Enhanced Osteoinduction by Biodegradable Gelatin-β-tricalcium Phosphate Sponge Capable for Bone Morphogenetic Protein Release

Masaya Yamamoto, Yoshitake Takahashi, Akishige Hokugo and Yasuhiko Tabata*

Department of Biomaterials, Institute for Frontier Medical Sciences, Kyoto University, Kyoto, Japan

Abstract: The objective of this study is to develop an osteoinductive scaffold which enables the controlled release of bone morphogenetic protein (BMP)-2. A biodegradable gelatin sponge incorporating 50 wt% of b-tricalcium phosphate (β-TCP) was fabricated to investigate the osteoinduction activity. The sponge prepared had an interconnected pore structure with an average pore size of 200 mm. The in vivo release test revealed that BMP-2 was released from the sponge for a time period longer than 28 days. Combination of the sponge capable for BMP-2 release and autologous bone marrow cells significantly induced bone regeneration at a segmental defect of rabbit ulnae. It is concluded that the gelatin sponge for BMP-2 release is a promising cell scaffold for osteoinduction *in vivo*.

Keywords:bone morphogenetic protein, controlled release, osteoinductive scaffold, gelatin sponge, β-TCP

Introduction

Bone tissue engineering has been attracted much attention as a new therapeutic technology which induces bone regeneration by making use of scaffolds, osteoinductive growth factors, and osteogenic cells, or their combination[1]. It is no doubt that combination of osteoinductive growth factors and scaffolds provides an appropriate osteoinductive environment for osteogenic cells.

Osteoinductive growth factors, such as bone morphogenetic protein (BMP), transforming growth factor-b, and basic fibroblast growth factor, have been used to promote bone regeneration[2]. Recently we have explored a biodegradable hydrogel of gelatin suitable for the in vivo controlled release of BMP-2. The hydrogel enabled BMP-2 to retain at the implanted site for extended time periods and consequently enhance the potential of bone induction, in marked contrast to free BMP-2[3].

Since it is basically expected that scaffolds for bone regeneration function as the substrate for the attachment and proliferation of osteogenic cells, 3-dimensional biodegradable materials with a porous structure have been extensively investigated. Among them, b-tricalcium phosphate (β-TCP) of bioactive ceramics has been employed as the cell scaffold for bone tissue engineering because of its bioabsorbability and inherent compatibility to natural bone tissue[4].

In this study, we have designed a biodegradable cell scaffold of organic-inorganic composite which has an ability for the controlled release of BMP-2. A biodegradable gelatin sponge incorporating β-TCP was fabricated to investigate the osteoinduction activity. The time profile of BMP-2 release was evaluated following the implantation of gelatin sponges incorporating BMP-2 into the back subcutis of mice. We also examine feasibility of combination of the sponge capable for BMP-2 release and autologous bone marrow cells in enhancing bone regeneration at a rabbit model of ulna defect.

Correspondence toYasuhiko Tabata, Ph.D., D. Med. Sci., D. Pharm. Institute for Frontier Medical Sciences, Kyoto University 53 Kawara-cho Shogoin, Sakyo-ku, Kyoto, 606-8507 Japan, E-mail: yasuhiko @ frontier.kyoto-u.ac.jp

Materials and Methods

A biodegradable gelatin sponge incorporating 50 wt% of β-TCP (gelatin- β-TCP sponge) was prepared by chemical crosslinking of gelatin with an isoelectric point of 9.0 (Nitta Gelatin Co., Osaka, Japan) with glutaraldehyde in the presence ofβ-TCP granules (2 mm in average diameter, Taihei Chemical Industries, Nara, Japan)[5]. To prepare gelatin-b-TCP sponges incorporating BMP-2, 20 μl of aqueous solution containing BMP-2 or ^{125}I-labeled BMP-2 was dropped onto the freeze-dried sponge, followed by leaving it at 4 °C overnight[5]. The gelatin-b-TCP sponges incorporating ^{125}I-labeled BMP-2 were implanted into the back subcutis of 6 week-age female ddY mice. At different time intervals, the radioactivity of gelatin sponge remained was measured on a gamma counter to assess the time profile of in vivo BMP-2 release.

A segmental bone defect (20 mm) was created at the ulnar bone of rabbits, followed by implantation of the autologous bone marrow cell-seeded gelatin sponges (20 x 5 x 5 mm^3) with or without BMP-2 incorporation. The BMP-2 dose was 17 mg / sponge. Bone regeneration was radiographically examined by Soft x-ray at 54 KVP and 2.5 mA for 20 sec.

Results and Discussion

The present study demonstrates that the in vivo osteoinductive activity of gelatin-β-TCP sponges incorporating bone marrow cells was greatly enhanced by the controlled release of BMP-2. Although it is known that gelatin is one of the substrate materials for cell adhesion and proliferation, the hydrogel dose not always function as a good scaffold of migration, proliferation, and differentiation of cells, because of no porous structure necessary for cell infiltration[3]. Therefore, in this study, a hydrogel with a porous sponge structure was fabricated by a homogenization process of aqueous gelatin solution. The pore size was adjusted at 180-200 mm since the pore size suitable for cell infiltration and ingrowth of host bone tissue is reported to be in the range of 100-350 mm[6]. On the other hand, when incorporated into gelatin-β-TCP sponges, BMP-2 was retained in the sponges in vivo for more than one month while a long-termed BMP-2 release was achieved (data not shown). These results indicate that the gelatin sponge obtained functions not only as the release carrier for growth

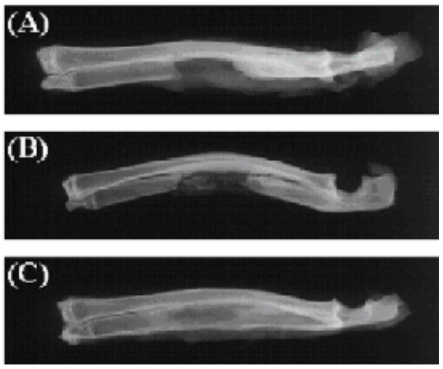

Fig.1. Radiographic pictures of ulna defects 6 weeks after application with gelatin-b-TCP sponges incorporating phosphate-buffered saline solution (A), bone marrow cells (B), and bone marrow cells and BMP-2 (C).

factor, but also as the scaffold of cell attachment and proliferation.

It is undoubtedly necessary for successful tissue regeneration to make use of cells constituting tissue to be regenerated. Considering the proliferation and differentiation potential of cells, stem cells are practically promising. Bone marrow cells have been clinically considered as one of stem cell sources, such as hematopoietic and mesenchymal stem cells (MSC). Indeed, MSC have been extensively investigated to apply the therapy of regenerative medicine because of its inherent potential to differentiate into the various cell lineages[7]. However, when seeded with autologous bone marrow cells and implanted into an ulna defect, the gelatin sponge dose not induce significant bone regeneration 6 weeks after implantation (Figure 1). On the contrary, the sponge was incorporated by BMP-2 for release and then seeded with bone marrow cells. Following implantation of the sponge prepared, significant bone regeneration was observed at the bone defect and the defect was completely filled with the bone newly formed (Figure 1). Thies et al reported that BMP-2 induced a high level of osteocalcin in bone marrow cells in a dose-dependent manner[8]. Therefore, it is reasonable to think that the gelatin-β-TCP sponge allows BMP-2 to achieve the slow release, resulting in biological stimulation of bone marrow cells in the sponge to induce bone regeneration.

In conclusion, the biodegradable gelatin-β-TCP sponge with an ability for BMP-2 release is a promising bone cell scaffold for osteoinduction *in vivo*.

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