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In vivo studies of poly(3-hydroxybutyrate-*co*-3-hydroxyhexanoate) based polymers: Biodegradation and tissue reactions

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Abstract

The in vivo tissue reactions and biodegradations of poly(3-hydroxybutyrate-*co*-3-hydroxyhexanoate) (PHBHHx), poly(lactide) (PLA), poly(3-hydroxybutyrate) (PHB), blends of PHBHHx (X) and poly(ethylene glycol) (PEG) (E) with ratios of 1:1 (E1X1) and 1:5 (E1X5), respectively, were evaluated by subcutaneous implantation in rabbits. Results revealed that the degradation rate increased in the order of PHB < PHBHHx < PLA. During the implantation period, crystallinity of PHBHHx increased from 19% to 22% and then dropped to 14%. Gel permeation chromatography (GPC) displayed increasing polydispersity and typical bimodal distribution from 3 to 6 months. The above results suggested that rapid PHBHHx degradation occurred in amorphous region rather than in crystalline region. While the in vivo hydrolysis of PHB was found to start from a random chain scission both in amorphous and crystalline regions of the polymer matrix, as demonstrated by its hydrolysis process accompanied by a decrease in molecular weight with unimodal distribution and relatively narrow polydispersity. Compared to pure PHBHHx, PHBHHx–PEG blends showed accelerated weight loss of PHBHHx with weak molecular weight reduction. In general, PHBHHx elicited a very mild tissue response during implantation lasting 6 months compared with relative acute immunological reactions observed among PHB and PLA objects, respectively. Pronounced tissue responses were observed in the capsule surrounding E1X1 and E1X5 as characterized by the presence of lymphocytes, eosinophils and vascularization, which might be resulted from the continuous leaching of PEG.
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1. Introduction

Bacterial poly(3-hydroxybutyrate) (PHB) is a well-known thermoplastic polyester that has shown excellent biocompatibility as evidenced by lack of toxicity[1–3], compatibility in contact with tissue [1,2] and blood [3]. However, high brittleness, poor processability and low degradation limited its application [4]. Recently, many studies have been published regarding the application of poly(3-hydroxybutyrate-*co*-3-hydroxyhexanoate)(PHBHHx) as a better potential implant material compared with PHB [5]. Improved properties of PHBHHx, such as mechanical properties, affinity to different types of mammalian cells and in vitro biodegradable ability, have proven to be better than those of PHB [5]. PHBHHx films containing high HHx content was also found to enhance smooth muscle cells' differentiation, which further revealed the possibility of PHBHHx to be used in blood vessel tissue engineering [6].

However, all these experiments were performed in vitro. Few studies concerning the in vivo biocompatibility or biodegradation of PHBHHx were conducted compared with that of PHB and poly(hydroxybutyrate-*co*-hydroxyvalerate) (PHBV) that had accumulated more in vivo data [1,2]. As many complicated and unexpected factors are involved in tissue reaction to polymeric materials, the effects of PHBHHx on tissues need to be examined to obtain an insight look of this cell-friendly material for application in biomedical implant areas.

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In this study, for the first time, PHBHHx and PHBHHx/PEG blends were implanted subcutaneously in rabbit, their in vivo biocompatibility and degradation were inspected, respectively.

2. Materials and methods

2.1. Polymeric materials preparation and implantation

Discs made of PLA, PHB, PHBHHx, blends of PHBHHx/PEG (Mw: 4000) with weight ratios of 1:1(ab. E1X1) and 1:5 (ab. E1X5), respectively, were fabricated by solvent-casting method as reported previously [7]. Polymeric materials (2.5%) in chloroform, refluxed at 80 °C for complete dissolution, were injected into a cylindrical mold with a diameter of $d = 25$ mm. The resulting discs (approximately 0.08–0.1 g/disc) were sterilized with ethylene oxide and implanted subcutaneously into the back of adult New Zealand white rabbits. Four discs of each polymer were implanted into different parts of a rabbit. No antibiotic administration was given to the animals at any time during the experiment. The rabbits were sacrificed after 1, 3, 6 months, and the surrounding tissues of the implants polymers were harvested.

2.2. Biodegradation test

2.2.1. Weight loss analysis

The explanted samples were washed with distilled water and allowed to dry in air to achieve a constant weight. For each polymer sample, eight discs were used and the degradation rate was determined by the ratio of weight loss to the initial weight of the samples.

2.2.2. Molecular weight analysis

The molecular weights of the polymers were determined by gel permeation chromatography (GPC) using high-performance liquid chromatograph (HPLC) equipped with an Evaporated Light Scattering Detector (ELSD). The GPC system was eluted with chloroform at a flow rate of 1.0 ml/min under 42 °C. Polystyrene was used as molecular weight standard and the molecular weight of the samples was calculated based on the standard curve.

2.2.3. Thermal analysis

Thermal analysis was performed using a 2910 Modulated DSC (TA, USA), a Dupont 2100 differential scanning calorimeter in the temperature range −50 to 190 °C at a heating rate of 10 °C/min. Samples were quenched in liquid nitrogen and then heating to 190 °C at 10 °C/min. The mass crystallinity of discs was approximated compared with that of totally crystalline PHB (146 J/g) [8] or PLA (93 J/g) [9], respectively.

2.3. Histological observation

Fixing in Bouin solution, the explanted tissues were embedded in paraffin; 8-μm thickness microtome sections were stained with hematoxylin and eosin (HE). The estimated parameters were used to evaluate the tissue formation dynamics surrounding the polymers, thickness of the fibrous capsules, its cellular composition, the number of fibroblast, and the period of development and maturation of collagen fibers around the implants.

3. Results

3.1. Physical appearance observation

The rabbits appeared to be healthy throughout the implantation period. No symptom such as necrosis, abscess or tumorigenesis was observed in the vicinity of the implants. Retrieved materials varied in their physical appearance after 6 months of implantation (Fig 1). PLA was degraded into small fragments while PHBHHx were in its initial shape. PHB was broken into several large pieces as evidenced by the sharp material interface. Capsule was found surrounding all three materials and PHBHHx had the thinnest one, indicating that PHBHHx caused the slightest tissue reaction.

3.2. Weight losses of discs in rabbit

PHBHHx lost its weight faster than PHB but slower than PLA after 6 months of implantation (Fig 2). Pure PHB and PHBHHx had almost no weight loss after 1-month implantation while PLA lost approximatley 5% of its original weight. Six months later, 10% and 6% weight loss were observed, respectively, in PHBHHx and

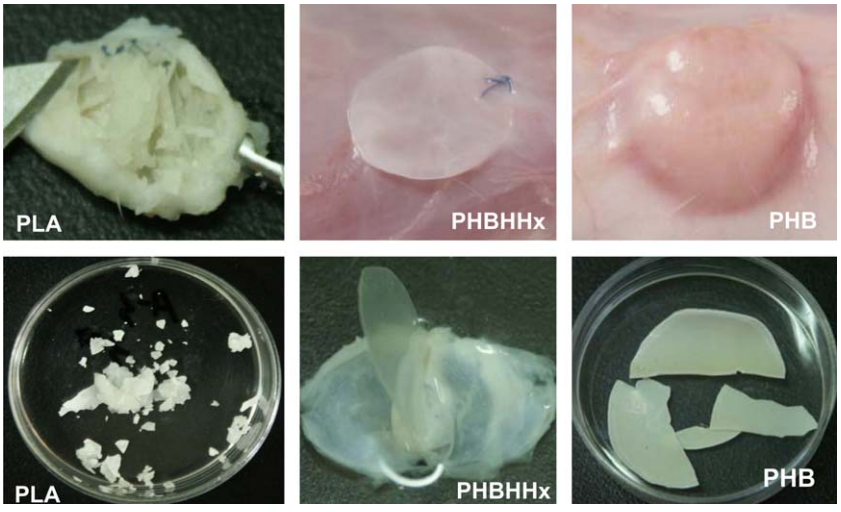

Fig. 1. Biomaterials explanted from rabbit after 6 months. Every four discs of same polymer were implanted subcutaneously in one New Zealand white rabbit without any antibiotic administrations over 6 months.

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