Improved processability of electrospun poly[(R)-3-hydroxybutyric acid] through blending with medium-chain length poly(3-hydroxyalkanoates) produced by Pseudomonas putida from oleic acid

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Polyhydroxyalkanoates are bacterial biopolymers having good biocompatibility and biodegradability. Poly[(R)-3-hydroxybutyric acid] is the least expensive/costly and most easily available member of this family. However, poly[(R)-3-hydroxybutyric acid] is very brittle because of its high crystallinity. Its poor processability poses limitations on its application. Blending of this material with another amorphous flexible polymer has been a common practice. In this research, we have improved the properties of poly[(R)-3-hydroxybutyric acid] through blending with a palm oil-based medium-chain length polyhydroxyalkanoate. Solution blending was carried out at four different ratios to produce electrospun fibres. As expected, the addition of medium-chain length polyhydroxyalkanoate has reduced the brittleness of poly[(R)-3-hydroxybutyric acid], through reducing the crystallinity. In addition, it has enabled the reduction of the diameter of the electrospun fibres and shifted it from micrometre towards nanometres, which can improve its porosity and permeability to make it a potential material in biomedical applications.

Keywords: Electrospinning, Nanofibres, Polyhydroxyalkanoate, Polyhydroxybutyric acid

Introduction

Owing to environmental pollution issues caused by petroleum-based plastics, various efforts have been devoted to find potential alternatives to produce polymeric materials from sustainable resources. Polyhydroxyalkanoates (PHAs), which are microbial polyesters that possess biocompatibility, biodegradability and plastic-like properties, have the potential to be an environmental friendly alternative in diverse fields of applications.\(^1\)–\(^3\) Polyhydroxyalkanoates could be produced by some micro-organisms when one or more of the essential nutrients such as nitrogen, phosphorous, oxygen or sulphur is not sufficiently available to them. Polyhydroxyalkanoates are produced as intracellular carbon and energy reserves.\(^4\) Polyhydroxyalkanoates could be classified based on the number carbon atoms in their monomers as short-chain length (scl) and medium-chain length (mcl). While scl-PHAs (3–5 carbon atoms) tend to be brittle and highly crystalline, mcl-PHAs (6–14 carbon atoms) are amorphous and elastic.\(^4\)

Poly-3-hydroxybutyric acid (PHB) is the most common and the least costly to produce in the PHA family. Its physical properties are comparable to some of the petroleum-based polyethylenes. Therefore, a number of studies were done to improve its properties through copolymerisation\(^5\) and blending with other polymers.\(^6\) Copolymers of PHB with 3-hydroxyvalerate and 3-hydroxyhexanoate are already commercially available. However, because of limitations in copolymerisation, blending it with other polymers has been widely investigated to produce new materials with desirable properties. Thus, blends of PHB with polycaprolactone,\(^7,8\) polylactic acid,\(^10,11\) chitosan\(^12\) and polyaniline\(^13\) are some of the systems that have been studied.

Electrospinning is a process which applies electrostatic force to produce ultrafine and nanofibres. Owing to its simplicity and ease of application, this technique has been widely explored. Electrospun fibres, because of their unique volume to surface ratio, display specific characteristics and have found a wide range of applications such as scaffolding and filtration.\(^14,15\) A simple electrospinning set-up consists of a high-voltage power supply, a pump to maintain a constant-controlled flow rate at the spinning time, a needle or capillary as
the spinneret and a piece of metal foil used as the collector. 

In recent years, electrospinning of PHB blends was widely carried out and the potential of nanofibres was investigated for scaffolding and other biomedical applications. In this study, we have looked into the electrospinning of PHB blended with mcl-PHA biosynthesised by *Pseudomonas putida* from palm kernel oil (PKO) as a substrate. Since both PHB and mcl-PHA possess good biocompatibility and biodegradability, their electrospun blends would possess all the advantages of pure PHB plus decreased crystallinity and improved porosity and permeability, due to reduction of fibre diameter, which are important factors in designing scaffolds. Also, research shows that less crystalline surfaces in scaffolds can improve scaffold–cell interaction of certain types of cells such as fibroblasts. For carrying out the electrospinning, instead of conventional DC current, we have used a semi-rectified DC. Blending was carried out at four different ratios of PHB/mcl-PHA, and the effect of the added mcl-PHA on morphology and microstructure of electrospun fibres was studied.

**Experimental**

**Materials and methods**

Poly[(R)-3-hydroxybutyric acid] ($M_w = 300,000$ g mol$^{-1}$) was purchased from Aldrich, USA. Chloroform and dimethylformamide (DMF), which were used as solvents for all the experiments, were purchased from Merck and Friedemann Schmidt, respectively.

Medium-chain length polyhydroxalkanoate was produced at the Biotechnology Laboratory of the Institute of Biological Sciences, University of Malaya, by using PKO as the fermentation substrate. Since *Pseudomonas putida* PGA1—which was used in this study—lacks the lipase gene, it could not consume the highly saturated PKO directly and required a saponification pre-treatment. Saponification was carried out by using an ethanolic solution of sodium hydroxide followed by drying at 65°C to obtain the sodium salt of the fatty acids which was subsequently used in batch feeding of the bacterial cell culture. The details of the process were explained in an earlier publication.

**Solution preparation**

Blends of PHB and mcl-PHA were prepared in weight compositional ratios of 100:0, 90:10, 80:20, 70:30 and 60:40, respectively. The blend was dissolved in a mixed solvents system consisting of nine parts chloroform and one part DMF. The solution was prepared in a flask fitted with a reflux condenser, and it was stirred at 60°C for 24 h using a magnetic stirrer.

**Electrospinning**

The experimental set-up consisted of a syringe pump (LongerPump, China), a 20-mL-luer-tip-glass syringe, a needle (Terumo needle, 0.91 × 30 mm) used as the spinneret, a transformer (capable of stepping up voltage of 24 kV) and a high-voltage capacitor (Maxwell Laboratories, Inc., USA), capacitance 0.22 µF, voltage: 50 kV). The high voltage generated by the transformer was passed through a rectifier to obtain a half-wave rectified output. The capacitor was used to reduce ripples and stabilise the half-wave rectified voltage. A piece of aluminium foil was used as the collector. The syringe pump was set at a feeding rate of 3 mL h$^{-1}$. The needle to aluminium foil distance was fixed at 18 cm, horizontally, while the needle was positively charged at 12 kV relative to the aluminium foil. The temperature of the polymer solution inside the syringe was maintained at 40°C using a custom-made heating coil jacket controlled by a rheostat. The whole set-up was placed inside a self-designed transparent acrylic box. Small holes on walls of the box were outlets for the evaporated solvent. A hygrometer (Hana Instruments, Romania) was used to monitor relative humidity and temperature changes during the process. The relative humidity during the spinning was in the range of 60–75%, and the temperature remained at 28 ± 2°C. The produced fibre mesh, collected on the aluminium foil, was dried in a vacuum oven for 48 hours at 40°C to remove any residual solvent.

**Measurements**

**Differential scanning calorimetry**

Differential scanning calorimetry (DSC) thermograms were recorded using a Mettler Toledo DSC equipped with an inter cooler. Specimens of each sample were sealed in aluminium pans while an empty pan was used as a reference. The weight of each sample was around 6 mg. All the experiments were carried out at heating rate of 20°C min$^{-1}$ from −50 to 200°C. Crystallinity was calculated based on the enthalpy of fusion ($\Delta H_f$) compared to a theoretical value (146 J g$^{-1}$) for 100% crystalline PHB ($\Delta H_m$) through equation (1)

$$X_c = \frac{\Delta H_f}{\Delta H_m}$$

**Field emission scanning electron microscopy**

The morphology of the electrospun fibres was studied using field emission scanning electron microscopy (FESEM) (FEI: QUANTA FEG 250). Diameter distribution patterns of fibres produced from different blending proportions were compared. Field emission scanning electron microscopy micrographs were analysed by an image analysis program (UTHSCSA Image Tool). For each sample, diameter measurement was carried out at 100 different random points. Fibre intersections and beads were avoided in measurements.

**Wide angle X-ray diffraction**

Wide angle X-ray diffraction (WAXRD) patterns of electrospun mats were obtained under ambient condition using an Empyrean diffractometer system operating at 45 kV and 30 mA; CuKa radiation ($\lambda = 1.54$ Å). Scans were performed in a $2\theta$ range of 10–40 Å.

**Results and discussion**

**Differential scanning calorimetry**

Figure 1 shows the changes in enthalpy of melt against weight percentage of mcl-PHA, measured based on DSC thermograms. As could be seen, there is a decrease in the enthalpy of fusion as the weight percentage of mcl-PHA increases. Therefore, the addition of mcl-PHA has
decreased the crystallinity. Owing to the plasticising effects of mcl-PHA, crystal packing in PHB is reduced significantly. For example, the sample containing 40\% of mcl-PHA has reduced the crystallinity of PHB from 47.2 to 28.7\%.

**FESEM microscopy**

Figure 2 shows FESEM micrographs of the samples. Field emission scanning electron microscopy images were taken at 2000 times magnification at a high-voltage field of 20 kV in a low-vacuum mode. Table 1 summarises the fibre diameter changes against the weight ratio of mcl-PHA based on 100 random measurements for each sample. As it could be seen in Fig. 2 and Table 1, the addition of mcl-PHA had a positive effect in decreasing electrospun fibre diameter. The lower limit of the fibre diameter for all the samples containing mcl-PHA shows a decrease compared to pure PHB. The plasticising effect of mcl-PHA has made the blend more flexible and stretchable to form fibres of smaller diameter using the same electrostatic field strength. For example, the average fibre diameter in the sample containing 40wt-% mcl-PHA has decreased about 460 nm compared to the pure PHB sample. Furthermore, PHB being rigid and brittle tends to form more uniform fibres, which have a lower standard deviation; while by increasing the weight ratio of mcl-PHA the distribution of fibre diameter has become broader. When the amount of mcl-PHA is increased, more beads were formed along the fibres. It could be due to the stretchable fibre which cannot maintain a constant diameter under the slight pulsation voltage of the semi-rectified DC during spinning time. Detzel et al.\(^\text{23}\) reported that at a higher voltage, more beads were formed, presumably the fibre flight time decreased with increasing voltage and the solvent could not have evaporated fast enough. In our case, the lower molecular weight mcl-PHA tends to lower the viscosity of the blend solution, making it easier to flow

<table>
<thead>
<tr>
<th>Sample</th>
<th>Fibre diameter (μm)</th>
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<tbody>
<tr>
<td></td>
<td>Mean</td>
</tr>
<tr>
<td>PHB</td>
<td>1.27</td>
</tr>
<tr>
<td>PHB/mcl-PHA 90 : 10</td>
<td>1.31</td>
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<tr>
<td>PHB/mcl-PHA 80 : 20</td>
<td>1.30</td>
</tr>
<tr>
<td>PHB/mcl-PHA 70 : 30</td>
<td>1.01</td>
</tr>
<tr>
<td>PHB/mcl-PHA 60 : 40</td>
<td>0.81</td>
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1. Changes of fusion enthalpy plotted against the weight percentage of mcl-PHA

2. Scanning electron microscopy images of electrospun PHB and its blends with mcl-PHA along with fiber diameter distribution: a PHB, b PHB/mcl-PHA 90 : 10, c PHB/mcl-PHA 80 : 20, d PHB/mcl-PHA 70 : 30, e PHB/mcl-PHA 60 : 40
under the same electrostatic field. The faster movement would reduce the flight time, and consequently, some formation of beads was observed.

Wide angle X-ray diffraction

Figure 3 shows the diffraction intensity profile of electrospun PHB and its blends with mcl-PHA. In all profiles, diffraction peaks for α-form crystals with 2θ, helix conformation could be observed at 2θ = 13.5° and 17°. Owing to the predominant presence of PHB in all samples and its high crystallinity, these two peaks are visible in all diffraction profiles. The diffraction peak observed at 2θ = 19.6° is assigned to β-form crystals with planar zigzag conformation. The presence of β-form crystals in PHB electrospun fibres has been previously reported by Ishii et al. β-Form crystals were not observed in samples containing 30 and 40% mcl-PHA. We assume that this is because of a smaller degree of chain orientation in amorphous areas, since β-form crystals are contributed from the amorphous chains between lamella crystals. It is also because of an increased proportion of the amorphous region in the sample, which leads to a lower degree of orientation.

Conclusion

Blending of poly[(R)-3-hydroxybutyric acid] and medium-chain length polyhydroxyalkanoate was successfully carried out at four different ratios followed by electrospinning using a semi-rectified DC high-voltage power supply. Scanning electron microscopy images have shown that the presence of medium-chain length polyhydroxyalkanoate can contribute to formation of smaller diameter nanofibres presumably by making the blend more flexible and stretchable. On the other hand, because of its amorphous structure, it cannot maintain the whipping instability, which leads to jet break and formation of beaded fibres at higher weight ratios of medium-chain length polyhydroxyalkanoate (30 and 40%). Differential scanning calorimetry results showed that the addition of medium-chain length polyhydroxyalkanoate to poly[(R)-3-hydroxybutyric acid] has led to a lower enthalpy of fusion and crystallinity. The addition of medium-chain length polyhydroxyalkanoate decreased fibre diameter and crystallinity, while presumably increasing porosity and permeability of the electrospun fibrous mesh. Considering the above-mentioned facts, we hypothesise that the blend of poly[(R)-3-hydroxybutyric acid]/medium-chain length polyhydroxyalkanoate should be promising biomaterial to be used in scaffolding purposes. Its decreased crystallinity could also be an advantage in some biomedical applications such as fibroblast tissue engineering.

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References