Available online at www.sciencedirect.com
ScienceDirect
Biomaterials 28 (2007) 1515–1522
Biomaterials
www.elsevier.com/locate/biomaterials

A platelet-derived growth factor releasing chitosan/coral composite scaffold for periodontal tissue engineering

Yufeng Zhang[1], Yining Wang[1], Bin Shi, Xiangrong Cheng*

Key Laboratory for Oral Biomedical Engineering of Ministry Education, School and Hospital of Stomatology, Wuhan University, Wuhan 430079, PR China

Received 25 September 2006; accepted 29 November 2006

Abstract

In recent years, functional biomaterial research has been directed towards the development of improved scaffolds and new drug delivery systems. The objective of this study was to develop growth-factor gene releasing coral composites as a regenerative material for periodontal regeneration. In this study, porous chitosan/coral composites combined with plasmid encoding platelet-derived growth factor B (PDGFB) gene were prepared through a freeze-drying process. These scaffolds were evaluated *in vitro* by analysis of microscopic structure and cytocompatibility. The expression of PDGFB and type-I collagen were detected with RT-PCR after human periodontal ligament cells (HPLCs) were seeded in this scaffold. Then these scaffolds were implanted subcutaneously into athymic mice. Results indicated that HPLCs showed much better proliferation properties on the gene-activated scaffolds than on the pure coral scaffolds, and the expression of PDGFB and type-I collagen up-regulated in gene-activated scaffold. After implanted *in vivo*, HPLCs not only proliferate but also increased the expression of PDGFB. This study demonstrated the potential of coral scaffold combined PDGFB gene as a good substrate candidate in periodontal tissue regeneration.

Keywords: Chitosan; Coral; Scaffold; Platelet-derived growth factor B; Gene transfer; Periodontium

1. Introduction

Periodontitis, which is a chronic inflammatory disease generally invading gingival, periodontal ligament, cementum and alveolar bone as well, can ultimately result in tooth loss [1]. To achieve periodontal regeneration, which is the ultimate goal of periodontal therapy, various procedures have been proposed so far [2], including guided tissue regeneration (GTR), application of enamel matrix derivative, bone substitutes and autogenous block grafting techniques [3,4]. However, attempts at regeneration of periodontal defects by these methods have not always yielded predictable results [5,6]. In bone replacement grafting, alveolar bone regeneration is seen in association with the formation of a long junctional epithelium, representing periodontal repair and not true regeneration. A Cochrane review of published studies [6] suggests that guided tissue regeneration can be effective at regenerating periodontal attachment to a limited extent, but the overall response rate is unpredictable. Recently, researchers have focused on the regeneration of periodontium using tissue engineering [7,8]. In order to develop techniques for the fabrication of new tissues to replace damaged or diseased tissues, tissue engineering is based on principles of cell biology, developmental biology and biomaterials [9].

Scaffolds are used to create the three-dimensional organization needed for appropriate cell interactions, to serve as vehicles to deliver and retain the cells at a specific site [10,11]. Natural coral (calcium carbonate), which is biocompatible and osteoconductive, has porous structure that is similar to those of human bone [12]. This porous structure can not only provide large internal surface for cell adhesion and migration but also contribute to the absorption of nutrients and metabolism. The applications of natural coral scaffolds for tissue engineering has been issued [13]. In the recent years, increased efforts have been focused on combination of growth factors with different scaffolds/matrixes to modify cell behavior. The growth

*Corresponding author. Tel.: +86 27 87646351.
E-mail address: Xiangrongcheng@hotmail.com (X. Cheng).
[1]Authors contributing equally to the work.

0142-9612/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.biomaterials.2006.11.040

factor delivery system helps the polypeptide to reach the desired defect site within the requisite time and further controls the release time course.

Growth factors are generally accepted to be essential mediators of tissue repair via well-established mechanisms of action that include stimulatory effects on angiogenesis and cellular proliferation, ingrowth, differentiation, and matrix biosynthesis. PDGF has demonstrated great potential in promoting gingival, alveolar bone, and cementum regeneration in a variety of wound healing models [4]. Previous studies demonstrated that sustained expression of PDGFB was effective in inducing periodontal regeneration [14–16]. However, direct application of PDGFB on patients often has limited success. A major problem that has not been overcome is how to localize the delivery of this short half-lived factor to target cells, for large doses of this factor in treatment can result in adverse side effects [17]. Gene transfer might be adapted as a means to provide sustained synthesis of bioactive transgenic products within periodontium. The delivery of the appropriate stimulatory factor(s) in this manner may enable synthesis of an improved periodontal repair tissue. DNA is a stable molecule with a long shelf life so storage is simplified, and it may be less expensive to manufacture than recombinant proteins [18]. But the major disadvantage is low efficiency of transfection.

In recent years, functional biomaterial research has been directed towards the development of improved scaffolds and new drug delivery systems. The potential of chitosan as a polycationic gene carrier has been explored in several research groups [19–21]. As a non-viral vector for gene delivery, chitosan offers certain advantages compared to viral vectors that can produce endogenous recombination, oncogenic effects and immunological reactions leading to potentially serious complications [22]. Moreover, advantages of this system include high-efficiency transfection and the lack of size limitations on the DNA transfection.

The objective of this study is to develop growth factor gene releasing coral composites as a regenerative material for periodontal regeneration. Therefore, in our study, we constructed chitosan/coral composites combined with plasmid encoding PDGFB gene and evaluated for cytocompatibility through seeding human periodontal ligament cells (HPLCs) into scaffold *in vitro*. Furthermore, HPLCs combined with scaffold were implanted into athymic mice to evaluate the biocompatibility *in vivo*.

2. Materials and methods

2.1. Materials

Chitosan (minimum deacetylation degree of 85%) was obtained from Sigma (St. Louis, MO, USA). 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was purchased from Sigma Chemical Com. Dulbecco's modified Eagle's medium (DMEM) and fetal bovine serum (FBS) were from Gibco Company. All other reagents were of analytical grade.

The plasmid DNA was the expression vector (pEGFP, Invitrogene, USA) consisting of the coding sequence of PDGFB (Genebank no: X02811) and the cytomegalovirus enhancer inserted at the upstream (pEGFP–PDGFB). We used enhanced green fluorescent protein (EGFP) for reporter gene. Therefore, we were able to visualize cells on each material by fluorescent microscopy. The plasmid was propagated in *Escherichia coli*, and then isolated and purified. The absorption ratio at the wavelength of 260 and 280 nm was measured for evaluation of plasmid concentration and purity.

2.2. Fabrication of porous coral scaffolds containing chitosan–DNA composite

Preparation of chitosan–DNA composite were fabricated according to the method described by Mao et al. [20]. Briefly, a chitosan solution (0.02% in 5 mmol sodium acetate buffer, pH 5.5) and a DNA solution of 150 μg/ml in 5 mmol of sodium sulfate solution were preheated to 55 °C separately. An equal volume of both solutions were quickly mixed together and vortexed for 30 s. The final volume of the mixture of chitosan and plasmid in each preparation was limited to below 500 μl in order to yield uniform solution. At the same time, sterile natural coral (RegeMed, China) was cut into $5 \times 5 \times 1$ mm pieces in shape and located into 24-well culture plates. After this process, 100 μl solutions was dropped onto coral scaffold, and then kept at 4 °C overnight. These complexes were then frozen by immersion into −80 °C for 2 h and transferred into a freeze-drying vessel (OHRIST BETA 1-15, Germany) for 48 h until dry.

Scaffolds were divided into two groups: group1, the pure coral scaffold group; group2, the coral scaffolds containing chitosan–DNA composite group.

2.3. Human periodontal ligament cells cultured into the scaffolds

HPLCs were isolated and cultured as described in our previous report [23]. These HPLCs were used at passage 2–4 in experiments. After 90% confluence, cells were digested by 0.25% trypsin and cell density was adjusted to 1×10^7 cells/ml. The scaffolds transferred into 24-well plastic culture plates, after prewetted with culture medium overnight, 100 μl cell suspensions were seeded into each scaffold. After 3 h, another 900 μl culture medium was supplied, and the culture was set at 37 °C in 5% CO_2 humidified atmosphere. Cell–scaffold complexes were also examined with a Zeiss LSM 510 2-photon confocal laser scanning microscope on 48 h after seeding.

The PDGFB secreted into culture medium was determined using a commercial PDGFB ELISA kit (R&D Systems Inc, Minneapolis) according to the manufacturer's instructions at day 3, 6, 9, 12, 18 and 24. Briefly, the culture medium was replaced with non-serum medium 24 h before assay, and then the supernatant was collected for evaluation. The mean values of concentration of PDGFB were compared by Student's *t*-test ($n = 4$), and P values equal to or less than 0.05 were considered significant.

2.4. Scanning electron microscopy (SEM) examination

The porous morphologies of the composite scaffolds were studied by SEM (Hitachi X-650). The group1 and group2 scaffolds without cells were frozen in liquid nitrogen for 5 min and then cut for SEM observation. The cells were fixed on scaffold after 2 days of culture with 2.5% glutaraldehyde in 0.1 mol/L sodium cacodylate buffer (pH = 7.3) for 3 h at room temperature, rinsed three times with PBS, and dehydrated in a grade ethanol series. Samples were then critically point dried, coated with gold and observed by SEM.

2.5. MTT assay

Scaffolds were transferred into 96-well plastic culture plates. After prewetted with culture medium overnight, 50 μl cell suspensions contain