Biomaterials 31 (2010) 7512–7518

Contents lists available at ScienceDirect

Biomaterials

journal homepage: www.elsevier.com/locate/biomaterials

The use of poly(3-hydroxybutyrate-*co*-3-hydroxyhexanoate) scaffolds for tarsal repair in eyelid reconstruction in the rat

Jing Zhou [a], Si-Wu Peng [a], Yuan-Yuan Wang [b], Song-Bin Zheng [a], Yang Wang [c], Guo-Qiang Chen [a,b,*]

[a] *Multidisciplinary Research Center, Shantou University, Shantou 515063, Guangdong, China*
[b] *Department of Pathology, Shantou Central Hospital, Shantou 515031, Guangdong, China*
[c] *Department of Biology, School of Life Sciences, Tsinghua University, Beijing 100084, China*

ARTICLE INFO

Article history:
Received 6 April 2010
Accepted 28 June 2010
Available online 20 July 2010

Keywords:
PHB
Polyhydroxyalkanoates
PHBHHx
Tarsal substitutes
Eyelid reconstruction

ABSTRACT

Tarsal repair is an important part for eyelid reconstruction. Presently traditional clinic treatments do not produce satisfactory repair effects. The key is to find a proper tarsal repair material. Microbial poly (3-hydroxybutyrate-*co*-3-hydroxyhexanoate) (PHBHHx) was studied for application as tarsal substitute in this study. PHBHHx scaffolds were implanted into tarsal defects of Sprague-Dawley rats. Eyelid samples of implanted materials and blank defect controls were collected for histological examination at weekly intervals post surgery. Results were compared among PHBHHx scaffolds, commercial acellular dermal matrices (ADM) and blank defect controls. Both PHBHHx scaffolds and ADM provided satisfactory repair results compared with the blank controls even though the implanted PHBHHx scaffolds showed a 2 weeks inflammation. Fibrous encapsulation and scaffold degradation were observed for the PHBHHx implants. Combined with its strong, elastic mechanical properties, the tissue compatible and biodegradable PHBHHx was proven to be a suitable candidate for tarsal repair.

© 2010 Elsevier Ltd. All rights reserved.

1. Introductions

An eyelid structure is supported by tarsus consisting of Meibomian glands and surrounding dense connective tissues [1]. Larger tarsal defects, especially those caused by trauma or tumor excision, are often difficult to repair [2]. Thus, tarsus repair has always been the key issue for eyelid reconstruction. An ideal tarsal substitute should have properties including thickness, surface characters, strength and flexibility similar to that of a natural tarsus. Moreover, it should be tissue compatible, easy to obtain and handle [3].

Frequently used grafts for repairing a defected tarsus are mainly divided into two types: one are homologous grafts such as hard-plate mucosa [4,5], allogenic acellular dermal matrix (ADM) [6] and nasal cartilage [7]; another are xenogenic substitutes including heterogenic sclera [8] and xenogenic acellular dermal matrix [9]. The homologous substitutes have the advantage of absence of immunologic rejection yet suffer from shrinkage, autolysis [10] and microbial infection [11]. They are often difficult to obtain and handle during surgery operation. In contrast, the xenogenic ones are easier to obtain and handle but often show low tissue tolerance [12]. Therefore, the development of a synthetic tarsal substitute is of great importance.

Polyhydroxyalkanoates (PHA) is a large family of biopolyesters produced by many microorganisms [13,14]. As one of the diverse PHA family members, poly(3-hydroxybutyrate-*co*-3-hydroxyhexanoate) (PHBHHx) shows adjustable mechanical strength [15,16], it has been produced in large scale, and has been used to develop several implant applications [17,18]. PHBHHx consisting of 12 mol% 3HHx showed ability to support *in vitro* and *in vivo* cell growth including tissue regenerations [19]. PHBHHx microparticles were shown to stimulate cell proliferation in murine fibroblast L929 [20]. Monomers compositions of PHBHHx showed effects on cell proliferation [21]. A significant biodegradation and mild subcutaneous tissue responses of PHBHHx discs were revealed [22]. Porous PHBHHx scaffolds promoted nerve tissue regeneration when used as nerve conduits [23]. In this paper we reported the use of PHBHHx for tarsal repair in rats.

2. Materials and methods

2.1. Animals used

36 Three months old clean Sprague-Dawley rats weighted 300–350 g were purchased from Animal Experiment Center, Shantou University (Shantou, China). They were raised in our special pathogen-free and air-conditioned animal facility. The rats were divided into 3 groups including test group with PHBHHx scaffolds implantation, control group implanted with ADM and blank group without implantation.

* Corresponding author at: Department of Biology, School of Life Sciences, Tsinghua University, Beijing 100084, China. Tel.: +86 10 62783844; fax: +86 10 62794217.
E-mail address: chengq@mail.tsinghua.edu.cn (G.-Q. Chen).

0142-9612/$ – see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.biomaterials.2010.06.044

2.2. Preparation of PHBHHx tarsal substitutes

1.2 g PHBHHx (M_w: 440,000 ± 10%; 12 mol% HHx) in powder form (supplied by Lukang Pharmaceutical Co. Ltd, Shandong, China) was dissolved in 20 mL 1,4-dioxane and refluxed to form a clear solution at 80 °C. The PHBHHx scaffolds were prepared using the reported method [24]. In details: the solution was poured into a 50 mL beaker wrapped with a layer of adiabatic foam and incubated in liquid nitrogen for 15 min. The beaker was vacuum lyophilized at −45 °C for 7 days. Finished scaffolds were cut into 5 mm × 5 mm cross sections with average thickness of 0.7 mm, and then vertically cut into 1.0 mm × 1.0 mm pieces. They were sterilized in 75% ethanol overnight, and balanced with normal saline (containing 40,000 IU penicillin sodium salt) before use.

Acellular dermal matrices (ADM) (0.7 mm thick, purchased from ReDerm, Jieya Company, Beijing, China) were lyophilized overnight [6], then they were cut into 1.0 mm × 1.0 mm pieces, then balanced with normal saline before use.

2.3. Scanning electron microscopy (SEM) examination

The PHBHHx scaffolds and ADM pieces were mounted on aluminum stumps, and then coated with gold in a sputtering device for 1.5 min at 10 mA and examined under a scanning electron microscope (SEM, JSM-6360LA, JEOL, Japan) [25].

2.4. Study on density and porosity of PHBHHx scaffolds

The estimation of scaffold density and porosity was conducted based on reported method [26]. Four cubic specimens (5.0 mm × 5.0 mm × 5.0 mm) cut from each scaffold sample were measured accurately by a micrometer caliper, and the weight was measured using an analytical balance. The average scaffold density D_f was calculated. The porosity ϵ was obtained from D_f and the polymer skeletal density D_p:

$$\varepsilon = \frac{D_p - D_f}{D_p} \quad (1)$$

where the skeletal density of PHBHHx was determined according to:

$$D_p = \frac{1}{\frac{1 - X_c}{D_a} + \frac{X_c}{D_c}} \quad (2)$$

where X_c was the degree of crystallinity of PHBHHx determined with differential scanning calorimetry as described previously [24]. For 12 mol% PHBHHx, due to *R*-3-hygdroxybutyrate (3HB) which is the dominant content affecting both the crystallinity and amorphous density, the density of amorphous polymer $D_a = 1.177$ g/mL and density of 100% crystalline polymer $D_c = 1.260$ g/mL [27].

2.5. Surgical operations

All the experiments were strictly conducted following the guidelines of Association for Research in Vision and Ophthalmology (ARVO) on the use of animals in research [28]. Rats were anesthetized with 100 mg/kg body weight chloral hydrate, followed by local anesthesia with 1% lidocaine hydrochloride near the middle point of the eyebrow. Longitudinal 5 mm skin and muscle incisions were made using a surgical knife with a #15 blade near the outer canthus of the upper eyelids, about 2 mm away from the palpebral margin. Tarsal areas of 1.0 mm × 1.0 mm were carefully separated from palpebral conjunctiva using ophthalmic microforceps and then excised by microscissors. Pieces of PHBHHx scaffolds or ADM were placed into those defect sites, and the skin was closed by 6-0 biodegradable sutures. The wounds were treated with pressure dressing. Observations of rats' activities were carried out every 24 h till 8th week post surgery. Recovery processes were recorded.

2.6. Histological examination

Rats of each group were euthanized by overdosed chloral hydrate at the 1st, 2nd, 4th and 8th week post surgery, respectively. Eyelid samples with implants were embedded in optimal cutting temperature (OCT) compound (Tissue-Tek, Sakura Finetek, USA) at −21 °C. 6 μm thick frozen sections were prepared and stained with HE. For each sample, 3 sections were chosen at 100 μm intervals in the central blocks. Cell types within the implanted sites were defined and then the number of each cell type was counted at a magnification of ×400. All fields of the view of each slide were chosen for examination.

2.7. Statistical analysis

All data were presented as the mean values plus standard deviation (SD) of three parallel studies. Statistical comparisons were performed using Student's *t*-test and carried out by Graph-Pad Prism5 (Graph-Pad, La Jolla, CA), with a confidence level of 95% ($p < 0.05$) considered statistically significance and 99% ($p < 0.01$) considered very significant.

3. Results

3.1. Characterization of experimental materials under SEM

Regular ladder-like tubing structures were observed in PHBHHx scaffolds under scanning electron microscopy (Fig. 1C1–C3). Directions of the tubes were uniformly oriented. Tube diameters were found to be between 50 and 80 μm. This result was consistent to that produced by the reported method [24,26]. The average density of 6 wt% PHBHHx scaffolds was 0.069 g/mL with a calculated porosity of 94.2% (Table 1). Meanwhile, multiple micropores with diameters around 5 μm were observed on tube walls in the scaffolds, which provided interconnectivity for cells grown inside

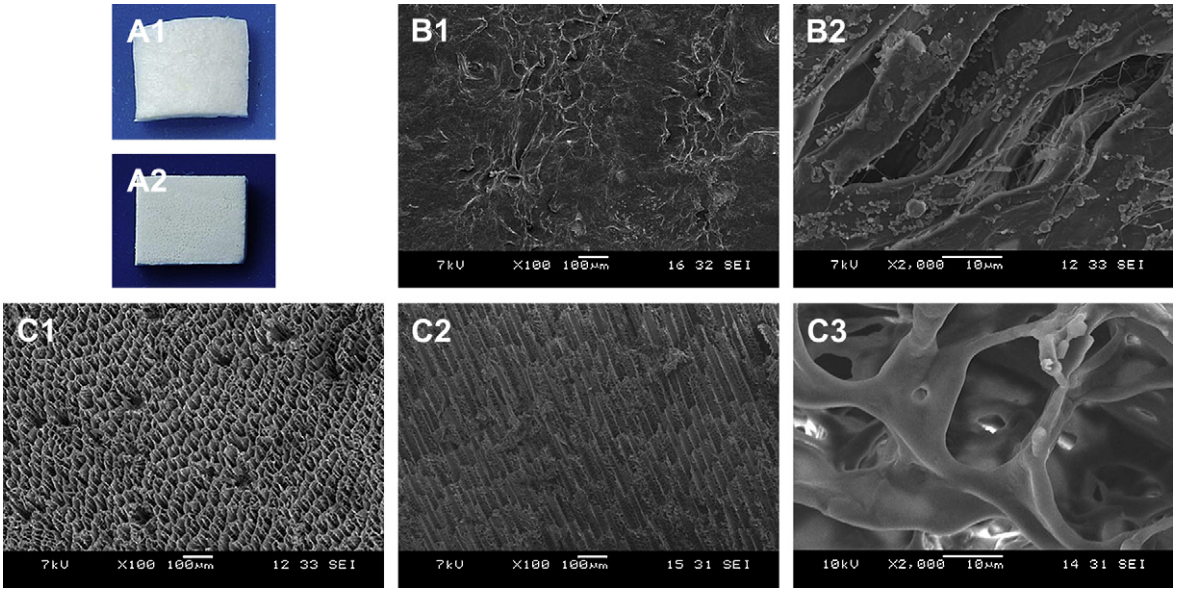

Fig. 1. Morphology of sections of PHBHHx scaffolds and basement membranes of acellular dermal matrices (ADM). (A1) Cross section of 6 wt% PHBHHx scaffolds; (A2) Implant of lyophilized xenogenic ADM; (B1–B2) SEM study of a ADM implant; (C1–C3) SEM study of a section of 6 wt% PHBHHx scaffold (C1) Cross sections; (C2–C3) Vertical sections. Scale bars are: (B1, C1, C2) 100 μm; (B2, C3) 10 μm.

Download English Version:

https://daneshyari.com/en/article/7858

Download Persian Version:

https://daneshyari.com/article/7858

Daneshyari.com