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Effect of composition of poly(3-hydroxybutyrate-co-3-hydroxyhexanoate) on growth of fibroblast and osteoblast

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Abstract

Films made of poly (3-hydroxybutyrate) (PHB), poly(3-hydroxybutyrate- co-3-hydroxyhexanoate) (PHBHHx) consisting of 5%, 12% and 20% hydroxyhexanoate (HHx), respectively, were evaluated for biomedical application in comparison with poly (L-Lactide) (PLA). With the increase of HHx content in PHBHHx, the polymer surface properties changed accordingly. P(HB-co-20%-HHx) had the smoothest surface while PHB surface was most hydrophilic among the evaluated PHB and all the PHBHHx. All PHBHHx also showed strong protein affinity and biocompatibility. It was found that fibroblast and osteoblast had different responses to these polymers: fibroblast cells favored P(HB-co-20%-HHx), yet osteoblast cells preferred P(HB-co-12%-HHx). PHB and all PHBHHx appeared to have better biocompatibility for fibroblast and osteoblast compared with PLA. Polymers possessing diferent surface properties may help meet different cellular requirements. Combined with their good mechanical properties for elongation and adjustable biocompatibility, PHBHHx may meet the needs of growth requirements of different tissues and cells.

Keywords: Poly (3-hydroxybutyrate-co-3-hydroxyhexanoate); PHBHHx; PHB; Biocompatibility; Osteoblast; Fibroblast

1. Introduction

Polyhydroxyalkanoates (PHA) have been considered as a polymer family that will extend significantly the range of biomaterials suitable for tissue engineering [1]. Many studies were conducted using poly (3-hydroxybutyrate) (PHB) and poly (3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) as biomaterial for in vitro and in vivo studies [2–11]. These results showed various degrees of biocompatibility and biodegradability in the long-gap repair in peripheral nerves [8], defect recovery of the osseous skull [9], neuronal rescue and regeneration after spinal cord injury [10].

Random copolyesters consisting of 3-hydroxybutyrate and 3-hydroxyhexanoate (PHBHHx) is a member of PHA family. PHBHHx was produced in large scale [12]. Some of its process properties, such as elongation at break, has been shown to improve over PHB that are also available in large scale [13]. PHBHHx was also evaluated for its biomedical applications [14–21]. In these studies, PHBHHx [14, 15], PHBHHx/PHB blend with different ratios [17–19], and PHBHHx with different surface treatment [14–16] were used. The results showed that these PHBHHx-based materials had good biocompatibilities for fibroblast [14–16], chondrocyte [17–19], nerve cells [20], and osteoblast [21].

To extend the series of PHBHHx-based materials, this paper, for the first time, aimed to evaluate PHBHHx consisting of different HHx content for biomedical application.

2. Materials and methods

2.1. Materials

P (HB-co-5%-HHx) (donated by OuYang SP, Tsinghua University, Beijing, China, Mw: 400,000), PHB

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(Shantou Lianyi Biotech Company, Guangdong, China, Mw: 400,000), P (HB-co-12%-HHx) (Shantou Lianyi Biotech Company, Guangdong, China, Mw: 400,000), P (HB-co-20%-HHx) (donated by Lu XY, Tsinghua University, Beijing, China, Mw: 400,000), and PLA (Biomedical Institute, Jinan, Shandong, China. Mw: 400,000) were used for films preparations. one gram polymers were dissolved in 50 ml chloroform. Subsequently, the 50 ml chloroform PHA solution was poured onto Petri dishes. The evaporation of solvent resulted in the formation of PHA films in the Petri dishes.

2.2. Methods

2.2.1. Contact angle measurement

Wettability was examined by measuring contact angles. The contact angles were measured for the films a Contact Angle Meter (JY-82, Chengde Test-Machine Factory, Chengde, China). Redistilled water of approximately 10 μl was gently plated on the surface of the films. At least three readings on different parts of the films were averaged for data collecting.

2.2.2. Scanning electron microscopy examination (sem)

Cell-seeded films were pre-treated as following: washed twice by phosphate buffered saline (PBS) and immersed in PBS containing 3% glutaraldehyde (pH 7.4) for 4 h. They were then dehydrated in increasing concentrations of ethanol (from 30%, 50%, 70%, 90%, and 95% to 100%), followed by lyophilization. Then samples (cell-seeded films with pre-treatment while non-cell-seeded films without pre-treatment) were then mounted on aluminum stumps, coated with gold in a sputtering device for 1.5 min at 15 mA and examined under a scanning electron microscope (KYKY-2800, Apparatus Factory, Chinese Academy of Sciences, Beijing, China).

2.2.3. Fibroblast 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 μg/ml streptomycin (Sanli Biotechnology, Beijing, China). Cells were incubated at 37°C in a 5% CO_2 incubator and the medium was changed every 2 days.

2.2.4. Osteoblast culture

Bone marrow cells were isolated from the femurs of young (3–5 days old) New Zealand white rabbits, as described by Maniatopoulos et al. [21,22]. The released cells were cultured for 1 week in DMEM supplemented with 15% fetal calf serum, 100 U/ml penicillin, and 100 μg/ml streptomycin. Cells were incubated at 37°C in a 5% CO_2 incubator and the medium was changed every 2 days.

2.2.5. Cell proliferation studies

Cell proliferation was assessed using a methylthiazol tetrazolium (MTT, Jingke Biotechnology, Beijing, China) assay. Briefly, 800 μl serum free medium and 80 μl MTT solution (5 mg/ml in PBS) were added to each sample, followed by incubation at 37°C for MTT formazan formation. The medium and MTT were replaced by 800 μl 10% sodium dodecyl sulfate (SDS) (Sigma, St. Louis, MO, USA) in 0.01 M HCl to dissolve the formazan crystals. After 20 h, the optical density (OD) at 570 nm was determined against SDS solution blank. Viable cell numbers on films were then determined from the standard curve based on their MTT absorbency.

2.2.6. Protein adsorption

Protein adsorptions by intensively washed with distilled water and dried polymer films were investigated by incubating the films in fetal bovine serum (FBS) for 2 h at 37°C. The analyses of adsorbed proteins on the surface of polymer films were carried out by ATR-FTIR qualitatively through the intensity of amide I and amide II bond.

2.2.7. Statistical analysis

Data were presented as means ±standard error of mean. Statistical comparisons were performed using Students *t*-test. *P*-values <0.05 were considered statistically significant ($n=4$).

3. Results

3.1. Surface hydrophilicity

Surface hydrophilicity was examined by measuring contact angle to water (Fig. 1). With increasing HHx content in PHBHHx contact angle increased from 68° to 85°, indicating a decreasing hydrophilicity on the polymer surface. While changing HHx content from 5% to 20% did not lead to dramatic change in surface hydrophilicity. All PHBHHx and PHB were more hydrophobic than PLA.

3.2. Surface morphology

Surface topology was examined by SEM (Fig. 2). With increasing HHx content, PHBHHx films became less porous on the surface. When HHx content increased to 20%, a dramatic change in the surface topology of PHBHHx was observed. The surface was very smooth. In comparison, PLA had almost the same smooth surface as P (HB-co-20%-HHx).