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Fine structure and enzymatic degradation of poly[(*R*)-3-hydroxybutyrate] and stereocomplexed poly(lactide) nanofibers

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Abstract

Fiber morphology and crystalline structure of poly[(*R*)-3-hydroxybutyrate] (P(3HB)) and stereocomplexed poly(lactide) (PLA) nanofibers were investigated by using scanning and transmission electron microscopies and X-ray and electron diffractions. In the P(3HB) nanofibers spun from less than 1 wt% 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP) solution, planar zigzag conformation (β-form) as well as 2_1 helix conformation (α-form) structure was formed. Based on the electron diffraction measurement of single P(3HB) nanofiber, it was revealed that the molecular chains of P(3HB) align parallel to the fiber direction. From the enzymatic degradation test of P(3HB) nanofiber, it was shown that β-form molecular chains are degraded more preferentially than α-form chains. Stereocomplexed PLA nanofibers were electrospun from 1 wt% poly(L-lactide)/poly(D-lactide) (PLLA/PDLA) solution in HFIP, which contains equal amounts of PLLA and PDLA. While as-spun stereocomplexed PLA nanofiber was amorphous, PLA nanofiber annealed at 100 °C contained only racemic crystal. It was supposed that the crystallization behavior of stereocomplexed PLA in the nanofiber is affected by the electrospinning process, which forcibly exerts the strain onto the polymer chains.
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1. Introduction

In these days with the increasing concern for the sustainable production and use of polymeric materials, development and popularization of biodegradable plastics are under way. In order to expand the application range of these polymers, many groups have developed the various forms of materials, such as fibers and films using various kinds of biodegradable polymers.

Recently, with the aim of developing porous materials or non-wovens, the formation of nanofibers with the diameter ranging from several tens to hundreds of nanometers is extensively studied (Reneker and Chun, 1996; Morota et al., 2004). Particularly, fabrication of scaffolds for cell culture using nanofibers of biodegradable polymers has been extensively studied (Murugan

and Ramakrishna, 2006). Nanofiber scaffolds have fine pores and grooves as small as a few micrometers wide. Such fine structural features facilitate the adhesion and proliferation of cells. It is required for nanofiber scaffolds to sustain sufficient strength to support regenerating tissue cells and to be degraded after the tissue regeneration is completed. To meet these demands, various kinds of biodegradable and biocompatible polymers have been processed into nanofibers. Furthermore, the fiber morphology, crystalline structure and degradation behavior of the nanofibers have been investigated (Buchko et al., 1999; Zong et al., 2002, 2003; Zeng et al., 2004).

Among the various kinds of biodegradable polymers, poly[(*R*)-3-hydroxybutyrate] (P(3HB)) and poly(lactide) (PLA) are attracting the largest attention for medical applications (Hasirci et al., 2001). P(3HB) is produced by many species of microorganisms as their intracellular energy and carbon storage substance (Doi, 1990). A wide variety of P(3HB) copolymers, classified as poly(hydroxyalcanoate)s (PHAs), can be produced by controlling the cultivation conditions of microorganisms. As PHAs show low degree of cytotoxicity and high mechanical

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strength, application of PHAs for medical uses, such as surgical sutures and wound dressings, have been attempted (Williams and Martin, 2002). Recently, the fabrication of the nanofibers of P(3HB) and its copolymers has been reported (Choi et al., 2004; Ito et al., 2005; Sombatmankhong et al., 2006). However, the information on the relation between the fine structure and degradation behavior of nanofiber has not been obtained yet.

PLA has long been utilized as medical materials such as sutures, implants, and so on (Ikada and Tsuji, 2000). Up to now, some researchers have prepared the nanofibers of PLLA (Zong et al., 2002; Inai et al., 2005; Moon et al., 2006) or poly(D,L-lactide) (PDLLA) (Zong et al., 2002) and investigated the fiber morphology, crystalline structure and mechanical properties. On the other hand, the formation of nanofiber of stereocomplexed PLA has not been reported yet. Stereocomplex is a kind of characteristic crystalline modification of PLA formed by the side-by-side arrangement of the molecular chains of enantiomeric PLAs, i.e. poly(L-lactide) (PLLA) and poly(D-lactide) (PDLA) (Ikada et al., 1987; Tsuji, 2005). As PLLA and PDLA molecular chains form left- and right-handed helix conformation, respectively, the side-by-side arrangement of both chains leads to the sterically stable racemic crystal (Okihara et al., 1991). As a result, the melting temperature increases to about 230 °C from the 175 °C for homopolymer (Ikada et al., 1987). Furthermore, it is well known that the enzymatic degradation of stereocomplex proceeds more slowly than PLLA (Tsuji and Miyauchi, 2001). This is advantageous to improve the stability of PLA material. However, PLA fibers that contain only racemic crystal have not been obtained yet.

In this paper, we investigate the fiber morphology, crystalline structure, and *in vitro* enzymatic degradation behavior of P(3HB) and stereocomplexed PLA nanofibers by using electron microscopies and diffraction methods. We will show that two kinds of molecular conformations are formed in P(3HB) nanofiber and that only the racemic crystals are formed in stereocomplexed PLA nanofiber. Furthermore, the changes in the fiber morphology and crystalline structure of P(3HB) nanofiber by the enzymatic treatment is investigated.

2. Experimental

2.1. Materials

Wild-type origin poly[(*R*)-3-hydroxybutyrate] (P(3HB)) with the M_w of 7.2×10^5 and M_w/M_n of 2.85, produced by ICI, was reprecipitated using chloroform as a solvent and *n*-hexane as a non-solvent before use. PLLA with the M_w of 6.2×10^5 and M_w/M_n of 2.1 was purchased from Polysciences, Inc. and used as received. PDLA with the M_w of 3.3×10^5 and M_w/M_n of 1.5 was synthesized according to the following procedure. The D-lactide monomer was obtained from Purac and recrystallized from anhydrous ethyl acetate. Bulk polymerizations were carried out in glass ampoules containing a magnetic stirring bar at 130 °C. Stannous octanoate in petroleum ether was used as the catalyst for the ring-opening polymerization. The ampoules were evacuated using a high vacuum pump and repeatedly flushed with high purity nitrogen to remove volatile impurities, solvents, and oxy-

gen. Then the ampoules were sealed with a blowtorch and heated to the reaction temperature. The products in the ampoules were dissolved in chloroform, precipitated in the excess of methanol, filtered, and dried.

2.2. Preparation of dope solution

1,1,1,3,3,3-Hexafluoro-2-propanol (HFIP) was used as solvent. Concentration of P(3HB) was varied from 0.5 wt% to 2.5 wt%. After 1 wt% solutions of PLLA and PDLA were separately prepared, equal volume of both solutions were mixed for several seconds by vortex mixer.

2.3. Electrospinning

Nanofibers of P(3HB) were prepared using an Esprayer ES-2000 electrospinning device (Fuence, Co. Ltd.). Dope solutions were extruded with a speed of 1.4 ml h^{-1} from a syringe needle with an inner diameter of 0.5 mm. Electrical voltage of 15 kV was applied to the syringe. Nanofibers were deposited onto a 10 cm × 10 cm aluminum substrate placed perpendicular to the needle. Distance between the needle tip and the substrate was set to 15 cm. The atmosphere of the spinning chamber was kept at less than 30% of relative humidity. PLLA, PDLA, and stereocomplexed PLA nanofibers were then annealed in an oven at 100 °C for 8 h. All nanofiber samples were stored at room temperature until use.

2.4. Scanning electron microscopy (SEM)

Nanofibers were deposited on a stub and then coated with Au. The thickness of Au coat was about 15 nm. SEM images of nanofibers were obtained using a field-emission scanning electron microscope (JSM-6330F, JEOL, Co. Ltd.) operating at an acceleration voltage of 5 kV of and an emission current of 12 μA. For estimating the average diameter of nanofibers, diameter was measured at more than 60 points on the printed SEM image.

2.5. Transmission electron microscopy (TEM)

Nanofibers were deposited on carbon-coated grids and shadowed with Pt–Pd alloy to obtain TEM images. No shadowings were applied to the samples for electron diffraction measurement. A JEM-2000FX II transmission electron microscope (JEOL, Co. Ltd.) operating at an acceleration voltage of 120 kV was employed to obtain the image and the diffraction pattern of single nanofiber.

2.6. Wide-angle X-ray diffraction (WAXD)

WAXD patterns of nanofiber mats were acquired under ambient condition using Rigaku RINT-2500 system operating at 40 kV and 200 mA. Measurements were performed on a Bragg–Brentano type 2θ/θ goniometer in a reflection mode. Ni-filtered Cu Kα radiation ($\lambda = 0.15418$ nm) was collimated with a (1/2)° divergence slit, (1/6)° scatter slit and 0.15 mm receiving slit. Scans were performed three times in a 2θ range of 10–40°