Carbon nanotube mediated miscibility of polyhydroxyalkanoate blends and chemical imaging using deuterium-labelled poly(3-hydroxyoctanoate)

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ABSTRACT

Biopolymers have potential as scaffolds supporting regrowth of damaged tissues, however their material properties may limit the range of applications. Blending polymers with different thermomechanical properties has been demonstrated to extend the range of possible applications for polyhydroxyalkanoate (PHA) polymers, while the addition of nanoparticles can be used to modulate miscibility which influences strength and flexibility of the blend. Here we report on the blending of Poly(3-hydroxybutyrate) and Poly(3-hydroxyoctanoate) which possess different thermomechanical properties, and the effect of single wall carbon nanotubes (SWCNT) on their miscibility, electrical conductivity and thermomechanical performance. The apparent perturbation of phase boundaries in nanocomposite films observed by Scanning Electron Microscopy (SEM) was complemented by chemical mapping of film cross sections containing a deuterium-labelled poly(3-hydroxyoctanoate) phase in the blend using Infrared Microspectroscopy (IRM), suggesting increased miscibility due to nanoparticle addition. The electrical percolation threshold in nanocomposite films was observed between 0.5 and 1 wt% SWCNT, where the surface resistivity was reduced by eight orders of magnitude compared to the insulating polymer blend. Addition of SWCNT did not impact significantly on mechanical properties of films containing up to 2.5 wt % SWCNT. A solvent cast bionanocomposite film containing optimally 1 wt% SWCNT yielded a material with improved electrical conductivity compared to the SWCNT-free blend and which supported growth of Olfactory Ensheathing Cells, providing a basis for developing biopolymer scaffolds capable of conducting electrical stimulation.

1. Introduction

Polyhydroxyalkanoates (PHAs) are a family of biopolyesters commonly produced by microorganisms utilising a variety of carbon based substrates to synthesise short chain length (*scl)* and medium chain length (*mcl*) PHAs [1,2]. Poly(3-hydroxybutyrate) (PHB) is the most commonly studied and applied member of the PHA family but its application is often limited by its brittle and crystalline nature [3,4]. Despite this, there are many reports on the usefulness of PHAs in biomedical devices and applications [5–8]. Blending PHB with *mcl*-PHAs or unrelated polymers may be employed to modulate material properties, however the drawbacks of blending immiscible polymers include mechanical weaknesses at fracture due to weak adhesion between polymer chains at the phase interface, which may be exacerbated by large domain size [9–13]. Theoretical studies have suggested that nanorods may be used as an effective emulsifying agent in such polymer solutions, slowing down the phase separation process and reducing the

length scale of the material domains upon evaporation [14]. Failures at the interface between polymer phases could be ameliorated by addition of carbon nanotubes, where the diffusion of chain segments across the interface is apparently enhanced and the propagation of cracking reduced. In a study of melt blended Poly(ε-caprolactone)/Polylactide, Multi Wall Carbon Nanotubes (MWCNT) were found to be selectively dispersed in the matrix phase and at the interface between two polymer phases [15]. More recently, MWCNT have been shown to reduce the macromolecular mobility of immiscible blend components, with selective localization of the nanoparticles in one of the polymer phases [16,17]. This selective dispersion of conductive nanoparticles, compared to more homogeneous distribution in monophasic composites, has been demonstrated to improve electrical conductivity, especially where the nanoparticles were confined to the interface between the polymer phases [18,19].

Miscibility of *scl-/mcl*-PHA solvent cast blends has been probed by Scanning Electron Microscopy (SEM) and thermomechanical analysis

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[20]. We have previously demonstrated chemical imaging of deuterium-labelled poly(3-hydroxyoctanoate) (D-PHO) using Infrared Microspectroscopy (IRM) to reveal phase separation in films containing equimolar amounts of PHB and PHO [20,21]. We hypothesized that addition of SWCNT to these polymer blends would enhance miscibility and therefore result in a biomaterial with more desirable thermomechanical properties that may prove suitable as scaffolds for tissue regeneration and engineering. Furthermore, an effective dispersion of the nanoparticles was anticipated to increase electrical conductivity in the films, opening opportunities for propagation of electrical stimulation through the material. This would support their potential as a scaffold in nerve repair, as reported in the application of electrical stimulus through biomaterial to enhance neural cell viability, proliferation and neurite outgrowth [22–26].

2. Materials and methods

2.1. Material and reagents

Chemicals, including PHB, were obtained from Sigma-Aldrich (St. Louis, MO, USA) and used as received. SWCNT nominally 1 nm diameter and 1–2 μm in length were obtained from CheapTubes.com. PHO and D-PHO were prepared and characterized as previously reported [21,27,28]. Briefly, the PHO biopolymer was produced by fed-batch biosynthesis using *Pseudomonas oleovorans* (ATCC 29347) in minimal medium with octanoic acid as the sole carbon source. Deuterated PHO (D-PHO) was biosynthesized from deuterated carbon source (octanoic acid-d_{15}, 98% D by ^{1}H NMR). Crude polymers were extracted from the freeze-dried biomass into chloroform and precipitated by addition of cold methanol. Polymers were then dried under vacuum to remove traces of solvent.

2.2. Preparation of film blends and nanocomposites

Films of PHA blends were prepared by solvent casting into PTFE-lined petri dishes (Welch Fluorocarbons, USA). Equal masses of PHB and PHO (or D-PHO) were dissolved in chloroform in sealed vials with stirring at 400 rpm and 60 °C. SWCNT were suspended in chloroform with stirring and aliquots were added to PHB/PHO solutions to achieve final concentrations from 0.1 to 10 wt% SWCNT. The nanocomposite mixtures were sonicated for 6 min at 3 W using a probe tip (Misonix 3000, NY, USA), with a 30 s on/off cycle to permit heat dissipation. Chloroform was slowly evaporated from a glass-covered petri dish to form films of even consistency, 75 ± 25 μm thickness as measured by digital calipers.

2.3. Infrared Microspectroscopy

Films containing a deuterated polymer phase (D-PHO) were microtomed to obtain sections (ca. 5 μm thickness) and mounted onto CaF$_2$ discs (Crystan Ltd., Poole, UK). The film cross sections were examined in transmission mode using a Bruker FT-IR spectrometer attached to a Hyperion 3000 IR microscope equipped with a 64 × 64 pixel focal plane array (FPA) imaging detector. With the microscope reflective IR Cassegrain objective and condenser (15×, 0.4 N.A.), each detector field of view corresponded to ca. 170 μm^2 on the sample. Larger sample areas were investigated by compiling several of these frames. All data were recorded using Bruker OPUS version 6.5 software, and data analysis was performed using Bruker OPUS 7.0 imaging software.

2.4. Scanning Electron Microscopy

SEM images of polymer blends were collected using an FEI Nova NanoSEM 230 operating at 3 kV or Hitachi S3400-N at 5 kV. Samples were prepared by cryomicrotoming (Leica EM FC6 Cryo-

Ultramicrotome) or freeze-fracturing after plunging into liquid nitrogen for at least 30 s prior. The exposed face of the film cross section was mounted on a stub using conductive carbon tape and coated with a layer of platinum (ca. 5 nm thick) prior to placing into the microscope chamber.

Cell growth on PHB/PHO films was tested using Murine Olfactory Ensheathing Cells (OECs) extracted from 5-week-old Wistar rats (University of NSW ACEC number-06/53A) and cultivated on Dulbecco's Modified Eagle's Medium (DMEM, Gibco-Invitrogen, Australia) containing 10% foetal bovine serum (FBS, Gibco-Invitrogen, Australia), 250 μg/mL penicillin, 250 μg/mL streptomycin and 1 μg/mL fungizone-amphotericin B (Gibco-Invitrogen, Australia). Cells were washed in PBS buffer then resuspended in fresh DMEM containing 10% FBS and inoculated at approximately 10^4 cells/well into multi-well plates containing nanocomposite films. Cells were incubated for 3–5 days prior to fixing in 2.5% glutaraldehyde solution and dehydrated in increasing concentrations of ethanol. Samples in 100% ethanol were then dried in liquid CO$_2$ at the critical point prior to for SEM analysis.

2.5. Resistivity measurements

Surface resistance of nanocomposite films was measured using a resistance meter (PRS-812) with concentric ring probe (PRF-912B) from Prostat Corporation (Bensenville, IL, USA). Average resistance was calculated from replicate measurements and from areas of different appearance in non-homogenous films where applicable (n = 5).

2.6. Thermomechanical measurements

Thermal properties were investigated by differential scanning calorimetry (DSC) using a DSC1 StarE with intracooler (Mettler-Toledo AG, Switzerland). Samples ca. 5 mg in aluminium pans were heated to 200 °C at 10 °C/min to remove thermal history then cooled to −70 °C at 10 °C/min to determine crystallisation temperature (T$_c$) and the degree of crystallinity X$_c$ (%) of the PHB phase. Melting temperatures (T$_m$) and glass transitions (T$_g$) were determined from second heating run to 200 °C. Crystallinity was calculated using equation (1), where ΔH_m is the melting enthalpy and ΔH_{100} is the theoretical enthalpy of fusion for 100% crystalline PHB (146 J/g) [29].

$$X_c = \frac{\Delta H_m}{\Delta H_{100}} \times 100 \tag{1}$$

Mechanical testing of rectangular film pieces ca. 10 × 30 mm was performed using an Instron 5543 tensiometer (Canton, USA). Samples were held between two pneumatic clamps positioned at 25–30 mm with a cross-head speed of 20 mm/min. Tensile strength and extension to break were calculated (n = 5) using Bluehill software (v4.2, Instron, Canton, USA).

3. Results and discussion

3.1. Chemical mapping of protonated/deuterated PHA blend miscibility

Solvent cast films of PHB and PHO blends have previously been shown to be immiscible using SEM and IRM techniques, particularly around equimolar ratios [20,21]. Here we investigated the addition of SWCNT to blends of equimolar PHB and D-PHO, using IRM to probe the effect on miscibility. The chemical maps obtained through the film cross sections show areas of high (pink/red) and low (blue/green) infrared absorbance corresponding to C−H stretching vibrations (2800–3050 cm^{-1}) primarily from the PHB phase, and C−D stretching vibrations (2050–2200 cm^{-1}) in the D-PHO phase respectively. The solvent cast blend without addition of nanoparticles shows domains of high C−H absorbance corresponding to low C−D absorbance, and *vice versa* (Fig. 1), indicating phase separation of PHB and D-PHO in the