibroblasts (a) Cell viability of the PLLA–PVA blend in comparison with the control, (b) SEM photographs at 1000×, and (c) SEM photographs at 5000X magnifications of the blend, respectively.

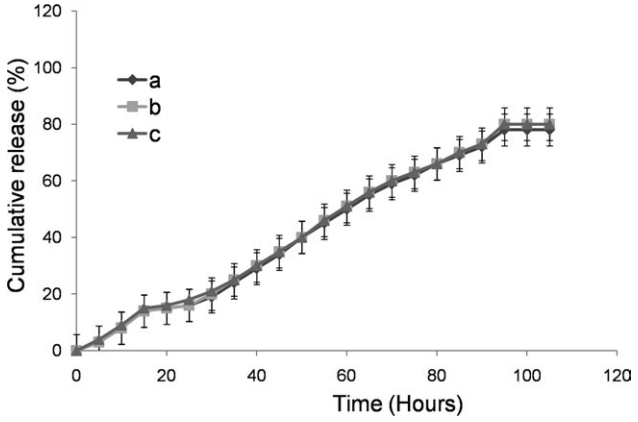

Figure 9. *In vitro* Curcumin release studies from Curcumin loaded (a) 0.5 wt %, (b) 1 wt %, and (c) 1.5 wt % PLLA-PVA nanofibrous mat.

data of MTT assay. Figure 8(b, c) shows the SEM images of the spindle shape fibroblasts maintained during the culture. Cells grew in the direction of the fiber orientation according to the architecture of the nanofibrous structure and some cells migrated underneath the fibers also. The MTT assay and SEM analysis supports that PLLA-PVA nanofibrous mat could be counted as a good biocompatible material, as superior cell attachment and proliferation was observed. These observations of the biocompatibility reflect the potential of the prepared scaffolds to be used for tissue engineering applications.

The release of Curcumin from Curcumin-encapsulated PLLA-PVA blend nanofibrous scaffolds was investigated. The *in vitro* release of Curcumin into PBS containing DMSO over a period of 4 days is shown in Figure 9. At the initial stage, loosely bound Curcumin on the surface resulted in small burst release effect. This initial small burst Curcumin release was later followed by more controlled and sustained release. The percentage cumulative Curcumin release at the end of fourth day period for 0.5, 1.0, and 1.5 wt % formulations was approximately 78, 80, and 80%, respectively.

CONCLUSIONS

In this work, biodegradable, biocompatible nanofibers of PLLA, PVA, and PLLA-PVA blend in the ratio of 1:9 were electrospun into nanofibers. The biocompatibility and *in vitro* release of Curcumin from the PLLA-PVA blend was investigated. The SEM micrographs show 100% bead free nanofibers in the above miscibility ratio of the blend. The FT-IR spectra clearly shows that there is an increase in the intensity of the —OH band and decrease in the intensity of C=O band when PVA is mixed with PLLA which in turn confirms strong hydrogen bonding between PLLA and PVA. The XRD data clearly reveals that both PLLA and PVA are semicrystalline polymers and the nanofibers showed diffraction peaks at $2\theta = 16.9^\circ$ and 19.7° which becomes amorphous on blending. A shift in the decomposition temperature as evidenced by the thermograms towards higher temperature in the PLLA-PVA blends further confirm the strong hydrogen bonding between PLLA and PVA. MTT assay of PLLA-PVA blend (1:9 ratio) shows good biocompatibility, good hydrophilicity, and cell compatibility which confirms the suitability of the blend for tissue engineering applications. *In vitro* release of Curcumin (0.5–1.5 wt %) from the PLLA-PVA blend shows ~78–80% release. Hence this study shows the potential of electrospinning technique for the preparation of PLLA-PVA biocompatible scaffolds for biomedical applications.

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