Available online at www.sciencedirect.com
ScienceDirect
Biomaterials 28 (2007) 3091–3100
Biomaterials
www.elsevier.com/locate/biomaterials

Osteochondral repair using the combination of fibroblast growth factor and amorphous calcium phosphate/poly(L-lactic acid) hybrid materials

Xin Huang[a], Disheng Yang[a], Weiqi Yan[b], Zhongli Shi[b], Jie Feng[b], Yanbo Gao[c], Wenjian Weng[c], Shigui Yan[a,*]

[a]*Department of Orthopaedic Surgery, The Second Affiliated Hospital, Medical College of Zhejiang University, Hangzhou 310009, China*
[b]*Institute of Orthopaedic Research, Zhejiang University, Hangzhou 310009, China*
[c]*Department of Material Science and Engineering, Zhejiang University, Hangzhou 310027, China*

Received 2 December 2006; accepted 9 March 2007
Available online 18 March 2007

Abstract

A novel amorphous calcium phosphate (ACP)/poly(L-lactic acid) (PLLA) material, which can experience morphological variations in the microstructure is supposed to be a suitable candidate as scaffold for cartilage tissue-engineering. The purpose of this study was to evaluate the efficacy of this scaffold combined with basic fibroblast growth factor (bFGF) to repair articular cartilage defects in a rabbit model. Forty-two osteochondral defects created in the femoral condyles were (a) left untreated, (b) treated by PLLA combined with bFGF, or (c) ACP/PLLA loaded with bFGF. The treatment of PLLA incorporated with bFGF improved defect filling compared with that left untreated, while the regenerated tissue was mainly fibrocartilage and showed little bone formation with only a small amount of collagen type II (Col II) and no aggrecan gene message measured. When implanted with ACP/PLLA and bFGF, most of the defects were filled with a well-established layer of cartilage tissue with abundance of cartilaginous extracellular matrix accumulation observed. Positive immunohistochemical staining of Col II was observed. High levels of Col II and aggrecan message were also detected by RT-PCR. These results indicate the feasibility of using the combination of ACP/PLLA with bFGF for cartilage repair.

Keywords: Cartilage tissue engineering; Amorphous calcium phosphate; Polylactic acid; Basic fibroblast growth factor; Gene expression

1. Introduction

It has been well established that articular cartilage has a limited capacity for self-repair due to the low mitosis of chondrocytes and avascular supply [1]. Cartilage lesions, whenever resulting from trauma, pathologic alterations or due to the aging-related degenerations, often fail to heal spontaneously and may lead to progressive destruction of the weight-bearing joint and the onset of osteoarthritis [2,3]. In recent years, autologous chondrocyte transplantation and autologous osteochondral grafting have been popularized in the clinical routine and have demonstrated the ability to promote the restoration of cartilage for patients with localized articular cartilage defects in some prospective randomized clinical trials [4–7]. However, the limited source of transplants and donor site morbidity seem quite inevitable, and have to be considered as a potential source for new clinical symptoms [8,9].

Based on the tissue-engineering approach, the use of bioactive agents such as growth factors combined with a three-dimensional scaffold in an appropriate microenvironment to help the body to heal itself has been a focus of attention [10–13]. The key issue in enhancing regeneration of the injured tissue is seeking an appropriate scaffold to provide a niche for cell recruitment to complete functional restoration. Currently, the most popular scaffolds used are bioactive polymers including chitosan [14], collagen [15,16], and hyaluronic acid [12,17]; these materials have the common disadvantages of poor mechanical properties and fast degradation, without adequate time to support cell proliferation and maintain extracellular matrix deposition. Synthetic biodegradable polymers such as poly(L-lactic

*Corresponding author. Tel.: +86 571 8776 7023; fax: +86 571 8702 2776.
E-mail address: sgyan_zrgk@yahoo.com.cn (S. Yan).

0142-9612/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.biomaterials.2007.03.017

acid) (PLLA) are widely used in tissue-engineering due to their good mechanical strength and adjustable degradation rate. However, their chemically hydrophobic and biologically inert properties are quite unfavorable for cell growth [18]. Therefore, modifying the synthetic polymer with bioactive bioceramics like calcium phosphate, to improve its biological activity seems quite important and a number of such hybrid materials have demonstrated good applications in cartilage tissue engineering [19,20].

It has been demonstrated that a variety of growth factors, including transforming growth factor-β (TGF-β) [10,21], basic fibroblast growth factor (bFGF) [12,13], bone morphogenetic protein (BMP) [22], and insulin growth factor (IGF) [23], have the potential to promote the healing been process of osteochondral defects. Among these, bFGF has aroused particular attention because it has been proven to be a chondrocyte mitogen and to have anabolic effects on bone and cartilage repair [24]. Furthermore, it can stimulate the synthesis of cartilaginous matrix of chondrocytes [25].

In the previous work, we have developed a novel material composed of PLLA with the deposition of amorphous calcium phosphate (ACP) particles. This porous ACP/PLLA hybrid scaffold has been demonstrated to be a good material for bone tissue engineering because it could enhance osteoblastic adhesion and differentiation [26], although its application in cartilage repair has never been evaluated. Furthermore, the ACP particles coated on the PLLA pore walls could experience a fast phase transformation and morphological variation to flake-like crystallites when soaked in phosphate-buffered solution (PBS) [26]. We hypothesized that such materials with morphological variation characteristics could be suitable candidates as growth factor carriers. In this work, we introduce this tissue engineered scaffold incorporated with bFGF to repair articular cartilage defect in a rabbit model and evaluate the efficacy of this biodegradable scaffold in delivery of bFGF for cartilage repair.

2. Materials and methods

2.1. Implant preparation

The porous three-dimensional scaffold was fabricated by a thermally induced phase separation technique as described previously [26]. Briefly, ACP powders were prepared by a low temperature co-precipitation method [27] by using $CaCl_2$ (AR, Nanjing Chem. Co.), Na_3PO_4 (AR, Nanjing Chem. Co.) and poly(ethylene glycol) (PEG) (AR, Shanghai Chem. Co.). PLLA ($M_w = 200{,}000$; supplied by Chengdu Institute of Organic Chemistry, Chinese Academy of Sciences) was dissolved in dioxane in the percentage of 5%. Pluronic F127 (Sigma) and ACP were added into the PLLA solution with the percentage of 50 wt% for ACP/(PLLA + ACP). The mixture was subjected to 1 h vigorous stirring, followed by 1 h freezing at -15° and lyophilization in a freeze-dryer for 3 days. The scaffold obtained had a micropore structure with the size of around 100 μm, and the pores were interconnected with each other (Fig. 1a). ACP particles existing as agglomeration were randomly distributed in the pore walls (Fig. 1b). ACP/PLLA material together with PLLA (as control) was made into cylinders with 4 mm in diameter and 5 mm in depth. The scaffolds were sterilized by ethylene oxide.

2.2. In vitro protein release

Recombinant human bFGF (10 μg, Invitrogen) was dissolved in PBS with a final concentration of 10 μg/ml. Each 60 μl bFGF solution was impregnated into ACP/PLLA scaffold and maintained at 37° for 1 h. The composites were placed in polypropylene tubes with 1 ml PBS (Gibco) and incubated statically at 37°. The PBS was entirely removed, frozen and replenished at 1, 3, 5, 8, 11 and 15 days. 60 μl bFGF solution, which was diluted into 1 ml PBS and frozen directly at -20° was acted as a positive control. PBS solution without bFGF was used as a negative control. bFGF concentrations were quantified using an ELISA kit obtained from R&D Systems (Quantikine human FGF basic immunoassay). The experiment was performed in triplicate, and data were represented as mean ± standard deviations (SDs).

2.3. Animal experiments

Twenty-one skeletally mature female Japanese white rabbits weighing about 3.0 kg were used in this study. All animals received bilateral knee joint surgery with a total of 42 osteochondral defects created. Among these, 18 animals with 36 defects were randomly divided into three groups and filled, respectively, with ACP/PLLA and bFGF composites (ACP/PLLA group, $n = 12$), PLLA and bFGF implants (PLLA group, $n = 12$), or left untreated as control (the control group, $n = 12$). Histological evaluation was carried out 4 ($n = 6$ in each group) and 12 weeks ($n = 6$ in each group) postoperatively. The remaining three animals were implanted with ACP/PLLA and bFGF on one side and PLLA together with bFGF on the other side to make an intraindividual comparison to further analyze gene expression 12 weeks after surgery. The experiments were conducted according to the guidelines for animal experiments of Zhejiang University Medical College Ethics Committee.

Prior to surgery, the animals were anesthetized by intramuscular administration of pentobarbital sodium (around 0.03 g/kg body weight, 25 g, Sigma). Besides, discontinuous small amounts of pentobarbital sodium were added intravenously according to the animal's response during the operation. The knee joint was exposed through a medial parapatellar incision and the patella was laterally dislocated to expose the medial condyle. With the knee maximally flexed, an osteochondral defect (4 mm in diameter and 5 mm in depth) was created in the weight-bearing

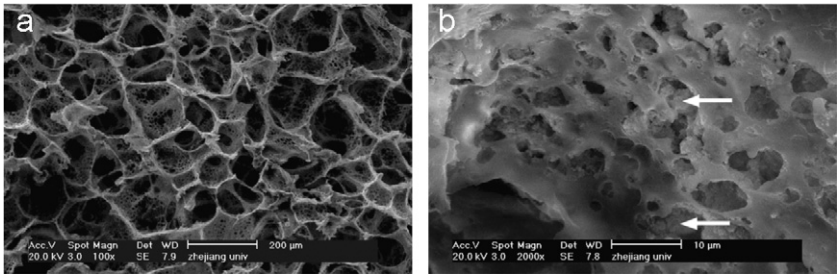

Fig. 1. SEM photographs of the porous ACP/PLLA scaffold. (a) The micropore structure of the scaffold. (b) ACP particles distributed in the pore walls. The white arrow symbol refers to the ACP particles.