Preparation and characterization of electrospun poly(3-hydroxybutyrate-co-3-hydroxyvalerate) fibrous mats

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Abstract. Poly (3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV), as a biodegradable polyester, was electrospun to obtain defect-free fibers with high surface-area-to-volume ratio. Several parameters such as solvent ratio, polymer concentration, applied voltage, flow rate, and tip-to-target distance were optimized to achieve defect-free morphology. The average diameter of the PHBV fibers was 1400 nm. In order to evaluate the final properties of PHBV nanofibers, the following characterization techniques were employed: scanning electron microscopy (SEM), Fourier transform infrared attenuated total reflectance (FTIR-ATR) spectroscopy, uniaxial tensile tests and dataphysics instruments.

1. Introduction

Electrospinning, which was introduced in early 1930s, is known as a novel and effective fabrication method for producing ultra fine fiber of nanometer to micrometer scale. Generally, electrospun nanofibrous mats with controllable diameters, high specific surface and porosity and three dimensional network structures, have the ability to closely mimic the biological function and structure of nature extracellular matrix (ECM). And electrospun nanofibrous scaffolds served as a three-dimensional template could provide favorable environment for directing cell adhesion, migration proliferation and differentiation, and, thus, promote tissue regeneration in vitro as natural extracellular matrix (ECM) does in vivo. As a consequence, electrospinning has been widely used for nanofibrous scaffold fabrication and have shown potential as effective tissue engineering scaffolds [1, 2]. Therefore, electrospun nanofibers are being explored as scaffolds for nanodevices, biosensors, drug delivery, and tissue engineering applications. Various polymers including synthetic ones such as PCL, polylactic acid (PLA), polyglycolic acid (PGA), PLGA, polystyrene, polyurethane (PU), polyethylene terephthalate, poly (L-lactic acid)-co-poly (e-caprolactone) (PLACL) and biological materials such as collagen, gelatin, chitosan have been successfully electrospun to obtain fibers with diameters ranging from 3 nm to 5 µm [3-6].

Poly (3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV), as a member of polyhydroxyalkanoate (PHA), is a well-known biodegradable, biocompatible, nontoxic, thermoplastic polyester produced by bacteria [7]. Besides that, PHBV possesses good oxygen permeability and mechanical strength, and...
the ultimate degradation product is (R)-3-hydroxybutyric acid which is a normal constituent of human blood. Therefore, PHBV has been extensively explored as biomaterials for in vitro and in vivo studies, and used for various biomedical devices such as sutures, prosthetic devices, drug delivery systems and surgical applications.

According to the investigations, Nonwoven electrospun PHBV fibrous mats have already been produced and revealed (in vitro) a better performance for chondrocytes and fibroblasts attachment, growth and proliferation when compared to their 2D film counterparts [8, 9]. In addition, electrospun PHBV fibers loaded with an angiogenesis factor have shown to accelerate the wound healing process [10].

Electrospinning of PHBV fibers has been previously reported using solvents like hexafluoroisopropanol (HFIP) [11], trifluoroethanol (TFE) [12], chloroform [13], and chloroform with DMF [14]. Many studies have reported PHBV fiber diameters between 1 and 4 μm using chloroform as solvent [13]. In this paper, the objective of this study was to obtain electrospun poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) fibers used dichloromethane as the solvents, which has low toxicity and residue. The study was aimed to make comparison between PHBV fibers and 2-D films, and then based on the conclusion of the experiment, the study may be explored further for culturing human skin fibroblast cells, which made PHBV fibers can be used for the skin tissue engineering.

It is important to underline that, to the best of our knowledge, dichloromethane as the solvents, which has not been related studies by far. Meanwhile, such full characterization of electrospun PHBV has not been reported yet.

2. Materials and methods

2.1. Materials
Poly (3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) with 1.09 mol% 3-hydroxyvalerate (HV) (Mw = 2.67×10^5), was purchased from Tianan Biologic Material CO., dichloromethane (CH2Cl2, 99.5%) was supplied by Shanghai Lingfeng Chemical Reagent CO. All reagents and solvents were used as-purchased without any further purification.

2.2. Fabrication of electrospun PHBV nanofibers and 2-D films
PHBV fibers and films were prepared by electrospinning and solvent casting, respectively. Electrospun PHBV was fabricated as follows. PHBV powder was dissolved in CH2Cl2 with a magnetic stirrer for 12 h to prepare homogeneous solution, the overall polymer amount was kept 10% wt/v. Electrospinning was carried out in air at room temperature, the solution was filled in a 10ml syringe equipped with a 22 G metallic needle, fixed in a digitally controlled syringe pump (KD scientific, MA, USA), and the flow rate was maintained at 1mL/h. A rectangular steel plate covered with aluminum foil was placed 14 cm away from the needle tip. Meanwhile, the tip of the needle was connected to a high voltage of 15 KV (GAMMA, ES, USA). All electrospun fibers collected were dried under vacuum for 24 h and stored in a desiccator for further characterization.

Two-dimensional PHBV films were cast by dissolving the PHBV in CH2Cl2 and kept overnight at room temperature and further vacuum-dried for 24 h.

2.3. Surface morphology
The surface morphology of the electrospun PHBV fibers and 2-D films was analyzed using scanning electron microscopy (FE-SEM, JSM 6701F, JEOL, Tokyo, Japan) at an accelerating voltage of 3 kV at various magnifications. The samples were sputter-coated with platinum prior to imaging. The fiber diameters were measured from different locations, and the fiber diameter versus number of fibers was plotted.

2.4. Porosity analysis
The percentage porosity of the electrospun PHBV fibers and the 2-D film were measured by using the following equations [15], the thickness of the fibrous mats was measured by a micrometer.

\[
\text{Porosity (\%)} = \left(1 - \frac{d}{D}\right) \times 100\
\]

\[
d: \text{mat apparent density (g/cm}^3\text{)}
\]

\[
D: \text{bulk density of PHBV (g/cm}^3\text{)}
\]

2.5. **Fourier transform infrared spectroscopy**

Infrared (IR) spectra of all mats were recorded on a Nicolet 8700 spectrophotometer, by ATR reflection method. The spectrum was acquired in thewavelength range of 650–4000 cm\(^{-1}\), spectral resolution 4 cm\(^{-1}\), scans 128.

2.6. **X-ray diffractometry**

X-ray diffraction analyses (XRD, Philips X'Pert) were performed in the following conditions: CuK\(\alpha_1\) = 1.5402 Å, 2\(\theta\) range 0°–50°, step size 0.020°, time per step 4s, scan speed 0.03°/s.

2.7. **Thermogravimetric analysis (TGA)**

Thermogravimetric measurements (TG) were carried out by simultaneous thermogravimetric and differential thermal analyses (TGA, Netzsch209 F1) in the following conditions: sample weight about 7 mg, N\(_2\) flow 20 cm\(^3\)/min, temperature range 20–600°C, heating rate 10°C/min. Residual mass and maximum degradation temperature (T\(\text{d}\)) were measured.

2.8. **Tensile properties**

The tensile properties of the PHBV fibers and 2-D films were evaluated using Universal Material Testing Machine. Fibers of 10 mm × 80 mm (n=5) and 2-D films of 10 mm×80 mm (n = 5) were used in this study. The ends of the samples were mounted on the gripping units of the tensile tester, and a load of 500N at an extension rate of 5mm/min was applied until failure.

2.9. **Measure of water contact angle**

For evaluation of wettability, the water contact angle of the electrospun PHBV fibers and 2-D films was measured at room temperature using a Kruss GmbH DSA 100 Mk 2 goniometer (Hamburg, Germany) followed by image processing of sessile drop with DSA 1.8 software. A droplet of deionized water (2.0 µL) was placed on the surface of a film at room temperature. More than five measurements were carried out for a single sample, and the resulting values are the averaged results.

3. **Results and discussion**

3.1. **Morphology of electrospun mats and 2-D films**

The structure and surface morphology of PHBV nanofibers and 2-D film were observed under a scanning electron microscope. Figure 1B showed that PHBV nanofibers have a uniform structure without any sign of beads formation. Figure 1C illustrated that the average diameter of the fibers obtained at 10% (w/v) polymer concentration was 1400 nm. Besides that, the nanofibrous scaffold had a pore size 4.2 µm and a porosity of 90%, whereas the 2-D film presented a pore size 75.42 nm and a porosity of 24.57%. In this study, it can be concluded that PHBV fibers have much higher porosity and pore size than 2-D films. As for the cell culture, controlled pore size and high porosity are essential for the transport of oxygen and nutrient supply to the cells and cellular growth. 2-D films may be allowed the nutrients and metabolic wastes pass through by the nanosized porosity, but, they seem too small to provide enough space for cell ingrowth. Most importantly, the average porosity of skin tissue engineering is 70%-80%. Therefore, PHBV fibers are more suitable for the potential skin tissue engineering. In addition, it should be noted that PHBV fibers with different fiber diameter, porosity and pore size can be collected by only varying the process parameters.
Figure 1. Scanning electron micrograph showing the surface morphology of (A) solvent cast PHBV 2-D film, (B) electrospun nonwoven PHBV fibers, and (C) histogram showing the PHBV fiber size distribution.

Figure 2. ATR spectra of electrospun PHBV nanofiber and solvent cast PHBV 2-D film.
3.2. **ATR spectroscopy of electrospun mats and 2-D films**

The spectra of the PHBV film and PHBV fiber were compared in figure 2, which confirmed the coincidence between 2-D film and nanofibers. Specially, all the characteristic PHBV vibrational modes were detected, in agreement with the related studies. Figure 2 showed the prominent characteristic of the ester carbonyl (C=O) of PHBV at 1720 cm\(^{-1}\), meanwhile, the absorption peak at 1282 cm\(^{-1}\) indicated the strong C-O stretching band, which further confirmed the presence of the ester carbonyl (C=O) group. In addition, the absorption peak observed at 1058-1282 cm\(^{-1}\) can be assigned to the stretching vibration of C-O-C and bending vibration of –CH, –CH\(_2\). The bands centered at 1184 cm\(^{-1}\), 1229 cm\(^{-1}\) were shown the crystalline phase of PHBV, revealling the high crystallinity of PHBV nanofiers and films.

3.3. **XRD analyses of electrospun mats and 2-D films**

In figure 3 diffraction patterns of electrospun mats and 2-D films are reported. It can be obtained that the crystallinity of electrospun mats and 2-D films are 66.36%, 55.74% respectively, using the Analysis Software of JADE.

In figure 3, PHBV fibers and 2-D films presented diffraction peaks at around 2\(\theta\) 14\(^{\circ}\), 17\(^{\circ}\), 21\(^{\circ}\), 22\(^{\circ}\), 26\(^{\circ}\), 27.5\(^{\circ}\) and 30.5\(^{\circ}\), ascribable to the helix lamellar \(\alpha\)-form conformation of the PHBV and to the (020), (110), (101), (111), (121), (040) and (002) crystallographic planes, respectively. But in constrast, PHBV 2-D films showed relatively weak diffraction peaks compared with fibers, which can be explained that the crystal structure of 2-D films was imperfect owning to the untrolled volatilerate rate of solvent that hinder the formation of nuclei and the growth of crystal grain. It has been reported that PHBV exhibits isodimorphism due to the comparable chain conformation of poly (hydroxybutirate) (PHB) and poly (3-hydroxyvalerate) (PHV).\(^{[16]}\) Because of the low HV content in the present work, it can be expected to crystallizes in the PHB lattice. Therefore, as to the fibers, the presence of \(\beta\)-form crystals indicates a high level of molecular stretching in the amorphous region between the \(\alpha\)-crystalline lamellae, which might develop first. Wang et al. stated that significant chain orientation of the PHB molecules is likely to take place in the entrance region from the Taylor cone to the ejecting jet, where a severe jet stretching is induced by the convergent flow and electric repulsion [17]. The resulting orientation readily induces the crystallization of the \(\alpha\)-form lamellae, and the interlaced chains between the crystalline lamellae are further elongated during jet whipping to develop the planar zigzag \(\beta\)-form crystals.

![Figure 3. XRD patterns of PHBV, fibers (a) and 2-D films (b).](image)

3.4. **Thermogravimetric analysis (TGA) of electrospun mats and 2-D films**

The TG and DTG curves of the electrospun mats and 2-D films were showen in figures 4A and 4B, respectively, showing that the thermal degradation of PHBV occurred in one step.
In figure 4A, it demonstrated that PHBV fibers had higher onset degradation temperature than 2-D films obviously. In figure 4B, the two derivative curves presented two distinctly distinguishable thermal decomposition (Td) peaks, in which the degradation temperature of PHBV fibers and 2-D films were 215.5°C~279.8°C and 199.7°C~247.7°C, the maximum decomposing temperature were 268.1°C and 235.7°C respectively. The reasons can be interpreted as follows, PHBV fibers were stretched in the process of electrospinning, which led to the internal structure of PHBV more regular and stable, thus, more energy should be needed to make the molecular chain start moving, and then decomposing. For the 2-D films, the fast and uncontrolled volatilization of dichloromethane made the molecular chain in a mixed and disorder arrangement, as a result, the decomposing temperature was lower compared with PHBV fibers. It was known that the decomposing temperature was high when the polymer had high crystallinity, which was corroborating the XRD results (figure 3). In short, PHBV fibers were equipped with excellent thermostability in contrast with 2-D films.

![Figure 4. TG (A) and DTG (B) curves of electrospun mats and 2-D films.](image)

3.5. Mechanical properties of electrospun mats and 2-D films

Table 1 showed that PHBV fibers exhibited higher tensile strength but lower Tensile modulus compared with 2-D film. The result can be explained as follows, the PHBV fiber was collected by rotating frame cylinder, therefore, the fibers obtained were equipped with orientation partly. The fast volatility of CH₂Cl₂, leading to 2-D films with low crystallinity when preparing the 2-D PHBV film may be the other reason. The increase in Young’s modulus for the PHBV film may be due to its greater thickness (0.2 mm) and lower porosity (24.57%) when compared with the fibers (0.12 mm and 90%). Moreover, the strain at failure (ε) suggested that PHBV fibers were more elastic than the 2-D films, which were suitable for skin tissue engineering. Venugopal et al. have previously shown the efficacy of elastic poly (caprolactone) and poly (caprolactone)-collagen nanofibrous coating for skin regeneration applications when compared with less elastic matrices [18]. Hence, the perfect tensile stress and elasticity made PHBV fibers possess more advantages applied in the skin tissue engineering.

| material          | σ (MPa) | E (MPa) | ε (%)  |
|-------------------|---------|---------|--------|
| PHBV fiber        | 2.91    | 76      | 18.25  |
| PHBV 2-D film     | 2.68    | 89      | 14.75  |

σ: Tensile stress.
E: Tensile modulus.
ε: Strain at failure.

3.6. Water contact angle of electrospun mats and 2-D films

The static WCA valued are shown in figure 5, which confirmed that PHBV films (5B) exhibited a less hydrophilic surface with 128.2° and the PHBV fibers (5A) with 111.1°. The phenomenon for PHBV films may be due to its low porosity (24.57%) when compared with PHBV fibers (90%),
moreover, there existed three-dimensional network structure for the PHBV fibers, the capillary effect may lead to the higher WCA. But for the skin tissue engineering, the hydrophilic scaffolds are beneficial to direct cell adhesion, migration proliferation and differentiation. The hydrophilicity of PHBV fibers had slight improvement compared with 2-D films, but the PHBV fibers need further modification which can make it serve as potential artificial skin scaffolds.

![Figure 5](image.png)

**Figure 5.** The static water contact angles (WCA) of PHBV fibers (A) and solvent cast PHBV 2-D film (B).

4. Conclusions

Electrospun PHBV fibers were optimized to obtain defect-free morphology with an average fiber diameter of 1400 nm, and possess marked similarities to the natural ECM. Regarding the mechanical properties, the PHBV fibers presented excellent values (2.91 Mpa, 76 Mpa, 18.25%) with respect to the PHBV 2-D films (2.68 Mpa, 89 Mpa, 14.75%). On the ground of experimental findings, it was possible to conclude that the obtained PHBV fibers featured excellent properties with respect to the PHBV 2-D film. In particular, the SEM and WCA values made it possible to develop PHBV fibers with a potential skin tissue engineering application.

Acknowledgments

The authors would like to acknowledge the research center for analysis & measurement of Donghua University. This work is supported by Fundamental Research Program of Science and Technology Commission of Shanghai Municipality (13NM1401502).

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