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The mechanical properties and in vitro biodegradation and biocompatibility of UV-treated poly(3-hydroxybutyrate-*co*-3-hydroxyhexanoate)

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Abstract

Strong mechanical properties and controllable biodegradability, together with biocompatibility, are the important requirement for the development of medical implant materials. In this study, an ultraviolet (UV) radiation method was developed to achieve controlled degradation for bacterial biopolyester poly (3-hydroxybutyrate-*co*-3-hydroxyhexanoate) (PHBHHx) which has a low biodegradation rate that limits its application for many implant applications required quick degradation. When UV radiation was applied directly to PHBHHx powder, significant molecular weight (Mw) losses were observed with the powder, Mw reduction depended on the UV radiation time. At the same time, a broad PHBHHx Mw distribution was the result of inhomogeneous radiation. Interestingly, this inhomogeneous radiation helped maintain the mechanical properties of films made of the UV-radiated powder. In comparison, the PHBHHx films subjected to direct UV radiation became very brittle although their degradation was faster than that of the PHBHHx powders subjected to direct UV radiation. After 15 weeks of degradation in simulated body fluid (SBF), films prepared from 8 and 16 h UV-treated PHBHHx powders maintained 92% and 87% of their original weights, respectively, while the untreated PHBHHx films lost only 1% of its weight. Significant increases in growth of fibroblast L929 were observed on films prepared from UV-radiated powders. This improved biocompatibility could be attributed to increasing hydrophilic functional groups generated by increasing polar groups C–O and C=O. In general, UV-treated PHBHHx powder had a broad Mw distribution, which contributed to fast degradation due to dissolution of low Mw polymer fragments, and strong mechanical property due to high Mw polymer chains. Combined with its improved biocompatibility, PHBHHx is one more step close to become a biomedical implant material.

Keywords: UV radiation; Biodegradation; PHBHHx; PHB; Mechanical property

1. Introduction

Polyhydroxyalkanoates (PHA) is a family of biopolyesters produced by bacteria [1–3]. Poly 3-hydroxybutyrate (PHB), poly (3-hydroxybutyrate-*co*-3-hydroxyvalerate) (PHBV), poly (3-hydroxybutyrate-*co*-3-hydroxyhexanoate) (PHBHHx) and poly (3-hydroxybutyrate-*co*-4-hydroxybutyrate) (P (3HB-4HB)) are the biopolyesters that are able to produce in large quantity [1–3]. Among them, PHBHHx was reported to have improved properties over those of PHB and PHBV, they are even comparable with those of conventional plastics such as PP, PE and PET [4].

Studies have been conducted to use PHA as implant materials for various applications, and no toxicity has been reported for PHA and their degraded products [5–22]. In these studies, investigations have showed that PHB and PHBV can be used to make matrices for in vitro proliferous cells [7–13]. Some studies also extended to cover more flexible poly(4-hydroxybutyrate) (P4HB) [14–16], poly(3-hydroxyoctanoate) (PHO) [17–19] and some of their blends [20,21]. At the same time, P (3HB-4HB) was also reported to be suitable for use as biodegradable drug carriers [22].

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PHBHHx was found to be a good biomaterial for growth of various cells including fibroblasts [23–25], osteoblasts [26–28], cartilage-derived chondrocytes [29–31], human umbilical vein endothelial cells (HUVECs) and rabbit aorta smooth muscle cells (SMCs) [32].

Although PHBHHx has found to have good biocompatibility, its in vitro and in vivo biodegradation are too slow for many clinical applications, PHBHHx films lost only 2–6% of its weights in a lipase containing phosphate buffered saline solution after 50 days [33]. In an in vivo study employing artificial esophagus made from PHBHHx, biodegradation was almost undetectable in dogs implanted with the artificial PHBHHx esophagus [5].

In this study, we aimed to develop a method that can accelerate the biodegradation of PHBHHx films and at the same time, the rapid degradable PHBHHx prepared in this way should also have good mechanical properties and good biocompatibility.

2. Experiment

2.1. Materials

2.1.1. Film preparation

PHBHHx containing 12% hydroxyhexanoate (donated by Shantou Lianyi Biotech Co., Guangdong, China) and poly (L-lactide) (PLA) (Biomedical Institute, Jinan, Shandong, China) were used for film preparation. In total, 1 g of PHBHHx was dissolved in 50 ml of chloroform under vigorous agitation for 2 h at 60 °C. The solution was then poured into Petri dishes. The dished were maintained at room temperature to allow complete evaporation of the chloroform. The evaporation of solvent resulted in the formation of films (about 100 μm in thickness) in the Petri dishes.

2.1.2. Simulated body fluid (SBF) preparation

SBF was used for in vitro hydrolytic degradation. The SBF was prepared by dissolving reagent-grade chemicals of NaCl, $NaHCO_3$, KCl, $K_2HPO_4 \cdot 3H_2O$, $MgCl_2 \cdot 6H_2O$, $CaCl_2 \cdot 2H_2O$, Na_2SO_4, and $(CH_2OH)_3CNH_2$ into distilled water and buffered with HCl to pH 7.4 at 37 °C. It had ion concentrations that were almost equal to those of human blood plasma [34].

2.2. Methods

2.2.1. UV treatment

Ultraviolet (UV) treatment on PHBHHx was conducted within a small area under two UV lamps at the same time. A 15 W UV lamp with a distance of 3 cm and a 30 W UV lamp with a distance of 60 cm were placed on the PHBHHx samples including films and powders. The UV-treated films and powder were dissolved in chloroform and cast into new films by the method 2.1.1. The PHBHHx were radiated (treated) with UV light for either 8 h or 16 h. Non-radiated films were used as controls in all investigations.

2.2.2. In vitro hydrolytic degradation of films

Films (each weighted about 100 mg) were submerged in SBF at 37 °C ($n = 6$). Samples were periodically removed, gently washed in distilled water, then dried in a vacuum by lyophilization prior to analysis. Weight losses after incubation were calculated and compared with those of the films prior to incubation.

2.2.3. Scanning electron microscopy (SEM)

The films were gold-coated and then examined under a scanning electron microscopy (KYKY-2800, Apparatus Factory, Chinese Academy of Sciences, Beijing, China) as described previously [33].

2.2.4. High performance liquid chromatography-gel permeation chromatography (HPLC-GPC)

HPLC-GPC was used for average molecular weight (Mw) analysis. The HPLC-GPC system consisted of a Spectra-System P2000 pump and AS3000 autosampler with a 50-ml, fixed loop injector, and a Thermo Hypersil ODS2, 250 × 4.6 mm, 5 μm column (Thermo Separation Products, Piscataway, NJ, USA). Chloroform was used as a flowing phase. Samples were added with 0.6 mg/ml and 1 ml/min for 40 min. Average Mw was determined based on the standard curve.

2.2.5. Tensile properties

Tensile strain analysis on PHBHHx films was performed with a strain rate of 10 mm/min (TRAPEZIUM, Shimazdu Corp., Kyoto, Japan). Maximum stress was determined as the stress values corresponding to 25% strain. Mechanical tensile data were calculated on an average of four specimens [28].

2.2.6. X-ray photoelectron spectroscopy (XPS) investigations

Effect of UV treatment on C–C and C=O proportions of PHBHHx were investigated by XPS. XPS were recorded on a USA PHI5300 ESCA/610SAM, XPS, using Al/MgK_α radiation as the excitation source [24].

2.2.7. Cell culture

The mouse fibroblast cell lines L929 (Chinese Academy of Preventive Medical Sciences, Beijing, China) were cultured in DMEM (Dubecco's Modified Eagle Medium, Invitrogen, California, USA) supplemented with 10% fetal calf serum (FCS), 100 U/ml penicillin, and 100 mg/ml streptomycin (Sanli Biotechnology, Beijing, China). Cells were incubated at 37 °C in a 5% CO_2 incubator. Cytocompatibility testing was performed as direct contact test. Discs of 14 mm diameter were cut out of the PHBHHx and PLA films, sterilized in 75% ethanol for an hour and fixed in 24-well culture plate. 10,000 cells were seeded onto the different materials and incubated for 3 days.

2.2.8. Mitochondrial metabolic activity studies

The metabolic activity of the cells was determined using a methylthiazol tetrazolium (MTT) (Jingke Biotechnology, Beijing, China) assay. The cells were rinsed with phosphate-buffered saline (PBS) twice. Then 900 μl of serum free medium and 100 μl of MTT solution (5 mg/ml in PBS) were added to each sample, followed by incubation at 37 °C for 4 h. After incubation, the MTT solution was removed, and the insoluble formazan crystals formed were dissolved in 1000 μl of dimethylsulfoxide (Sigma). The absorbance was measured at 550 nm using a 96-well plate spectrophotometer [24].

2.2.9. Statistical analysis

Data were presented as means ± SD. Statistical comparisons were performed using the Student's t-test. P values <0.05 were considered statistically significant.

3. Results

3.1. Effect of UV radiation on PHBHHx molecular weights and biodegradation

Films prepared using PHBHHx with an original Mw of 526 kD were found to become brittle after being subjected to UV radiation (treatment) for 8 and 16 h, respectively, the reason can be easily attributed to the dramatic reduction in Mw of PHBHHx after UV treatment (Table 1). Weight average Mw of PHBHHx films subjected to 8 or 16 h of UV treatment were reduced from 526 to 10.2 kD or 7.3 kD, with a slightly increase in polydispersity from 2.0 to 2.4 or 2.1. Importantly, films made of PHBHHx powders