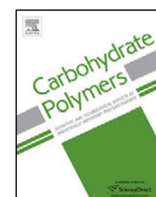
Carbohydrate Polymers 172 (2017) 255–264

Contents lists available at ScienceDirect

Carbohydrate Polymers

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



Study of a new biodegradable hernia patch to repair abdominal wall defect in rats

Jing Qiao, Zhiwen Jiang, Yan Yang, Jinhua Chi, Xuesong Qiao, Baoqin Han*, Wanshun Liu

Laboratory of Biochemistry and biomedical materials, College of Marine Life Sciences, Ocean University of China, Qingdao 266003, PR China

ARTICLE INFO

Article history:
Received 7 January 2017
Received in revised form 13 April 2017
Accepted 10 May 2017
Available online 11 May 2017

Keywords:
Hernia repair
Chitin
Tissue regeneration
Bio-degradability
Neovascularization
Fibrosis

ABSTRACT

A novel biodegradable chitin hernia patch was prepared by acetylation of chitosan fabric in our study. Physicochemical properties, cell compatibility and biodegradability of the chitin patch were quantified. Histopathological study of the functional experiment showed that this newly designed hernia patch promoted collagen deposition and neovascularization by significantly promoting the secretion of FGF1 and TGF-β1 in the early postoperative ($P < 0.01$). Chitin patch caused less inflammation by inhibiting excessive expression of IL-6 and TNF-α when compared to the polypropylene mesh ($P < 0.01$). Acceptable fibrosis was consistent with the results of immunohistochemistry studies. The density of FGF1 and TGF-β1 positive cells in the chitin patch group at 7 d was reduced to a lower level at 15 d ($P < 0.01$). With regeneration of the defect abdominal wall, chitin patch degraded gradually, avoiding foreign body response and chronic complications. Our studies demonstrated that the newly designed chitin patch showed good promise for the hernia treatment.

© 2017 Elsevier Ltd. All rights reserved.

1. Introduction

Worldwide over 20 million surgeries are performed annually for hernia repair with implantation of a surgical mesh (Kingsnorth, 2004). Prosthetic meshes for hernia repair have been improved dramatically. Permanent synthetic meshes, such as polypropylene meshes, are the most commonly used in order to achieve the maximum reinforcement of the affected area. However, this type of mesh, rather than benefiting the patients, causes greater foreign body reaction (FBR) and exacerbated fibrosis, which lead to higher rate of chronic pain and restricted movement (Calvo et al., 2016; Eberli et al., 2010). Other non-biodegradable synthetic patches, such as polyester and expanded poly (tetrafluoroethylene), are also associated with complications including intestinal erosion (Shrivastava, Gupta, Gupta, & Shrivastava, 2013), serious infection and high recurrence following long-term degradation (Brown & Finch, 2010), as well as limited physical activity of the patient due to the retention of patching material after hernia healing. These outcomes always result in a revision or removal of the implant. In contrast, biological materials, such as dermal matrix and small intestinal submucosa (SIS), are appropriate for contaminated wounds and easy to be colonized by the host (Eberli et al., 2010; Valentin et al., 2010). However, the application of biological hernia scaffolds is limited by their complicated acellular processes, loss of strength with remodeling, and high cost (Valentin et al., 2010). Further efforts made in designing materials to improve the applicability and functionality appear to be a promising strategy. Based on this concept, developments for different biomedical devices for tissue regeneration have been widely reported (Borzacchiello et al., 2011; Giordano et al., 2011). A novel approach for hernia repair, designing a device to provide temporary support for the defect abdominal wall and regenerate new abdominal tissue with full biodegradability could be a promising alternative to meet current clinical needs for hernia repair.

Chitosan has received considerable attention as a suitable scaffold material in neural, vascular, ligament and tendon tissue regeneration because of its unique biological properties, including fine biocompatibility and anti-inflammatory activity, as well as unique biodegradability (Badhe et al., 2016; Majima et al., 2005). Additionally, the physical and chemical properties of chitosan can be tailored for specific applications by tuning of the acetylation percentage (Kim et al., 2008). Current research showed that chitosan coated polypropylene mesh for abdominal wall repair function very well (Udpa et al., 2013), indicating that chitosan based biomaterial may be an ideal candidate for hernia repair. However, chitosan based materials are associated with chronic inflammation as a long-term outcome for *in vivo* implantation (Xie, Lucchesi, Teach, & Virmani, 2012). This defect was also reported in the study of chitosan fiber woven hernia patch (Ren, 2014). To overcome this challenge, appropriate chemical modification on chitosan should be performed.

* Corresponding author.
E-mail address: baoqinh@ouc.edu.cn (B. Han).

http://dx.doi.org/10.1016/j.carbpol.2017.05.035
0144-8617/© 2017 Elsevier Ltd. All rights reserved.

A novel biodegradable chitin based hernia patch was prepared by acetylation of chitosan fabric. The objective of the present study was to evaluate the biocompatibility and biodegradability of this new hernia patch. In addition, its effects and molecular interactions in repair of the rat abdominal wall partial-thickness defect model were studied.

2. Experimental

2.1. Materials and reagents

Chitosan fabric with a deacetylation degree of 95% and molecular weight of 340 kDa was obtained from Qingdao Biotemed Biomaterial Co., Ltd (Qingdao, China). Polypropylene mesh (PP mesh) was from JNJ (USA) with the trade name PROLENE®. Rat Anti-FGF1 antibody, rat Anti-TGFβ1 antibody, SABC (rat IgG)-POD Kit and the following enzyme-linked immunosorbent assay (ELISA) Kits for rat interleukin-6 (IL-6), rat tumor necrosis factor alpha (TNF-α), rat fibroblast growth factor 1 (FGF1) and rat transforming growth factor beta-1 (TGF-β1) were purchased from Boster Biological Engineering Co., Ltd (Wuhan, China). Masson Stain Kit was purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). Calcein-AM/PI Double Stain Kit, sodium pentobarbital and 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) were from Sigma Chemical Co. (St. Louis, MO, USA). Fetal bovine serum (FBS) and DMEM medium were from GIBCO BRL (Grand Island, NY, USA). Cell culture plates were from Corning INC. (USA).

2.2. Animals and cell lines

Adult male Sprague-Dawley (SD) rats, weighing 220 ± 20 g, were supplied by the Laboratory Animal Center of Shanxi Medical University in China, with certificate of SCXK (Shanxi) 20150001. All animals were kept under a 12 h light-dark cycle at consistent temperature (25 ± 1 °C) and relative humidity (60–70%). Experiments were performed in accordance with the ethical guidelines of the Shandong Province Experimental Animal Management Committee and were in complete compliance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Mouse fibroblast cells (L929) were obtained from Cell Bank of Chinese Academy of Sciences and were grown and subcultured at 37 °C in a humidified incubator with 5% CO_2. The DMEM medium was supplemented with 10% FBS, 100 U/ml penicillin and 100 μg/ml streptomycin. Cells in exponential growth period were used in all experiments. Cell viability was determined by trypan blue exclusion, and ranged from 95% to 98%.

2.3. Preparation and properties analysis of the chitin patch

Chitosan fabric was acetylated with acetic anhydride in methanol (East & Qin, 1993). A Nicolet Nexus-470 (Nicolet Co. USA) Fourier-transformed infrared spectroscopy (FTIR) spectrometer was utilized to characterize chemical structure of the chitin patch. Elemental analysis was used to determine the degree of acetylation with an elemental analyzer (Heraeus, Germany) at Nanjing University. Heavy Metal Content (HMC) and Intracellular Toxin Content (ITC) were measured according to the national standard of China. The typical longitudinal tensile load-displacement curve of the chitin patch with specification of 15 × 25 mm was determined with a AGS-X mechanical testing instrument (Shimadzu Instruments Manufacturing Co., Ltd, China).

2.4. Cytocompatibility test

Chitin patch leaching solution was extracted in DMEM medium with 10% FBS (37 °C, 72 h). L929 cells were seeded in 96 well plates with density of 4×10^4 cells/ml and 200 μl/well and incubated for 24 h. The original culture medium was then replaced by 200 μl of chitin patch leaching solution for each well of the experimental group. Cells treated with complete medium served as control group. Cell morphology in each well was recorded with the T1-SM 100 inverted microscope (Nikon Co. Japan) at time points of 24, 48, and 72 h. At the established time points, 20 μl of MTT (5 mg/ml) solution was added to each well and incubated at 37 °C for 4 h. After that the supernatant was removed, and 100 μl of dimethylsulfoxide was added to each well to dissolve the blue formazan crystal. The absorbance of each well was measured at 492 nm with an ELISA microplate reader (Thermo, USA). Cytocompatibility of chitin patch leaching solution on L929 was calculated using the percentage of relative proliferation rate (RPR) according to the formula below:

$$\text{RPR}(\%) = \left(\frac{\text{Absorbance of the experimental groups}}{\text{Aborbance of the control group}}\right) \times 100$$

Effects of the chitin patch on cell membrane permeability were evaluated by co-culture with L929 cells. Chitin patches with diameters of 6 mm were placed at the bottom of 96 well plates, and sterilized with ethylene oxide at room temperature for 24 h. L929 cells were subsequently seeded with density of 4×10^4 cells/ml and 200 μl/well. A blank control was established with routine culturing. After 24, 48, and 72 h, RPR was detected by an MTT assay as mentioned above. Cell morphology in each well was recorded with the T1-SM 100 inverted microscope (Nikon Co. Japan) at 72 h after Calcein-AM/PI double staining (Krystofiak, Matson, Steeber, & Oliver, 2012).

2.5. In vivo degradability analysis

Biodegradability of chitin patch was studied *in vivo* with subcutaneous and intramuscular implantation in rats. SD rats were anesthetized with sodium pentobarbital (3% in saline) injected intraperitoneally at a dose of 1 ml/kg. Then the back and legs of rats were shaved to expose skin with iodophor disinfection, which was removed by 75% alcohol. Incisions of 10 mm on the back skin and skin accompanied with a layer of muscle on legs were made. Chitin patches (5 × 5 mm) were implanted subcutaneously and intramuscularly through the incisions. Skin closure was performed over the patch by two-layered suture. The implanted samples were morphologically observed at 14, 30, 60, 90, 180, 270, 360 and 450 d respectively after surgery. At the established time point, six rats were sacrificed to observe the degradation of the implants and the samples were then used for histological analysis.

2.6. Establishment and in situ repair of the abdominal wall defect model in rats

The experiment was carried out on adult male SD rats, which is a frequently used animal model for the evaluation of abdominal muscle behavior (Gruber-Blum et al., 2014; Valentin et al., 2010). The partial-thickness abdominal wall defect model has been well established and showed high accuracy and reproducibility for evaluation of muscular tissue remodeling since introduced (Badylak, Kokini, Tullius, Simmons-Byrd, & Morff, 2002; Petter-Puchner, Fortelny, Mittermayr, Öhlinger, & Redl, 2005). To minimize pain, anesthesia was induced with a 3% saline solution of sodium pentobarbital at a dose of 1 ml/kg by intraperitoneal injection. Under sterile surgical techniques, 10 × 20 mm defects were created in the lateral wall of the abdomen comprising the plane of the external oblique, the internal oblique and transversal abdominis muscles, sparing

Download English Version:

https://daneshyari.com/en/article/5156706

Download Persian Version:

https://daneshyari.com/article/5156706

Daneshyari.com